Establishment Success and Potential Weediness of Oilseed Rape (*Brassica napus* L.) under Ruderal Conditions in View of GMO Risk Assessment

**Dissertation**

zur Erlangung des naturwissenschaftlichen Doktorgrades im Fach Biologie (Fachbereich 2) an der Universität Bremen

vorgelegt von

Jana Seeger

Bremen, 12.12.2013

1. Gutachterin: Prof. Dr. Juliane Filser
2. Gutachter: Prof. Dr. Martin Diekmann

Datum des Promotionskolloquiums: 14.02.2014
# Table of contents

ABBREVIATIONS ........................................................................................................... 1  
SUMMARY ..................................................................................................................... 5  
ZUSAMMENFASSUNG ................................................................................................... 7  

1. GENERAL INTRODUCTION ......................................................................................... 9  
1.1 OVERVIEW ............................................................................................................ 9  
1.1.1 Transgenic OSR and ruderal populations ......................................................... 9  
1.1.2 Aims and outline ............................................................................................... 11  
1.2 BIOLOGY OF BRASSICA NAPUS ........................................................................... 15  
1.2.1 General aspects ............................................................................................... 15  
1.2.2 Characteristics of feral populations ................................................................. 17  
1.2.3 Seed bank dynamics ......................................................................................... 18  
1.3 BRASSICA NAPUS AS A GM PLANT ..................................................................... 19  
1.3.1 Relevance as a GM plant ................................................................................ 19  
1.3.2 Risks ................................................................................................................ 21  
1.3.3 Transgene escape via feral populations .......................................................... 22  

2. ESTABLISHMENT AND REPRODUCTIVE SUCCESS OF BRASSICA NAPUS (L.) UNDER RUDERAL CONDITIONS ............................................................................. 25  
2.1 INTRODUCTION .................................................................................................... 25  
2.2 METHODS ............................................................................................................. 28  
2.2.1 Site characterization ....................................................................................... 28  
2.2.2 Cultivars .......................................................................................................... 29  
2.2.3 Design ............................................................................................................. 30  
2.2.4 Estimates of establishment success and reproductive potential ....................... 32  
2.2.5 Data analysis .................................................................................................... 33  
2.3 RESULTS .............................................................................................................. 34  
2.3.1 Factors influencing establishment success on ruderal sites ............................. 35  
2.3.2 Seed density ..................................................................................................... 40  
2.3.3 Differences between sowing events ............................................................... 40  
2.3.4 Ruderal vs. agricultural sites ........................................................................... 42  
2.4 DISCUSSION ....................................................................................................... 42  
2.4.1 Factors influencing establishment success on ruderal sites ............................. 42  
2.4.2 Ruderal vs. agricultural sites ......................................................................... 45  
2.4.3 Differences between sowing events ............................................................... 46  
2.4.4 Seed density ..................................................................................................... 46  
2.4.5 Conclusions ..................................................................................................... 47  
REFERENCES .............................................................................................................. 48  

3. ESTABLISHMENT AND REPRODUCTIVE SUCCESS OF OILSEED RAPE (BRASSICA NAPUS L.) AND WEEDY RELATIVES ON POOR-QUALITY RUDERAL SOILS ........................................................................... 52  
3.1 INTRODUCTION .................................................................................................... 52  
3.2 METHODS ............................................................................................................. 55  
3.2.1 Site description ............................................................................................... 55  
3.2.2 Design ............................................................................................................. 56  
3.2.3 Seed origin and sowing .................................................................................. 57  
3.2.4 Estimates of establishment success and reproductive potential ....................... 58  
3.2.5 Seed viability ................................................................................................... 58  
3.2.6 Data analysis .................................................................................................... 59  
3.3 RESULTS .............................................................................................................. 61  
3.3.1 Plant species differences ................................................................................ 61  
3.3.2 Substrate effects ............................................................................................... 64  
3.3.3 Substrate effects on plant species differences .................................................. 66
6.2 IMPLICATIONS FOR GMO RISK ASSESSMENT

6.2.1 Limitations of this study
6.2.2 Consequences for the fitness of transgenic lines
6.2.3 Relevance of establishment on ruderal sites
6.2.4 Conditions and traits which can increase weediness
6.2.5 Mitigation approaches
6.2.6 Consequences for modelling, monitoring and management

6.3 CONCLUSIONS AND OUTLOOK

6.3.1 Main findings
6.3.2 First insights
6.3.3 Resulting research needs
6.3.4 Implications for GMO risk assessment

RESUME

APPENDIX I: FAILED EXPERIMENTS

I.1 IS DWARFED OILSEED RAPE (BRASSICA NAPUS L.) LESS COMPETITIVE THAN CONVENTIONAL OSR?

I.1.1 Objective
I.1.2 Methods
I.1.3 Results and discussion

I.1 THE EFFECT OF COLLEMBOLA ON OSR SEED PERSISTENCE

I.2.1 Objective
I.2.2 Methods
I.2.3 Results and discussion

APPENDIX II: METHODS AND SITE DATA

II.1 EXPERIMENTAL SITES CHAPTER 2
II.2 SOIL ANALYSES

II.2.1 Chapter 2
II.2.2 Chapter 3
II.2.3 Chapter 4
II.2.4 Chapter 5
II.2.5 Standard methods
II.2.6 Nutrient content of soil samples

II.3 RAILWAY TRACKS AND MINI-SITES

II.4 ADDITIONAL SITE CONDITIONS CHAPTER 2
II.5 CLIMATE DATA OF THE STUDY PERIOD

II.5.1 Chapter 2
II.5.2 Chapter 3
II.5.3 Chapter 4
II.5.4 Chapter 5

II.6 LIST OF CENSUSES CHAPTER 2

II.7 SPLIT-PLOT DESIGNS

II.8 SITE DESCRIPTION CHAPTERS 3 AND 4

II.9 CENSUSES CHAPTER 3

II.10 TETRAZOLIUM TEST FOR SEED VIABILITY

II.10.1 Test protocol

II.11 DEFAUNATION OF SOILS

II.12 ATTEMPTED RESOWING (SUBSTRATE COMPARISON CHAPTER 4)

II.13 CENSUSES CHAPTER 4
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>cultivar Artus</td>
</tr>
<tr>
<td>AAS</td>
<td>atomic absorption spectrophotometry</td>
</tr>
<tr>
<td>AF</td>
<td>agricultural fields</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>B</td>
<td>boron</td>
</tr>
<tr>
<td>BCH</td>
<td>Biosafety Clearing-House</td>
</tr>
<tr>
<td>BfN</td>
<td>Bundesamt für Naturschutz (a federal agency)</td>
</tr>
<tr>
<td>BMELV</td>
<td>Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz (a federal ministry)</td>
</tr>
<tr>
<td>B. napus</td>
<td>Brassica napus</td>
</tr>
<tr>
<td>B. nigra</td>
<td>Brassica nigra</td>
</tr>
<tr>
<td>B. rapa</td>
<td>Brassica rapa</td>
</tr>
<tr>
<td>Bt</td>
<td>Bacillus thuringiensis</td>
</tr>
<tr>
<td>BÜK</td>
<td>Bodenübersichtskarte (a soil survey map)</td>
</tr>
<tr>
<td>C</td>
<td>carbon, unless otherwise denoted</td>
</tr>
<tr>
<td>°C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>Ch.</td>
<td>chapter</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>cm</td>
<td>centimetre</td>
</tr>
<tr>
<td>CO₂</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>CORINE</td>
<td>coordination of information on the environment</td>
</tr>
<tr>
<td>ctr</td>
<td>unmown control plots</td>
</tr>
<tr>
<td>D</td>
<td>disturbed</td>
</tr>
<tr>
<td>d.f.</td>
<td>degrees of freedom</td>
</tr>
<tr>
<td>dd</td>
<td>denominator degrees of freedom from Wald test</td>
</tr>
<tr>
<td>DM</td>
<td>dry mass</td>
</tr>
<tr>
<td>dn</td>
<td>degrees of freedom from Wald test</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DWD</td>
<td>Deutscher Wetterdienst (German Weather Service)</td>
</tr>
<tr>
<td>e.g.</td>
<td>for example</td>
</tr>
<tr>
<td>E</td>
<td>east</td>
</tr>
<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
</tr>
<tr>
<td>EPSPS</td>
<td>5-enolpyruvyl-shikimate-3-phosphate synthase</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>F</td>
<td>variance ratio from ANOVA, unless otherwise denoted</td>
</tr>
<tr>
<td>F*</td>
<td>F value of the Wald test</td>
</tr>
<tr>
<td>F1</td>
<td>first filial generation</td>
</tr>
<tr>
<td>F2</td>
<td>second filial generation</td>
</tr>
<tr>
<td>FIA</td>
<td>flow injection analysis</td>
</tr>
<tr>
<td>Fig.</td>
<td>figure</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
</tbody>
</table>
Abbreviations

G  grassland sites
$g^{-1}$  per gram
$g^*m^{-2}$  gram per square metre
GC  gas chromatography
GenStat  General Statistical package (statistical software)
GLMM  generalized linear mixed model
GLU  glufosinate
GLY  glyphosate
GM  genetically modified
GMHR  genetically modified herbicide resistant
GMO  genetically modified organism
GMP  genetically modified plant
gpi  green partners international
h  hour
ha  hectare
HH  high quality sites with high vegetation cover
HL  high quality sites with low vegetation cover
H$_2$O  water
HR  herbicide resistant
H-test  Kruskal-Wallis H-test
IBM  International Business Machines Corporation
i.e.  that is
IL  Illinois
Inc.  Incorporated
Int.  International
ISAAA  International Service for the Acquisition of Agri-Biotech Applications
IWM  integrated weed management
K  potassium
km$^{-2}$  per square kilometre
K$_2$O  potassium oxide
L  litre
L.  Linnaeus
LBEG  Landesamt für Bergbau, Energie und Geologie (a state office)
LH  low quality sites with high vegetation cover
LL  low quality sites with low vegetation cover
LQ  lower quartile
Ltd.  Limited
LWK  Landwirtschaftskammer (a chamber of agriculture)
m  metre
m$^2$  square metre
m$^2$  per square metre
mg  milligram
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgO</td>
<td>magnesium oxide</td>
</tr>
<tr>
<td>mio</td>
<td>million</td>
</tr>
<tr>
<td>ml</td>
<td>millilitre</td>
</tr>
<tr>
<td>mm</td>
<td>millimetre</td>
</tr>
<tr>
<td>Mon810</td>
<td>genetical modification event Mon810 (Bt-corn)</td>
</tr>
<tr>
<td>n</td>
<td>number of replicates, unless otherwise denoted</td>
</tr>
<tr>
<td>N</td>
<td>nitrogen</td>
</tr>
<tr>
<td>N₂</td>
<td>nitrogen gas</td>
</tr>
<tr>
<td>n.s.</td>
<td>not significant</td>
</tr>
<tr>
<td>NOₓ</td>
<td>nitrogen oxide</td>
</tr>
<tr>
<td>NPK</td>
<td>nitrogen, phosphorus, potassium</td>
</tr>
<tr>
<td>NV</td>
<td>non-viable</td>
</tr>
<tr>
<td>O₂</td>
<td>oxygen gas</td>
</tr>
<tr>
<td>OECD</td>
<td>Organisation for Economic Co-operation and Development</td>
</tr>
<tr>
<td>OSR</td>
<td>oilseed rape</td>
</tr>
<tr>
<td>p.</td>
<td>page</td>
</tr>
<tr>
<td>p</td>
<td>significance level</td>
</tr>
<tr>
<td>P</td>
<td>phosphorus, unless otherwise denoted</td>
</tr>
<tr>
<td>PE</td>
<td>polyethylene</td>
</tr>
<tr>
<td>pers. comm.</td>
<td>personal communication</td>
</tr>
<tr>
<td>pH</td>
<td>negative logarithm of the hydronium ion concentration</td>
</tr>
<tr>
<td>PH</td>
<td>low quality sites with high vegetation cover</td>
</tr>
<tr>
<td>P₂O₅</td>
<td>phosphorus pentoxide</td>
</tr>
<tr>
<td>PR45DO3</td>
<td>name of a semi-dwarf hybrid OSR cultivar</td>
</tr>
<tr>
<td>PVC</td>
<td>Polyvinyl chloride</td>
</tr>
<tr>
<td>qual.</td>
<td>soil quality</td>
</tr>
<tr>
<td>r</td>
<td>correlation coefficient</td>
</tr>
<tr>
<td>RH</td>
<td>ruderal site with high soil quality</td>
</tr>
<tr>
<td>RL</td>
<td>ruderal site with low soil quality</td>
</tr>
<tr>
<td>R. raph.</td>
<td>Raphanus raphanistrum</td>
</tr>
<tr>
<td>R. raphanistrum</td>
<td>Raphanus raphanistrum</td>
</tr>
<tr>
<td>R. solani</td>
<td>Rhizoctonia solani</td>
</tr>
<tr>
<td>RT</td>
<td>railway track</td>
</tr>
<tr>
<td>s</td>
<td>second</td>
</tr>
<tr>
<td>S</td>
<td>soil quality</td>
</tr>
<tr>
<td>s.c.</td>
<td>substrate comparison experiment</td>
</tr>
<tr>
<td>s.m.</td>
<td>simulated moving</td>
</tr>
<tr>
<td>SE</td>
<td>standard error</td>
</tr>
<tr>
<td>spp.</td>
<td>species, not identified</td>
</tr>
<tr>
<td>SOM</td>
<td>soil organic matter content</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences Incorporated (statistical software)</td>
</tr>
<tr>
<td>ssp.</td>
<td>subspecies</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>Description</td>
</tr>
<tr>
<td>---------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Sz-online</td>
<td>Süddeutsche Zeitung online (an online newspaper)</td>
</tr>
<tr>
<td>TM</td>
<td>transgenic mitigation</td>
</tr>
<tr>
<td>T-test</td>
<td>Student's T-test</td>
</tr>
<tr>
<td>TransGen</td>
<td>Transparenz Gentechnik (a genetic engineering website)</td>
</tr>
<tr>
<td>TZ</td>
<td>tetrazolium chloride</td>
</tr>
<tr>
<td>U</td>
<td>undisturbed plot</td>
</tr>
<tr>
<td>UFOP</td>
<td>Union zur Förderung von Öl- und Proteinpflanzen (an agricultural union)</td>
</tr>
<tr>
<td>U.S.</td>
<td>United States</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>µm</td>
<td>micrometre</td>
</tr>
<tr>
<td>UQ</td>
<td>upper quartile</td>
</tr>
<tr>
<td>U-test</td>
<td>Man-Whitney U-test</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>V</td>
<td>vegetation cover</td>
</tr>
<tr>
<td>VC</td>
<td>vegetation cover</td>
</tr>
<tr>
<td>veg.</td>
<td>vegetation cover</td>
</tr>
<tr>
<td>vs.</td>
<td>versus</td>
</tr>
<tr>
<td>WDG</td>
<td>water-dispersable granule</td>
</tr>
<tr>
<td>WHC or WHC&lt;sub&gt;max&lt;/sub&gt;</td>
<td>maximum water-holding capacity</td>
</tr>
<tr>
<td>w/w</td>
<td>mass fraction (mass/mass)</td>
</tr>
<tr>
<td>yr&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>per year</td>
</tr>
<tr>
<td>Zn</td>
<td>zinc</td>
</tr>
<tr>
<td>Ø</td>
<td>diameter</td>
</tr>
<tr>
<td>χ&lt;sup&gt;2&lt;/sup&gt;</td>
<td>chi-square value of the Kruskal-Wallis H-test or Friedman test</td>
</tr>
</tbody>
</table>
Summary

Genetically modified (GM) herbicide-resistant (HR) lines of oilseed rape (OSR) are pending approval for cultivation in the EU. A comprehensive risk assessment is therefore needed to evaluate whether these or future GM lines pose a threat to the environment. The probability for an unintended spread of transgenes is high: OSR can persist in subsequent crops as a volunteer plant and grow outside cultivation in feral (wild) populations. Feral plants typically grow on disturbed ruderal sites with little competing vegetation. Monitoring programmes need to include these plants as potential vectors for uncontrolled transgene spread. However, we have little information on population dynamics of feral OSR. It is contentious whether the usually short-lived feral populations can grow self-sustained without external seed input.

The aim of my thesis is to provide baseline data on the establishment success and reproductive output of ruderal OSR populations and to determine environmental factors of relevant impact focusing on the effects of soil quality. Establishment success of OSR was low under natural ruderal conditions in a three-year field experiment with up to 28 sites in Northern Germany. Major mortality factors were competing vegetation, herbivory and disturbance, while high soil quality, fertilisation and the creation of disturbed microsites were beneficial.

Substantial reproductive output was observed on a ruderal site in the absence of competing vegetation and when herbivores were excluded. In this field experiment, the cultivated species OSR and Brassica rapa and their weedy relatives Raphanus raphanistrum and Brassica nigra were grown together in containers (65 L) with different substrates. OSR produced more seeds than its weedy relatives. Potential weediness thus appears to be high. OSR showed potential for self-sustained population increase even on low-quality soils but was less successful on sandy than on more humic soil. To predict the overall influence of soil quality, indirect effects via competing vegetation need to be included. Microsites for establishment were more quickly invaded by competing vegetation if soil quality was very high. OSR should thus perform best on soils with intermediate quality.

Dwarfing, intended as a method of transgenic mitigation, reduces OSR fitness under competitive conditions but might lead to higher fitness under stressors relevant on ruderal sites, i.e. mowing and low soil quality. A third field experiment therefore compared the fitness of a dwarfed OSR cultivar with that of a tall cultivar on a ruderal site. The semi-dwarf hybrid PR45D03 produced less seeds per plot than the tall cultivar Artus in containers with ruderal
soils of low to high quality. The tall cultivar was more likely to be damaged by simulated mowing, but fully compensated or even over-compensated for its higher loss in biomass. Therefore, dwarfing could be used to mitigate transgene escape, but would not prevent it as the semi-dwarf hybrid still showed substantial reproductive output under all conditions.

The potential for seed dormancy is important for long-term establishment success of OSR. OSR seeds were buried in minicontainers in up to nine sites in two consecutive years. The persistence of dormant OSR seeds in the seed bank decreased with soil pH but was not affected by soil water-holding capacity and organic matter content. Soil fungi caused some mortality in seeds at dry conditions. Meso- and small macrofauna had a positive effect on seed persistence at more humid conditions and low organic matter content.

The observed overall low establishment success could not explain the high frequency of feral OSR populations in Bremen. This suggests very high levels of seed input to ruderal environments or systematic dispersal to suitable sites. The potential for weediness under favourable conditions was however high. Transgenic OSR is thus likely to reproduce and persist in disturbed ruderal habitats in case of GMHR OSR cultivation. This study provides baseline data for more accurate predictions of feral OSR population dynamics. Weediness of feral OSR will however be restricted to very specific conditions. A well-targeted monitoring should concentrate on frequently disturbed sites with intermediate soil quality and sites with acidic pH (~5) which are sheltered from major herbivores. Monitoring and risk assessment should concentrate on conditions which could create more invasive genotypes.
Zusammenfassung


Zusammenfassung

Zwergwuchs, eine Methode zur transgenen Schadensminderung, verringert die Fitness von Raps unter Konkurrenzbefindungen, könnte aber unter Stressfaktoren, die auf Ruderalflächen relevant sind, wie Mahd und geringe Bodenqualität, zu höherer Fitness führen. In einem dritten Freilandexperiment wurde daher die Fitness einer zwergwüchsigen Rapssorte mit der einer hochwüchsigen Sorte auf einer Ruderalfläche verglichen. Der Halbzwerghybride PR45D03 produzierte in Containern mit ruderalen Böden von geringer bis hoher Qualität weniger Samen pro Aussaat-Quadrat als die hochwüchsige Sorte Artus. Die hochwüchsige Sorte wurde mit höherer Wahrscheinlichkeit bei der simulierten Mahd beschädigt, kompensierte aber ihren höheren Biomasseverlust vollständig oder zeigte sogar eine Überkompensation. Zwergwuchs könnte zur Minderung der Ausbreitung von Transgenen verwendet werden, würde sie aber nicht verhindern, da der Halbzwerghybride dennoch unter allen Bedingungen das Potential für eigenständiges Populationswachstum zeigte.


1. General Introduction

1.1 Overview

1.1.1 Transgenic OSR and ruderal populations

Worldwide cultivation of genetically modified (GM) crops has risen from 1.7 million ha in 1996 to 170 million ha in 2012. These are mainly located in the U.S. (69.5 mio. ha), Brasil (36.6 mio. ha), Argentina (23.9 mio. ha), Canada (11.6 mio. ha) and India (10.8 mio. ha) (ISAAA 2013a). Germany ceased GM crop cultivation in 2012 – the GM potato Amflora has been withdrawn from the market after two years of cultivation, and approval for cultivation of the pest-resistant Bt-maize MON810 has been suspended since 2009 (TransGen 2013a).

Soybean, cotton, maize and oilseed rape (OSR) are the worldwide prevailing GM crops by acreage. By far the most dominant GM trait is herbicide resistance, followed by stacking of herbicide resistance with insect resistance and insect resistance alone (ISAAA 2013a). A comprehensive risk assessment (Hilbeck et al. 2011) is an important prerequisite for safe use of this new technology.

Herbicide-resistant (HR) OSR has received much attention in the debate over safety of GM plants (Chapman & Burke 2006, Devos et al. 2004, Senior & Dale 2002). OSR cultivars resistant to the broad-spectrum herbicides glyphosate and glufosinate are grown throughout North America, in South America and in Australia (TransGen 2012a). So far, the applications for cultivation in the EU have not been granted (1.3.1). The introduction of GMHR OSR could have undesired consequences for the environment, the economy and possibly human health. These risks need to be weighed against realized benefits (see 1.3.1 and 1.3.2 for details on both). Possible environmental effects of GMHR OSR cultivation reach from molecular and physiological to landscape-scale processes (Züghart & Breckling 2003). Indirect negative effects on invertebrates have already been demonstrated (Bohan et al. 2005, Hawes et al. 2003). Recent research has further raised concern regarding the toxicity of glyphosate-based herbicides to vertebrates and humans (e.g. Paganelli et al. 2010, Mesnage et al. 2013). Another problem lies in weed species shifts (Bohan et al. 2005) and evolution. Several weed species have already evolved resistance to glyphosate (Powles 2008), which may explain an increased use of herbicides in GMHR cropping systems (see chapter 6.3.4). HR transgenes can further be introgressed into weedy relatives through hybridisation (Warwick et al. 2008). Possible consequences of such crop-to-wild gene flow are increased weediness or the
extinction of wild relatives (Ellstrand 2001, 2003). Presence of HR traits might also increase weediness of the GMHR crop itself.

One aim of the EU is to ensure that other types of agriculture can co-exist next to GM crops (European Commission 2003, 2010). The effective implementation of measures to ensure co-existence is a problem yet unsolved, as are questions of responsibility and liability for the resulting costs (Messéan et al. 2009). The risk of transgene escape in OSR is overall high (Devos et al. 2004, Jørgensen et al. 2009). Adventitious presence in non-GM OSR can occur through various pathways (Messéan et al. 2009): Cross-pollination between OSR fields is a moderate risk which can be controlled through spatial separation. The highest risk is posed by so-called volunteer populations of OSR in subsequent crops. Volunteers pollinate with non-GM crops and are difficult to control. The crop can further hybridise with wild relatives (FitzJohn et al. 2007, Warwick et al. 2008, (1.2.1)), leading to moderate risks of transgene spread in areas where relatives are abundant in fields (Messéan et al. 2009). OSR seeds are dispersed accidentally in large numbers (Bailleul et al. 2012, Lutman et al. 2005), giving rise to feral (wild) populations on ruderal sites (Breckling & Menzel 2004, Crawley & Brown 2004). The probability that feral plants cause crop impurities is rated as low due to their low density in comparison with crops and volunteers (Messéan et al. 2009, Squire et al. 2009). However, they provide avenues for the uncontrolled and unmonitored spread of transgenes in the environment (Squire et al. 2009).

Monitoring the effects of released GMOs on the environment is mandatory as outlined by EU Directive 2001/18/EC (European Parliament and Council 2001) and Decision 2002/811/EC (European Council 2002). According to the EFSA (2010), “the ability of the GM plant to form feral populations and hence the potential impacts on the receiving environment should be considered” where appropriate. Menzel (2006) previously made a convincing case for including ruderal and urban OSR populations in monitoring programmes. Züghart & Breckling (2003) recommend a monitoring of wild OSR populations within a radius of 5 km surrounding deliberate release sites and at large distances. Yet, an exhaustive monitoring of all ruderal populations will hardly be feasible. Monitoring should focus on representative and relevant sites with favourable environmental conditions (Züghart et al. 2008). It will be crucial to identify regions with high risk of transgene spread and local hotspots with a high likelihood of persisting feral plants.
In this thesis, I concentrate on ruderal sites such as dump sites, road verges and industrial wasteland. OSR is frequently found on such sites in urban environments (Menzel 2006). Ruderal vegetation is the foremost herbaceous vegetation of sites with strong anthropogenic changes and/or disturbance, if these are neither used for agriculture nor for forestry (translated from Brandes 2007). The flora of fresh soil depositions in Bremen is dominated by annual and biennial species, with considerable numbers of rare species and neophytes (Müller & Kuhbier 2008). Urban soils show a high spatial and vertical heterogeneity (Meuser 2010). The pH is comparatively alkaline due to calcium leakage from e.g. cement and paving stones. Street verges often reach pH 9 as a result of de-icing salt. However, ash and deposition of airborne particles can lead to acidification of the soil surface, so that acidic soils are also common, e.g. in the vicinity of trees (Gilbert 1994, Hiller & Meuser 1998). Nutrient status seems equally variable: While airborne particles, sludge, manure and fertiliser can lead to eutrophication comparable to agricultural soils or higher (garden soils!) (Hiller & Meuser 1998, Pietsch & Kamieth 1991), many urban soils are unfertilised, show a low content of organic matter and thus a low supply of nitrogen and phosphorus (Fellenberg 1991, Pulford 1991).

Ruderal OSR populations need to be considered when schemes to limit transgene spread are devised. Transgenic mitigation (TM) links transgenes to genes conferring a fitness disadvantage. Dwarfing has been developed as a TM trait and can reduce the fitness of OSR volunteers growing under competitive conditions (Al-Ahmad et al. 2006). Reuter et al. (2008) reveal that existing fitness comparisons do not account for conditions commonly found in urban environments, where most ferals grow on sites with little competing vegetation. Reuter et al. (2008) propose that dwarfed OSR could be more likely to escape damage, e.g. by mowing. In addition, dwarfed OSR might be more tolerant to drought or more nitrogen-use efficient, as has been shown for dwarfed varieties of wheat (Blum & Sullivan 1997, Singh & Arora 2001) and sunflower (Angadi & Entz 2002).

1.1.2 Aims and outline

The aim of this thesis is to provide baseline data on the establishment success of OSR feral plants and to identify factors of relevant impact. Some work has already been done on the distribution (Schafer et al. 2011, Schoenenberger & D'Andrea 2012) and dynamics of feral populations and wild relatives (Dietz-Pfeilstetter et al. 2006, Elling et al. 2009, Kawata et al. 2009, Knispel & McLachlan 2010, Menzel 2006), population dynamics along road verges (Claessen et al. 2005a, 2005b, Crawley & Brown 1995, 2004) and invasiveness of natural habitats (Crawley et al. 1993, 2001). These studies have mostly drawn conclusions from the
observation of existing populations, but do not expressively link seed input and establishment success: How much propagule pressure is necessary to successfully establish and maintain a feral population, and what are the major obstacles? Are OSR populations self-sustaining (e.g. Elling et al. 2009, Pessel et al. 2001), or do they depend on renewed seed input by humans (e.g. Crawley & Brown 2004)? To my knowledge, the only study which has addressed these questions by dispersing seeds and recording plant establishment and reproductive output with varying environmental factors has been conducted in natural habitats (Crawley et al. 1993, 2001). While the authors included a gravel and a bracken site, extensive comparable studies are still lacking in ruderal environments, where feral plants can be very successful (e.g. on railway tracks, see Menzel 2006). We also do not know how to evaluate observed establishment success: On the one hand, we can calculate rates of population increase to predict self-sustained growth. On the other hand, we do not know how these compare to the performance of weedy plants, which persist in the environment and even cause weed problems. In my thesis, I therefore address these questions with sowing experiments on ruderal sites, using conventional cultivars but discussing my results in the context of GM lines. I wish to contribute to a better understanding and predictability of population dynamics on ruderal sites and to an evaluation of weediness outside cultivated areas. This can also help to identify traits which could increase weediness of OSR, which is relevant both for risk assessment and transgenic mitigation (TM) strategies.

The thesis starts with an overview and an outline, followed by more detailed background on the study organism and aspects relevant for transgene dispersal. I then describe several sowing experiments (chapters 2-4) which quantify establishment success and address the question whether populations are self-sustaining. In addition, these experiments determined the relevance of various environmental factors (Fig. 1.1) with a strong emphasis on soil quality. The effects of soil quality on feral plants have been largely uninvestigated in previous research and are considered as negligible, unless indirect effects on vegetation are included (Crawley et al. 1993). A third major aspect I analysed experimentally is the performance of different genotypes (Fig. 1.1), seeking to set establishment success of OSR in relation to the success of weedy relatives (chapter 3) and to discuss the suitability of dwarfing as a mitigation strategy for transgene dispersal (chapter 4). I further buried OSR seeds in the soil (chapter 5) to determine factors relevant for long-term survival in the seed bank. A short overview of the different experiments is given below. Results are set in perspective by a consolidating general discussion.
In chapter 2, I determined establishment success over two years under natural conditions on ruderal sites. Varying site properties and small experimental manipulations provided insights into the influence of several factors on OSR establishment and reproductive output, mainly aiming to identify the relevance of competing vegetation, small-scale disturbance and soil quality (Fig. 1.1).

Fig. 1.1: Overview of topics covered by this thesis with reference to the respective experimental chapter. Crossed arrows denote failed experiments (discussed in Appendix I), Ap. = Appendix, Ch. = chapter, p. = page.

As establishment success in chapter 2 was smaller than expected, I carried out a second sowing experiment on a former dump site for construction rubble (chapter 3). The main focus of this experiment was to find out whether cultivated species (OSR and Brassica rapa) are less successful than weedy relatives (Brassica nigra & Raphanus raphanistrum). Plants were sheltered from slug and rabbit herbivory to ensure successful establishment. I studied the
effects of soil quality in more detail by planting the seeds in containers with different substrates ranging from sand to humous soil.

The experiment in chapter 4 was also carried out on the dump site and mainly addressed the question whether dwarfing is an appropriate method of transgenic mitigation under stressors relevant on ruderal sites. I compared plant fitness of conventional OSR and a semi-dwarf hybrid variety on substrates of low quality and under two different mowing regimes. I also intended to assess relative fitness in the presence of competing vegetation, where dwarfing should be detrimental, but the experiment failed (Appendix I.1).

The previous experiments indicated that persistence in the soil seed bank is likely crucial to long-term establishment success. In the final experiment, I investigated abiotic and biotic factors driving the persistence of OSR seeds (chapter 5). I buried seeds in various soils in the field to determine the impact of soil quality, mainly characterised by soil water-holding capacity, pH and organic matter content. The roles of fungi and of the meso- and small macrofauna were investigated by excluding these organisms from subgroups of buried seeds. I attempted to elucidate the responsible mechanisms for an observed faunal effect in a laboratory experiment (Appendix I.2), which unfortunately failed.

The following main hypotheses were examined in the different experiments of my study:

*Establishment success on ruderal sites is high enough to facilitate self-sustained population growth.*

Self-sustained population growth can be expected when the seed production per seed sown is higher than one. This hypothesis was tested in chapter 3. Supplementary data on establishment success are presented in chapter 2. See 6.1.2 for a general discussion.

*Soil quality (water-holding capacity (WHC) and soil organic matter content (SOM)) affects the establishment success of OSR.*

I expected the percentage of fruiting individuals, the seed production per plant and the composite variable seed production per seed sown to be higher on soils with high WHC and high SOM (tested in chapters 2 and 3). As high WHC likely favours seed-rotting fungi, I predicted lowest seed persistence at high WHC (tested in chapter 5). See 6.1.3 for a general discussion.
Establishment success on ruderal sites is higher for the semi-dwarf hybrid PR45D03 than for the tall cultivar Artus in the absence of competing vegetation.

This hypothesis is based on the assumption that low soil quality and mowing favour dwarfed cultivars, leading to a higher seed production per plot. See chapter 4 for results and 6.1.5 for a general discussion.

Establishment success on ruderal sites is lower for crop Brassicaceae than for wild relatives.

Crop plants are often thought to be less well-adapted to non-agricultural environments than wild plants. To test the hypothesis, the seed production per seed sown of the crop species *B. napus* and *B. rapa* was compared with that of the two weedy relatives *B. nigra* and *R. raphanistrum*. See chapter 3 for results and 6.1.5 for a general discussion.

### 1.2 Biology of *Brassica napus*

#### 1.2.1 General aspects

**OSR as a crop plant & general biology**

OSR (*Brassica napus* L. ssp. *oleifera*) is an annual cruciferous crop plant of the Brassicaceae family with worldwide distribution. It is Europe’s most important oil crop and was cultivated as a winter variety on 1.42 million ha in Germany in 2013 (UFOP 2013). Oil from its seeds is used in human nutrition, for industrial purposes or as fuel, and the plant may serve as animal fodder or as a pollen and nectar source for bees (Hofmeister & Garve 1998). OSR is well-adapted to the climate of Middle and Northern Europe (Cramer 1990). The optimum sowing period for winter OSR in Germany is late August. It overwinters best as a plant rosette with 8-12 leaves and a well-developed tap root. Temperatures as low as -20 °C can then be tolerated. Regrowth starts in spring and the stem is elongated to up to > 2 m height. The root can reach up to 1.5 m depth. Stem elongation and the formation of flowers require a vernalisation period of at least 3 weeks with temperatures close to 0 °C. An OSR stand flowers for 3-5 weeks (Cramer 1990) within the period of March to July (Menzel 2006). Pollination, by wind or insects (1.3.3), leads to the development of pods with on average 15-20 spherical seeds (Diepenbrock 2000). OSR follows a competitive-ruderal strategy (BfN 2013b) and is dispersed through humans and animals (1.3.3). Dry seeds contain approximately 22% protein and 39-45% oil, mainly composed of triacylglycerides (Cramer 1990, Gulden *et al.* 2008). Breeding programmes have managed to reduce components which are harmful and of little...
nutritional value: Since the late 1970s, 0-cultivars with little erucic acid have been cultivated, and presently cultivated 00-varieties also show low levels of glucosinolates (Gressel 2005).

**Habitat requirements**

Cramer (1990) recommends medium-textured, nutrient- and humous-rich calcareous soils, sandy loam, loam and humous loam for cultivation. Unsuitable soils are extremely light, shallow soil, extremely heavy clay soil, water-logged or stony soil. Yield is reduced at pH < 5.5 or > 8.3 as well as on saline soils (Gulden et al. 2008). OSR is a crop with high nutrient requirements (Grant & Bailey 1993). Yield can usually be increased through nitrogen fertiliser (Grant & Bailey 1993, Rathke et al. 2006) and the addition of phosphorous (Holmes & Ainsley 1978, Lickfett et al. 1999). Yield response of OSR to K fertiliser is mostly less pronounced, but sulphur deficiencies are frequently limiting (Grant & Bailey 1993). Sandy soils are unsuitable for production due to water shortage (Gulden et al. 2008). Seed production per plant can be reduced by up to 48% through drought (Champolivier & Merrien 1996, Richards & Thurling 1978). Yet, OSR shows a high degree of phenotypic plasticity and possibly adaptations to its environment, enabling growth under unfavourable conditions (Gulden et al. 2008, Menzel 2006, Reuter et al. 2008).

**Related species**

OSR is thought to have developed from hybridisation between Brassica rapa and Brassica oleracea and is closely related to several other Brassicaceae (Fig. 1.2). It can hybridise with 23 related species (reviewed in FitzJohn et al. 2007). Observations of spontaneous

![Phylogenic relationships between Brassica napus and several other Brassica species](image)

**Fig. 1.2:** Phylogenic relationships between Brassica napus and several other Brassica species (adapted from U 1935 in Gressel 2005), n = number of chromosomes, chromosomes from each of the genomes A, B and C are given.
hybridisation are comparably rare, but have repeatedly been made for *B. rapa* (hybrids were also found in natural populations) as well as for *B. oleracea*, *B. juncea*, *Hirschfeldiana incana*, *Raphanus raphanistrum* and *Sinapis arvensis*. Successful backcrosses and F2 production have been found with 18 species (FitzJohn *et al.* 2007). The parental species *B. rapa* is the most successful hybridisation partner (FitzJohn *et al.* 2007, Scheffler & Dale 1994). The likelihood of gene flow and stable introgression is extremely variable and depends on many factors, but the available data suggest that in some environments, introgressed plants will be as fit as the wild parent and sometimes as fit as OSR (Jørgensen *et al.* 2009). Accordingly, stable introgression of the glyphosate resistance gene from *B. napus* into weedy *B. rapa* has already occurred over six years under commercial field conditions in the absence of selection pressure (Warwick *et al.* 2008).

### 1.2.2 Characteristics of feral populations

OSR has retained many weedy characteristics which facilitate growth outside cultivation: rapid growth and resource capture, high reproductive capacity, high individual plasticity and unspecialized pollination mechanisms including self-pollination (Gressel 2005). In consequence, OSR frequently occurs in subsequent crops as a volunteer plant or outside cultivated fields in feral populations. Densities of feral plants reach 1-10,000 plants*km⁻²* (Squire *et al.* 2010). They typically grow on disturbed sites in agricultural and ruderal areas, such as road verges (Fig. 1.3), railway tracks, industrial wasteland, harbours, dump sites and field margins (Crawley & Brown 2004, Menzel 2006, Nishizawa *et al.* 2009). Possible sources of these populations are given in 1.3.3.

Most feral populations contain 1 to 100 flowering plants, but population sizes may exceed 1,000 individuals (Squire *et al.* 2010). Nevertheless, most feral OSR populations go extinct within 3-4 years (Crawley & Brown 2004, Menzel 2006). Studies in Northern Germany found that only 13% (Menzel 2006), 30-58% (Elling *et al.* 2009) or 12-80% (Dietz-Pfeilstetter *et al.* 2006) of ruderal populations re-occurred in the following year. Major mortality factors of OSR outside cultivation are competing vegetation, vertebrate and mollusc herbivory (Crawley *et al.* 1993, Crawley & Brown 1995) and mowing (Pessel *et al.* 2001). Urban populations of OSR most frequently occur on sites with little competing vegetation (Reuter *et al.* 2008). Still, a few populations persisted for 6-8 years on road verges or railway tracks (Menzel 2006, Pessel *et al.* 2001). Overall, it remains contentious whether feral populations are self-sustaining: While recruitment in many OSR populations depends on renewed input through
seeds spilled in transport (Crawley & Brown 1995, 2004), other findings show that populations can survive without external seed input (Bond et al. 2004, Elling et al. 2009, Pessel et al. 2001). Studies predict that long-term persistence is possible in the form of a meta-population under frequent disturbance (Claessen et al. 2005a), with seed immigration from nearby populations (Claessen et al. 2005b) or recruitment from the soil seed bank (Pessel et al. 2001).

### 1.2.3 Seed bank dynamics

**Dormancy and persistence**

While ripe OSR seeds show almost no primary dormancy and germinate readily at sufficient moisture, a combination of water stress and darkness can induce secondary dormancy and facilitate persistence in the seed bank (Pekrun et al. 1997, Schlink 1994). Buried OSR seeds can survive for up to 11 years (Lutman et al. 2003). While such long persistence is only achieved by an average of 1.8% of the seeds, as much as 1-45.4% outlast shorter periods of 10-16 months (Chadoeuf et al. 1998, Hails et al. 1997, Schlink 1994, Walker et al. 2004). The high variability partly arises from differences between cultivars (Gruber et al. 2004c). Persistence generally increases with burial depth and tends to decrease with longer duration of seed storage (Schlink 1994).

**Microbial-induced mortality**

Seeds in the seed bank may lose viability through attack by fungi (e.g. Blaney & Kotanen 2001b, 2002, Gallery et al. 2010) and possibly bacteria (Chee-Sanford et al. 2006). OSR seeds are susceptible to fungal attack during storage (Pronyk et al. 2006, Tańska et al. 2011), and germinability in *Brassica spp.* can be reduced by certain seed- or soilborne fungi (Chirco & Harman 1979, Shiraishi et al. 2003). Microorganisms might well affect the long-term survival of buried dormant OSR seeds. If this is true, other environmental factors, e.g. soil moisture (Blaney & Kotanen 2001b, Kienwick 1964), can potentially impact seed survival indirectly by affecting fungal or bacterial growth. Plant-pathogenic fungi are known
to be suppressed by fungivorous soil fauna, e.g. by earthworms (Stephens et al. 1994),
nematodes (Lootsma & Scholte 1997), oribatid mites (Enami & Nakamura 1996) and
collembolans (Innocenti et al. 2011, Lartey et al. 1994). Fungal-induced seed mortality can
indeed be reduced in the presence of Collembola (Mitschunas et al. 2006). Seed mortality can
thus be expected to vary considerably under different environmental conditions. So far, we
know little about the relevant factors.

Seed predation
Mortality of seeds in the seed bank may further be caused by post-dispersal seed predators
(e.g Blaney & Kotanen 2001a, Crawley 2000, Hulme 1998b, Lundgren 2009). Birds feed on
OSR seeds lying on the ground (Twigg et al. 2008), and seed-feeding rodents have also been
noticed (Crawley & Brown 1995). The latter can dig up buried plant seeds, but these still
appear to be relatively safe from predation (Hulme 1998a, Thompson 1987). However,
earthworms, carabid beetles and their larvae consume seeds and are potentially active
(2004c) suspected that buried OSR were affected by soil fauna in their study.

1.3 Brassica napus as a GM plant

1.3.1 Relevance as a GM plant
Transgenic HR OSR was first introduced in Canada in 1995 and has rapidly been adopted in
North America (Beckie et al. 2006). GM OSR lines constituted 30% of the worldwide OSR
cultivation area in 2012 and were grown on 8.4 mio. ha in Canada and also in the U.S.,
Australia and Chile (TransGen 2013b). Transgenes in presently cultivated OSR confer
resistance to the broad-spectrum herbicides glyphosate (GLY) or glufosinate (GLU). These
herbicides can thus be used post-emergence during the whole growing season without causing
crop damage, and are considered as more cost-effective and flexible than weed control
methods in non-HR crops (pre-emergence weed control, tillage and selective herbicides)
(GMO Safety 2012).

The most common GMHR system using glyphosate-based herbicides is Roundup Ready®
(GMO Safety 2012) by Monsanto. Glyphosate (N-(phosphonomethyl)glycine) prevents plant
growth by inhibiting the enzyme EPSPS, which is unique to plants and certain micro-
organisms. In consequence, the production of essential aromatic amino acids is inhibited
General Introduction

(Dill et al. 2010, Giesey et al. 2000). Cultivated GMHR crops carry genes from *Agrobacterium sp.* which encode an EPSPS insensitive to GLY (Feng et al. 2010).

Liberty Link® (Aventis CropScience) is the predominant GMHR system with glufosinate-based herbicides (GMO Safety 2012). GLU inhibits an enzyme involved in nitrogen metabolism, glutamine synthetase, leading to a toxic build-up of ammonia. In GMHR crops, expression of a gene from the bacterium *Streptomyces sp.* results in N-acetylation of glufosinate, thus preventing herbicidal activity (Feng et al. 2010, Green & Owen 2010). GLU-resistant crops have been less successful than GLY-resistant ones, mostly due to higher costs and less flexible timing of herbicide applications (Green & Owen 2010).

Experience from a decade of planting GMHR OSR in Canada suggests that some benefits can indeed be realised with the adoption of HR varieties (Beckie et al. 2006). They have led to an average 10% increase in yield and enhanced seed oil quality. Herbicide use per ha was lower in HR than in non-HR OSR due to lower application rates, fewer applications and less need for herbicide combinations. GLY and GLU in HR crops can replace weed management with pre-emergence, soil-incorporated herbicides, so that herbicides can be applied on demand. HR crops consequently facilitated an increase in conservation and zero-tillage systems and reduced fuel consumption and carbon dioxide emissions (Beckie et al. 2006, Brookes & Barfoot 2005). Reduced tillage in HR crop systems will likely reduce erosion, loss of soil moisture and possibly soil compaction (Cerdeira & Duke 2006), although it may also increase soil-borne pathogens (Bockus & Shroyer 1998). Moreover, GLY and GLU are often described as herbicides with comparably low toxicity to invertebrates and higher organisms (Duke 2010, Giesey et al. 2000, Hoerlein 1994) and no risk to human health at relevant levels of exposure (Hack et al. 1994, Williams et al. 2000). Contrasting evidence is given in 1.3.2. Both herbicides are rapidly degraded in the soil (Giesey et al. 2000, Hoerlein 1994). Toxicity of GLY indeed seems to be comparatively low (Peterson & Hulting 2004), but could be higher than previously determined (1.3.2). EFSA has further voiced concern regarding possible risks of GLU to mammals (EFSA 2005).

Benefits of GMHR crops (Beckie et al. 2006, Brookes & Barfoot, Cerdeira & Duke 2006) can however be offset by the development of HR resistant weeds, which pose an increasing threat to the efficiency of HR crop systems (1.3.2 and 6.3.4). For instance, some Canadian farmers had to revert to tillage to control HR OSR volunteers in subsequent crops (Mauro & McLachlan 2008).
Several other GM traits have been approved for commercialisation in OSR to date, i.e. altered fertility for breeding purposes (e.g. male sterility) and various modifications to improve product quality (BCH 2013). Transgenic cultivars containing lauric acid or resistant to the herbicide bromoxynil were cultivated in the U.S. and Canada for brief periods (Beckie et al. 2006, Murphy 2010). Various other traits are targeted in research and development, e.g. resistance to fungal pathogens (Stahl et al. 2006) and insects (Stewart et al. 1997), drought tolerance and improved nitrogen efficiency (TransGen 2012a, 2012b). GM OSR lines have not yet been authorised for cultivation in the EU, but two glufosinate-resistant lines are pending approval. Granted authorisations for three other herbicide-resistant lines only permit the import of seeds and processing to food and feed. Most of these lines also carry a modified fertility system (GMO Compass 2013, GMO Safety 2012).

1.3.2 Risks

There is evidence for various unintended consequences which may arise from the cultivation of GMHR OSR. Socio-economic aspects should be taken into consideration (Aheto et al., Mauro & McLachlan 2008). Co-existence problems in Canada include the adventitious presence in seed lots (Beckie et al. 2006, Friesen et al. 2003) and considerable economic losses in organic OSR farming and honey sales to the EU (Smyth et al. 2002).

GMHR OSR cropping will also affect the environment: Field trials in the UK showed negative effects on the abundances of bees and butterflies, likely due to the reduction of dicotyledonous weeds (Bohan et al. 2005). Predators and parasitoids were also less abundant in GMHR spring OSR fields (Hawes et al. 2003). Recent studies further show that vertebrates suffer carcinogenic effects and/or embryo malformations from glyphosate or glyphosate-based herbicides, which may possibly explain malformations of human embryos in Latin America (George et al. 2010, Paganelli et al. 2010). Sub-agricultural dosages of Roundup® are toxic to human cells and damage cell DNA (Benachour & Séralini 2008, Gasnier et al. 2009). Toxic adjuvants considerably add to glyphosate toxicity (Mesnage et al. 2013). Safety of GM food with pesticide residues thus needs to be re-evaluated (Mesnage et al. 2010).

Changes in weed communities are also indicated: Cultivation of GMHR OSR suppresses dicotyledonous weeds but increases the biomass and species number of monocotyledonous weeds (Bohan et al. 2005, Squire et al. 2009). Moreover, 24 weed species have already evolved resistance to glyphosate (Heap 2013), including very troublesome weeds such as johnsongrass (Sorghum halepense) and Palmer amaranth (Amaranthus palmeri). The majority
of these species have evolved GLY resistance through selective pressure in GLY-resistant crops (Powles 2008, Reddy & Norsworthy 2010). Wild relatives, especially *Brassica rapa*, may acquire HR traits via transgene introgression (1.2.1). GMHR OSR itself can also cause weed problems (Mauro & McLachlan 2008). Any fitness advantage seems to be restricted to environments with application of the target herbicide (reviewed in Devos *et al.* 2012). Invasion of natural habitats appears unlikely (Crawley *et al.* 2001), but there is a high potential for weediness in agricultural and ruderal environments. Consequences of HR-resistant weeds for weed management are described in 6.3.4.

### 1.3.3 Transgene escape via feral populations

**Vectors of seed and pollen dispersal**

Anthropogenic seed dispersal is likely the major source of OSR feral populations. Their occurrence is often correlated with transportation routes of trucks or railways (Crawley & Brown 2004, Knispel & McLachlan 2010, Yoshimura *et al.* 2006). Substantial numbers of OSR seeds are deposited in motorway tunnels (von der Lippe & Kowarik 2007). As many as 404 OSR seeds*m^-2_ may be spilled onto road verges by grain trailers during harvest (Bailleul *et al.* 2012), and up to 10,000 seeds*m^-2_ can be scattered on fields after harvest (Lutman *et al.* 2005). Seeds may occasionally be further dispersed via drafts of passing vehicles (Garnier *et al.* 2008), verge mowers or agricultural vehicles. Seeds are spilled in port areas and from freight trains (Schoenenberger & D'Andrea 2012, Tamis & Jong 2010). Feral populations in agricultural areas in France predominantly originated from seed dispersal from adjacent fields and from persistent seed banks (Pivard *et al.* 2008). Local recruitment and long-distance transport accounted for a mere 10% and 15% of feral populations. OSR can also be distributed when field soil is transferred to road building and construction sites in rural and urban areas (Menzel 2006, Wilkinson *et al.* 1995). Fireworks can contain live OSR seeds (Menzel 2006). Furthermore, seeds from OSR and wild relatives may be sown in forage seed mixtures and game cover crops (Elling *et al.* 2009). Viable OSR seeds can further be dispersed via faeces of deer (Guertler *et al.* 2008), birds (Twigg *et al.* 2008) and sheep (Stanton *et al.* 2003) as well as via coats and hooves of wild boar and deer (Schmidt *et al.* 2004).

OSR is predominantly self-pollinated, with average outcrossing rates of one third, varying from 12% to 47% (Becker *et al.* 1992). Its pollen are dispersed by insects and, to a lesser extent, by wind (Cresswell *et al.* 2004, Ramsay *et al.* 2003). Honey bees, bumblebees, other hymenopterans and pollen beetles play a major role (Chifflet *et al.* 2011, Cresswell *et al.*

**Feral and volunteer populations as stepping-stones**

Modelling clearly shows that spread of transgenes will only remain below impurity thresholds if fields are rigorously controlled for OSR volunteers (Begg et al. 2006, Colbach 2009, Middelhoff et al. 2011) – otherwise, impurities (0.9% threshold) of higher than 60% of harvests are predicted six years after a single year with GM cultivation (Reuter et al. 2011). Feral populations could also play an important role despite their moderate size, as they provide refuges for transgene persistence in the environment, gene stacking and the evolution of new genotypes (Squire et al. 2009). Problematic genotypes could quickly re-colonize fields, especially as feral populations often occur in close proximity to fields (Squire et al. 2009). Feral OSR populations may serve as stepping-stones for gene flow, increasing the potential for hybridisation with wild relatives which repeatedly occur on the same sites in Northern Germany (Elling et al. 2009, Menzel 2006). Menzel (2006) identified several ruderal centres of potential hybridisation in Bremen in old industrial and harbour areas. The related agricultural weeds *Sinapis arvensis* and *Raphanus raphanistrum* have become less frequent within fields due to herbicide application and can now be found more often outside of agriculture. In addition, the flowering periods of wild relatives show a longer overlap with feral than with cultivated OSR (Menzel 2006).

There are already several examples of transgene escape via volunteer and feral plants: GM OSR volunteers can persist for seven (Beckie & Warwick 2010) to ten (D'Hertefeldt et al. 2008) years after cultivation. Transgene persistence over time is favoured by persistence in the seed bank (1.2.3). Transgenic *B. napus* grows along road verges in Canada and the U.S., partly far from cultivation areas (Knispel & McLachlan 2010, Schafer et al. 2011), or in countries like Japan and Switzerland where GM OSR is only imported but not cultivated (Aono et al. 2006, Schoenenberger & D'Andrea 2012). Some volunteers (Hall et al. 2000) and feral plants (Aono et al. 2006, Knispel et al. 2008, Schafer et al. 2011) even show resistance against multiple herbicides. Transgenic OSR has already hybridised with *B. rapa* on a road verge in Canada (Yoshimura et al. 2006).
Mitigation strategies

Several strategies have been proposed to reduce the unintended spread of transgenes. Devos et al. (2004) list practical measures, which include isolation distances between fields (see also Damgaard & Kjellsson 2005) and discarding of border rows to limit pollen dispersal. Seed dispersal can be reduced e.g. by cleaning machinery and sealing transport vehicles. Farmers should attempt to maximize germination of scattered seeds, e.g. by avoiding tillage for at least 3–4 weeks after harvest (see also Gruber et al. 2004a), and should vigorously control emerging volunteers. Furthermore, use of cultivars with low dormancy would reduce seed bank persistence over time (Gruber et al. 2004b).

In addition, molecular strategies for containment of transgenic crops are under development (Daniell 2002, Gressel 2005). These include approaches to disrupt transgene escape via sexual reproduction, e.g. male sterility (Daniell 2002) and cleistogamy (self-pollination in closed flowers, Fargue et al. 2006) in OSR. Another strategy, transgenic mitigation (TM), tightly links the target transgene in a tandem construct with a TM gene conferring a fitness disadvantage. Effective TM traits do not diminish the success of crops but reduce the fitness of crop volunteer or hybrid offspring, thus keeping spread of the transgene to a minimum by negative selection pressure (Gressel 2005). Dwarfing has been developed as a TM strategy in OSR (Al-Ahmad et al. 2006).
2. Establishment and reproductive success of *Brassica napus* (L.) under ruderal conditions

**Abstract**

In case of cultivation of genetically modified oilseed rape (OSR, *B. napus* L.), feral (wild) populations provide possible avenues for uncontrolled transgene escape and persistence in self-sustaining populations. The aim of my study was to investigate the population dynamics of these feral plants and to identify factors of relevant impact. I performed a gradually simplified sowing experiment on ruderal sites, assessing the effects of site vegetation cover, small-scale disturbance, soil quality and seeding rate. I further assessed differences between cultivars and sowing events\(^1\).

Establishment success was generally low, but higher in urban than in abandoned agricultural areas. Site vegetation cover had no significant effect, but a disturbance of 30*30 cm plots clearly enhanced the establishment success of seedlings and reproducing individuals. Seedling emergence and the number of pods per plant were larger on high-quality sites in the sowing of spring 2007. In the following sowing (fall 2007), I found no effect of soil quality, most likely since other factors such as precipitation and increasing vegetation cover were limiting. However, artificial fertilisation of plots on low-quality sites significantly increased the percentage of fruiting plants. A higher seeding rate increased the number of emerged seedlings but did not affect the establishment of reproducing individuals.

This study identified soil quality and disturbance as factors which influence feral plant establishment and found that OSR is probably more microsite- than seed-limited under stressful ruderal conditions. My results further stress the importance of considering urban OSR populations in risk assessment. Yet, the overall low establishment success cannot explain the high frequency of feral populations observed in previous studies in Bremen. It seems likely that many feral populations may have originated from topsoil imported from former agricultural areas to construction sites: In this case, seeds would automatically be dispersed to appropriate habitats.

2.1 Introduction

*Brassica napus* is a major oilseed crop cultivated on 31 million ha worldwide, 9.2 million ha of which are sown with genetically modified (GM) varieties (data from 2012, TransGen

\(^1\) In this text, the term “event” stands for different sowing dates and does not refer to a specific genetic modification event.
Establishment success of OSR under ruderal conditions

2013a). An approval for cultivation in the EU has been sought, but not yet granted, for GM lines with a resistance against the herbicide glyphosate (TransGen 2013b). Assessments of whether these lines would pose a risk to the environment need to consider the fact that oilseed rape (OSR) frequently grows outside cultivated fields in so-called feral populations (e.g. Crawley & Brown 2004, Gressel 2005, Menzel 2006, Squire et al. 2010). Mounting evidence suggests that seeds spilled from trucks in transport (e.g. to processing mills) contribute to long-distance dispersal (Crawley & Brown 1995, von der Lippe & Kowarik 2007) and have already generated feral GM OSR populations in countries where these lines are not cultivated (Kawata 2009, Nishizawa et al. 2009, Schoenenberger 2012). Successful containment of GM OSR is therefore nearly impossible. While feral populations are at present unlikely to cause major crop impurities in case of GM cultivation, they may well create refuges for the development of new genotypes (Squire 1999): Feral plants facilitate hybridisation between cultivars and with wild relatives (Elling et al. 2009, Hansen et al. 2001), as well as the development of multiple herbicide resistance (Aono et al. 2006, Knispel et al. 2008). Such hybridisations could lead to new invasive genotypes (Ellstrand 2003). Volunteer OSR populations already occur as weeds in subsequent crops (Gulden et al. 2008), and escaped herbicide transgenes persist in a weedy relative in Canada (Warwick et al. 2008).

Population dynamics and the factors determining the survival of feral OSR are still poorly understood. Some studies support the view that these usually short-lived populations largely persist through renewed external seed input (Crawley et al. 2001, Crawley & Brown 1995). Other findings confirm persistence (most likely self-sustained) over up to 6-8 years and substantial seed production in some cases (Bond et al. 2004, Dietz-Pfeilstetter et al. 2006, Menzel 2006, Pessel et al. 2001, Squire et al. 2010). Feral populations are not restricted to the agricultural environment but are a common feature of ruderal habitats, including urban areas (Dietz-Pfeilstetter et al. 2006, Elling et al. 2009, Menzel 2006). These areas indeed harbour potential centres for hybridisation with wild relatives and therefore require the attention of GM risk assessment (Breckling & Menzel 2004). Von der Lippe & Kowarik (2007) found that more OSR seeds were deposited in a tunnel leading out of Berlin than in a tunnel leading into Berlin. While the authors suspect seed spillage from trucks to be the source of most of these seeds, long-distance dispersal of seeds from feral populations from within the city may have made a contribution. Dispersal of OSR genotypes developing in urban areas could thus occur via seeds adhering to or using the draft of vehicles, albeit probably at low rates (Garnier et al. 2008).
One factor known to determine the survival of OSR feral plants is the presence of competing vegetation: OSR is generally restricted to disturbed sites such as road verges, railway tracks, field margins and industrial wasteland (Menzel 2006, Pessel et al. 2001, Pivard et al. 2008). Long-term survival of roadside OSR populations appears to depend on the renewed creation of gaps in the vegetation cover (Crawley & Brown 1995, 2004) – otherwise, rapid colonisation with perennial vegetation seems to cause local extinction within three to four years. Yet, it is unclear whether disturbances at small scales, comparable to those frequently created by burrowing animals, might already suffice to facilitate establishment.

Ruderal sites may resemble agricultural conditions insofar as disturbance may occasionally or regularly create competition-free microsites. However, abiotic conditions will often be harsher than on fertilised and possibly irrigated agricultural soils. OSR is considered as a crop with comparably high nutrient requirements (Grant & Bailey 1993). Soil moisture also affects total yield or yield components (e.g. Clarke & Simpson 1978, Rood & Major 1984). A negative influence of ruderal soil conditions may therefore be expected, unless the ruderal site receives high nutrient input. However, a systematic evaluation of the contribution of soil conditions to establishment success of feral plants is still lacking. Some studies give observational clues, showing that OSR populations can occur on dry, meagre soils (Menzel 2006), or might establish on gravel (Dietz-Pfeilstetter 2004).

My study is the first to investigate the survival and reproductive potential of conventional OSR in urban ruderal habitats in relation to the number of seeds dispersed. Due to the frequent occurrence of OSR populations in urban Bremen (Menzel 2006), I expect OSR to perform better than in natural habitats where comparable studies (Crawley et al. 1993) observed an average of only 0.7% adult individuals (% of seeds sown). My study further examined the influence of various factors on establishment success. I conducted sowing experiments with two winter OSR cultivars on sites differing in vegetation cover and soil quality, including three sowing events to test for seasonal and successional effects. The main hypotheses were that establishment success is 1) negatively affected by competing vegetation and positively affected by small-scale disturbance and 2) larger on soils with high quality (nutrient-rich with high water-holding capacity) than on soils with low quality. Furthermore, I compared the performance in ruderal versus agricultural environments to determine if ruderal populations can be equally successful as ferals in agricultural areas.

---

2 Calculated from Table 1 for the control line
2.2 Methods

2.2.1 Site characterisation

Ruderal sites

Sites used for the sowing experiment were road verges, dump sites, allotments and industrial wasteland in Bremen, Northern Germany (see Appendix II.1, Fig. II.1 - II.3 for site locations). Some had previously supported OSR populations (Menzel 2006), but I excluded sites where OSR grew at present to avoid confusion of naturally occurring and experimentally sown plants. Criteria for selection were that the vegetation cover had to be disturbed at least in patches to facilitate OSR establishment, while future major disturbances had to be unlikely so that plants could survive till maturity. Whenever possible, I selected sites which were not in close proximity of rabbit holes or droppings to avoid rabbit grazing. Four site types were differentiated, each of which was represented by seven replicate sites (Fig. II.4-II.7 in Appendix II.1): low-quality sites with relatively high (LH) or low vegetation cover (LL), and high-quality sites with either high (HH) or low vegetation cover (HL). Quality of sites was defined by soil organic matter content, which was < 1.6% DM (DM = dry mass) for low-quality and > 1.7 for high-quality sites (see Appendix II.2.1 for the analysis of soil parameters). High-quality sites showed a significantly higher water-holding capacity (WHC\text{\textsubscript{max}}), soil organic matter content (SOM) and nutrient content (% C (carbon), % N (nitrogen), mg P*100 g\textsuperscript{-1} DM (phosphorus), mg K*100 g\textsuperscript{-1} DM (potassium)) than low-quality sites (Table 2.1, T-tests or U-tests p ≤ 0.05, n = 14), but did not differ in pH. In addition to these experimental sites, I included several railway tracks and mini-sites, which are presented in the Appendix (see II.3 and III.1.1).

Abandoned agricultural sites

The agricultural sites selected for the experiment represent small abandoned niches, e.g. field margins, in cultivated agricultural fields or fresh fallows which may harbour feral OSR. I chose borders of arable fields or grasslands (Fig. II.8 and II.9 in Appendix II.1) which were ploughed prior to sowing and then exempted from cultivation practices. Agricultural fields showed a significantly lower pH and P content than comparable ruderal HL sites (Table 2.1, T-test p ≤ 0.05, n = 6-7), but other soil variables were similar (T-test or U-test n.s.). The chosen grassland sites had a significantly higher N content (T-test, p = 0.039, n = 4-7) and tended to have a higher WHC\text{\textsubscript{max}} and SOM (T-test, p ≤ 0.1, n = 4-7) than high-quality ruderal sites with high vegetation cover (HH).
Table 2.1: Soil properties of the ruderal and agricultural sites in March 2007 (0-15 cm depth). Means ± SE, n = 7 for ruderal site types, n = 6 for agricultural fields (AF) and n = 4 for grassland sites (G).

<table>
<thead>
<tr>
<th>site qual. veg.</th>
<th>pH</th>
<th>WHC&lt;sub&gt;max&lt;/sub&gt;</th>
<th>SOM</th>
<th>P*</th>
<th>K*</th>
<th>% N</th>
<th>% C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ruderal sites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL low low</td>
<td>6.5 ± 0.3</td>
<td>22.6 ± 0.8</td>
<td>0.5 ± 0.1</td>
<td>1.8 ± 0.7</td>
<td>0.7</td>
<td>2.5 ± 0.6</td>
<td>0.001 ± 0.001</td>
</tr>
<tr>
<td>LH low high</td>
<td>6.1 ± 0.2</td>
<td>25.6 ± 0.9</td>
<td>0.9 ± 0.1</td>
<td>2.1 ± 0.6</td>
<td>0.6</td>
<td>2.9 ± 0.5</td>
<td>0.017 ± 0.007</td>
</tr>
<tr>
<td>HL high low</td>
<td>6.6 ± 0.1</td>
<td>52.0 ± 9.0</td>
<td>4.5 ± 0.9</td>
<td>13.1 ± 0.8</td>
<td>0.8</td>
<td>18.2 ± 5.5</td>
<td>0.169 ± 0.043</td>
</tr>
<tr>
<td>HH high high</td>
<td>6.1 ± 0.2</td>
<td>44.9 ± 6.9</td>
<td>4.4 ± 1.0</td>
<td>9.8 ± 1.9</td>
<td>1.9</td>
<td>16.1 ± 4.8</td>
<td>0.144 ± 0.041</td>
</tr>
<tr>
<td><strong>agricultural sites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AF rich low</td>
<td>5.4 ± 0.3</td>
<td>49.4 ± 8.5</td>
<td>6.3 ± 2.4</td>
<td>7.9 ± 2.0</td>
<td>2.0</td>
<td>14.6 ± 4.2</td>
<td>0.236 ± 0.091</td>
</tr>
<tr>
<td>G rich high</td>
<td>5.8 ± 0.1</td>
<td>66.0 ± 6.1</td>
<td>8.4 ± 1.6</td>
<td>9.2 ± 3.7</td>
<td>3.7</td>
<td>26.0 ± 10.2</td>
<td>0.336 ± 0.077</td>
</tr>
</tbody>
</table>

* [mg/100 g dry mass]
qual. = soil quality
veg. = vegetation cover
WHC<sub>max</sub> = maximum water-holding capacity
SOM = soil organic matter content
P = phosphorus
K = potassium
N = nitrogen
C = carbon

Additional variables with potential influence on establishment success were recorded repeatedly throughout the experiment. Cover of competing vegetation was estimated on the 23*23 cm sowing plots on each census of seedling emergence in intervals of 10%. The degree of herbivory was determined by the percentage of completely defoliated seedlings. External disturbance of plots was recorded and the mean number of plots with > 30% disturbance per treatment per site was calculated as a disturbance index. These data are presented in Tables II.1 and II.2 in Appendix II.4, and weather conditions for Bremen are reported in Appendix II.5 and II.5.1.

2.2.2 Cultivars

I chose two non-GM winter OSR cultivars, Artus and Smart, which are common in feral populations in Northern Germany (Dietz-Pfeilstetter et al. 2006, Elling et al. 2009). Artus was of particular interest as it is near-isogenic to the GM cultivar Avalon<sub>LL</sub> (Gruber et al. 2004). Smart was the cultivar most frequently found in ruderal populations near Osnabrück (Elling et
2.2.3 Design

The experiment comprised three sowing events in order to assess variation between seasons and years. The most extensive sowing was conducted in spring 2007 (1-13 April 2007) on 28 experimental ruderal sites and 10 abandoned agricultural sites (freshly ploughed field borders). I contrasted the results of the spring sowing with a fall sowing in the same year (28 August – 2 September 2007). Winter OSR usually germinates in fall, yet some feral plants are thought to emerge in spring (Menzel 2006). A third sowing in fall 2008 (3-5 September 2008) was included to assess differences between years. Both fall sowings included a limited number of sites as those least suited for establishment were gradually excluded from the experiment. In some cases, sites which turned out to be unsuitable were replaced with new ones.

In the first sowing in spring 2007, I compared the effects of soil quality, vegetation cover, local disturbance and cultivar in a full four-factorial design. Five largely homogenous blocks were defined for each site and subdivided into subplots for different sowing events (Fig. 2.1), randomly arranged within each block. Within subplots, sowing plots (23*23 cm) were marked

---

Fig. 2.1: Set-up of a replicate experimental site with five blocks. The spatial arrangement of blocks depended on site shape and homogeneity. Three subplots in each block were established (one for each sowing event 1-3). In each subplot, the cultivars Artus and (in the first sowing) Smart were sown into square plots (represented by the small white and grey boxes) which were either disturbed or undisturbed.

---

3 Imbibed seeds are coated with chemicals, e.g. fungicides.
and randomly assigned to one of four treatments: Cultivar Artus sown into undisturbed (UA) or disturbed (DA) plots or cultivar Smart sown into undisturbed (US) or disturbed (DS) plots. Disturbance was achieved by manually removing all vegetation and larger roots in a 30*30 cm square and ploughing the soil with a spade to a depth of approximately 20 cm, thus causing vegetation gaps comparable to those created by burrowing vertebrates. 60 seeds were sown per plot in a random distribution. Seeds were mixed with 200 ml soil from the respective site which was then sprinkled evenly onto the plot.

Only cultivar Artus was used for further experiments due to time constraints. A distinction between sites with low or high vegetation cover was no longer feasible for the second and third sowing, as only a limited number of sites were kept as potentially suitable for establishment, and some new and more promising sites of differing successional stage were included. This was done in order to ensure that analysis of the tested factors was not unduly confounded by major mortality factors such as external disturbance and rabbit grazing. Thus, both fall sowings were carried out with a simplified design: A full two-factorial design concentrating on the effects of disturbance and soil quality (nine low-quality vs. nine high-quality ruderal sites) in fall 2007, and a sowing on only one low-quality and five high-quality ruderal sites in fall 2008 when the only treatment kept was disturbance.

**Fertilisation**

Effects of fertilisation were assessed in the fall sowing 2007 on eight low-quality ruderal sites. In three blocks per site, I added one replicate plot each which received NPK compound fertiliser (Floraplus Premium, gpi, Gladbeck) directly before sowing as well as 3 and 6 months after sowing. The fertiliser was pulverised and evenly broadcast on the soil surface of the respective 23*23 cm plots with a salt shaker, at the maximum recommended dosage of 90 g*m$^{-2}$ (equivalent to 10.8 g*m$^{-2}$ N as ammonium nitrate, 10.8 g*m$^{-2}$ P$_2$O$_5$, 15.3 g*m$^{-2}$ K$_2$O, 1.8 g*m$^{-2}$ MgO, 0.018 g*m$^{-2}$ B, 0.009 g*m$^{-2}$ Zn).

**Seed density**

Additional treatments were added for the fall sowing 2007 on four sites (a railway track, an arable field, a high-quality and a low-quality ruderal site) to determine whether seed density affects OSR establishment success. I chose seven densities (4, 8, 16, 30, 60, 120 and 200 seeds) and replicated each density on three undisturbed 23*23 cm plots. Replicates were randomly distributed among the five blocks.
2.2.4 *Estimates of establishment success and reproductive potential*

Emerging seedlings were generally counted 2-4 and 6-9 weeks after the first substantial rainfalls following the sowing date, except in 2008 when the second census was omitted due to time constraints (Fig. 2.2, Table II.3 in Appendix II.6). Seedling emergence was analysed as the maximum number of seedlings or plants found over the course of those censuses and may slightly underestimate true emergence, as some seedlings had either not yet emerged on the first census or had already been consumed by herbivores on the second one. In cases when stalks of grazed seedlings were still visible, they were counted as emerged seedlings. Reproducing individuals were assessed on two to three dates in the period from May to July both in 2008 and 2009 (see Appendix II.3). Spring-sown plants did not flower in the same growing season (2007) but overwintered and flowered in the following spring (2008). I recorded the percentage of flowering and fruiting individuals (% of seeds sown)\(^4\) and estimated the number of pods per reproducing plant (number of infructescences multiplied by

---

\(^4\) I considered a plant as having flowered if buds, flowers or pods were found during any of the censuses. Plants were counted as fruiting if developing or mature pods were present.
the mean number of pods per infructescence). If plants had more than three infructescences, pod number was counted for one infructescence of intermediate size. The pod number per plant was assessed for at maximum ten plants per treatment and site. Seed number per pod was not assessed as pod removal might have influenced population dynamics of F1 plants. If plants had reproduced in the previous year, I also searched for F1 individuals in and within a distance of 1 m from those plots. Pod numbers per plant are only presented for the first sowing, as other sowing events and experiments yielded only few reproducing plants.

2.2.5 Data analysis

I generally included all plots in the analyses, even if they had been subjected to external disturbances such as animal burrowing, construction work or mowing. These factors are part of the relevant factors influencing the survival of OSR in ruderal habitats. As this could however have confounded my analyses of other factors, all analyses were additionally carried out for a dataset excluding all plots which showed external disturbance on more than 30% of the plot area. Whenever analyses from the two datasets differed, this is reported in the results section, except when loss of significance through exclusion of disturbed plots resulted rather from reduced power through loss of replicates than from a change in mean values. For analysis, percentage data were arcsine-square-root-transformed to normalise the distribution and improve variance homogeneity, and count data were usually log-transformed after adding a small number to avoid zero values. Data are displayed as original values. Results were considered as significant at $p \leq 0.05$. All data analyses were carried out in SPSS 19.0 (SPSS Inc., Chicago, IL, USA), except for split-plot ANOVAs which were run in Genstat 8.1 (VSN Int. Ltd., Hemel Hempstead, Hertfordshire, UK).

Parametric analyses were preferred whenever the assumptions were met. I tolerated moderate deviations from normality if sample sizes were equal owing to ANOVA’s robustness (Quinn & Keough 2007). Seedling emergence on ruderal sites was analysed with a full factorial split-plot ANOVA from which non-significant interaction terms were excluded (spring and fall 2007) or with a Mann-Whitney U-test (fall 2008). Data distributions allowed only for one-factorial analyses for the remaining variables of plant establishment (one-way ANOVA or Mann-Whitney U-test). Generalized linear models were not suitable due to variance heterogeneity whenever transformation proved to be ineffective in stabilising variance. As one-factorial analyses do not account for the nested character of the split-plot design, tests for factors varying between sites (soil quality, vegetation cover) were made with mean values per site, averaged over all within-site treatments (disturbance, cultivar) and blocks, to avoid
pseudoreplication (Crawley 2005, Appendix II.7). Within-site treatments were analysed with all data points which represent true replicates for these factors (see Appendix II.7.1 for further details). The effect of fertilisation was also analysed with U-tests including all data points from the three blocks per site which had received this treatment.

Differences between sowing events were tested with the non-parametric Friedman test, including only sites which were sown in the respective two compared sowing events and using data from cultivar Artus only. Effects of site environment were analysed by comparing high-quality ruderal sites with agricultural sites in one-factorial analyses, both over all sites high-quality sites with high vegetation cover (HH) and agricultural fields versus low-quality sites with low vegetation cover (LL). For sites with low vegetation cover, I used only disturbed plots from both environments as there were no undisturbed plots in agricultural fields.

The effect of seed density on variables of establishment success was assessed with regression analysis which included data from all sites. I used means of the three replicate plots for the respective density within a site to improve normality. A linear regression was performed to relate log-transformed seed density with the log-transformed number of emerged seedlings. No appropriate or significant regression model was found for the number of flowering and fruiting individuals, which did not meet the assumptions of homogenous variances and normality of residuals.

A summary of the design and all results is displayed in Tables III.2 – III.5 in Appendix III.1.

2.3 Results

I first present results for the factors influencing establishment success, followed by results on the seed density manipulation and on differences between sowing events. Finally, I compare establishment on ruderal sites versus agricultural sites.

---

5 I did not include the effects of cultivar and disturbance in the analysis, as data distributions were not suitable for a full factorial analysis and the effects of these factors were of little interest.

6 Linear, log-linear and quadratic regression was tested as well as generalized linear regression models with Poisson or negative binomial distribution, and a Spearman rank correlation.
2.3.1 Factors influencing establishment success on ruderal sites

Vegetation cover

I detected no overall effect of vegetation cover on seedling emergence, reproducing individuals or pod production (Fig. 2.3, Tables 2.2-2.4), but a significant interaction with disturbance for emerged seedlings (Table 2.2). Looking at undisturbed plots only, emergence seemed to be negatively affected by high vegetation cover, but not significantly (Fig. 2.3, three-way split-plot ANOVA). A converse trend was seen on disturbed plots, which indicated a higher percentage of flowering (Appendix III.1, Fig. III.1) and fruiting individuals (Fig. 2.3) on sites with high vegetation cover (U-tests, p = 0.094 and p = 0.052 respectively, n = 14). Plants on sites with low vegetation cover appeared to produce more pods per fruiting plant than plants on sites with high vegetation cover, but the effect was not significant (Table 2.3).

Disturbance

The disturbance treatment showed an overall effect on seedling emergence in spring 2007, yet this effect depended on site type (Fig. 2.3.a, Table 2.2): Four-way split-plot ANOVA revealed an interaction with initial site vegetation cover and soil quality. Low-quality sites with high vegetation cover (LH) showed a significantly higher percentage of seedlings on disturbed than on undisturbed plots (one-way ANOVA, $F_{1,139} = 25.99; p < 0.001, n = 70$), while disturbance had no significant effect on sites with low vegetation cover or high-quality sites (one-way ANOVAs). No distinct effect was seen in the sowing of fall 2007, but the sowing of fall 2008 yielded significantly more seedlings on disturbed than on undisturbed plots. Reproducing plants showed a distinct positive effect of disturbance: There was a significantly higher number of flowering individuals on disturbed plots of all three sowing events (Table 2.3, U-tests, $p < 0.001, n = 30-280$). This was reflected in the fruiting individuals, which reached 1.00% on disturbed and 0.02% on undisturbed plots in fall 2007 (Fig. 2.3.e, Table 2.4) and did not develop on undisturbed plots at all in fall 2008 (Fig. 2.3.f, Table 2.4). In spring 2007, the positive effect of disturbance on fruiting individuals was only significant for sites with high vegetation cover (U-test, $p = 0.006, n = 140$). The number of pods per plant, however, was significantly higher on undisturbed plots (Table 2.3, one-way ANOVA, $F_{1,23} = 8.10; p = 0.009, n = 7-17$).
Establishment success of OSR under ruderal conditions

Fig. 2.3: Seedling emergence and fruiting individuals [% of seeds sown] for the three sowing events, spring 2007 (plots a and d), fall 2007 (plots b and e) and fall 2008 (plots c and f). Note that fruiting individuals are presented on a log-scaled y-axis. Data of the spring sowing include the cultivars Artus and Smart sown on low-quality vs. high-quality sites with low or high vegetation cover and disturbed or undisturbed plots. Data of the fall sowings comprise cultivar Artus only, and sites were no longer divided into low and high vegetation cover sites. For the sowing of fall 2008, sites were also not divided into low-quality and high-quality soils. D = disturbed plots; U = undisturbed plots. Mean ± SE, n = 35 (spring 07), n = 45 (fall 07) and n = 30 (fall 08).

Cultivar

The two cultivars showed a comparable seedling emergence (Fig. 2.3.a, Table 2.2). Yet, Artus had significantly more flowering and fruiting individuals than Smart (Fig. 2.3.d, Tables 2.3 & 2.4). However, exclusion of externally disturbed plots reduced the effect to a trend (U-tests: p = 0.082 (flowering) and p = 0.064 (fruiting), n = 233-235), indicating that Smart may have suffered more from external disturbance. The pod production per fruiting plant appeared to be higher for Smart, but the difference was not significant (Table 2.3, one-way ANOVA).
**Table 2.2:** Effects of soil quality (S), vegetation cover (V), plot disturbance (D) and cultivar (C) on seedling emergence [% of seeds sown] according to split-plot ANOVA or Mann-Whitney U-test. Results refer to cultivar Artus (fall 2007 & fall 2007) or to both cultivars, Artus and Smart (spring 2007). Separate analyses were made for the three sowing events, spring 2007, fall 2007 and fall 2008. Significance levels (p), degrees of freedom (d.f.) or sample size of factor level (n) and variance ratios (F) are displayed. Interactions between factors could only be tested for seedling emergence – those with no significance or trend were excluded from the model. Significant effects (p ≤ 0.05) are shown in bold type.

<table>
<thead>
<tr>
<th>effect</th>
<th>d.f.</th>
<th>F</th>
<th>p</th>
<th>d.f.</th>
<th>F</th>
<th>p</th>
<th>n</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>1</td>
<td>4.62</td>
<td>0.042</td>
<td>0.042</td>
<td>1</td>
<td>0.91</td>
<td>0.354</td>
<td>0.354</td>
<td>—</td>
</tr>
<tr>
<td>V</td>
<td>1</td>
<td>0.45</td>
<td>0.510</td>
<td>0.45</td>
<td>1</td>
<td>0.45</td>
<td>0.510</td>
<td>0.510</td>
<td>—</td>
</tr>
<tr>
<td>Mainplot error</td>
<td>25</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>16</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Block (Site)</td>
<td>112</td>
<td>1.62</td>
<td>—</td>
<td>72</td>
<td>1.03</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>19.00</td>
<td>&lt;0.001</td>
<td>—</td>
<td>30</td>
<td>—</td>
<td>&lt;0.001</td>
<td>0.005</td>
<td>—</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>0.91</td>
<td>0.341</td>
<td>0.91</td>
<td>1</td>
<td>0.91</td>
<td>0.341</td>
<td>0.341</td>
<td>—</td>
</tr>
<tr>
<td>V × D</td>
<td>1</td>
<td>4.91</td>
<td>0.027</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>S × D</td>
<td>1</td>
<td>28.50</td>
<td>&lt;0.001</td>
<td>—</td>
<td>—</td>
<td>n.s.</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Subplot error</td>
<td>416</td>
<td>—</td>
<td>—</td>
<td>89</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>559</td>
<td>—</td>
<td>—</td>
<td>179</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

1 arcsine-square-root-transformed for analysis
a split-plot ANOVA (four-way for spring 07 and two-way for fall 07)
b Mann-Whitney U-test

**Soil quality**

Seedling emergence was consistently higher on high-quality than on low-quality sites (Fig. 2.3.a & 2.3.b), amounting to 28% and 15% respectively in spring 2007. While this effect was significant for the sowing of spring 2007, it could not be confirmed statistically for the sowing of fall 2007 (Table 2.2). Neither the percentage of flowering nor of fruiting individuals varied significantly with soil quality. However, fruiting plants on high-quality sites developed nearly 100 times more pods per plant (Fig. 2.4) than plants on low-quality sites (sowing of spring 2007, Table 2.3, one-way ANOVA, F₁,11 = 11.32, p = 0.007).

Experimental fertilisation of plots on low-quality sites, sown in fall 2007, did not influence seedling emergence or the percentage of flowering individuals (Table 2.5, U-test). However, fruiting individuals were significantly more numerous on fertilised plots (U-test, p = 0.040, n = 24).
Establishment success of OSR under ruderal conditions

Table 2.3: Pods per fruiting plant and flowering individuals [% of seeds sown] counted for the different factor levels of soil quality, vegetation cover, disturbance and cultivar for each sowing event. Results refer to cultivar Artus (fall 2007 & fall 2007) or to both cultivars (spring 2007). Pods per plant are only shown for spring 2007. Significant differences between treatments according to one-way ANOVA (log-transformed pods) or Mann-Whitney U-test (arcsine-square-root-transformed percentage of flowering individuals) are denoted by asterisks (* for p ≤ 0.05; ** for p ≤ 0.01; and *** for p ≤ 0.001). See Fig. III.1 in Appendix III.1 for a full factorial display of flowering individuals. Note that while tests for factors disturbance and cultivar incorporated all data points, tests for factors soil quality and vegetation cover were based on the mean values per site (averaged over blocks & treatments within blocks) to avoid pseudoreplication. Mean ± SE and number of replicates per factor level (n).

<table>
<thead>
<tr>
<th>Factor</th>
<th>pods per plant</th>
<th>flowering individuals [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>spring 2007</td>
<td>fall 2007</td>
</tr>
<tr>
<td></td>
<td>n   Mean  SE</td>
<td>n   Mean  SE</td>
</tr>
<tr>
<td>soil quality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>low</td>
<td>7  3 ± 1</td>
<td>14 0.20 ± 0.08</td>
</tr>
<tr>
<td>high</td>
<td>5 276 ± 192</td>
<td>14 0.21 ± 0.10</td>
</tr>
<tr>
<td>vegetation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>high cover</td>
<td>8 8 ± 3</td>
<td>14 0.24 ± 0.10</td>
</tr>
<tr>
<td>low cover</td>
<td>4 336 ± 235</td>
<td>14 0.17 ± 0.08</td>
</tr>
<tr>
<td>disturbance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>disturbed</td>
<td>17 145 ± 88</td>
<td>280 0.34 ± 0.07</td>
</tr>
<tr>
<td>undisturbed</td>
<td>7 713 ± 273</td>
<td>280 0.08 ± 0.03</td>
</tr>
<tr>
<td>cultivar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artus</td>
<td>17 215 ± 130</td>
<td>280 0.30 ± 0.06</td>
</tr>
<tr>
<td>Smart</td>
<td>7 542 ± 202</td>
<td>280 0.11 ± 0.04</td>
</tr>
</tbody>
</table>

Table 2.4: Effects of soil quality (S), vegetation cover (V), plot disturbance (D) and cultivar (C) on flowering individuals [% of seeds sown] according to Mann-Whitney U-test. Results refer to cultivar Artus (fall 2007 & fall 2007) or to both cultivars, Artus and Smart (spring 2007). Separate analyses were made for the three sowing events, spring 2007, fall 2007 and fall 2008. Significance levels (p) and sample size per factor level (n) are displayed. Significant effects (p ≤ 0.05) are shown in bold type. Note that tests for factors D and C incorporated all data points, but that tests for factors N and V were based on the mean values per site (averaged over blocks & treatments within blocks) to avoid pseudoreplication (see 2.2.5).

<table>
<thead>
<tr>
<th>Factor</th>
<th>spring 2007</th>
<th>fall 2007</th>
<th>fall 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n  p</td>
<td>n  p</td>
<td>n  p</td>
</tr>
<tr>
<td>S</td>
<td>14 0.958</td>
<td>9 0.628</td>
<td>— —</td>
</tr>
<tr>
<td>V</td>
<td>14 0.220</td>
<td>— —</td>
<td>— —</td>
</tr>
<tr>
<td>D</td>
<td>280 0.094</td>
<td>90 &lt;0.001</td>
<td>30 0.005</td>
</tr>
<tr>
<td>C</td>
<td>280 0.012</td>
<td>— —</td>
<td>— —</td>
</tr>
</tbody>
</table>

1 arcsine-square-root-transformed for analysis

Fig. 2.4: Well-developed fruiting individual on a high-quality site (sown in spring 2007).
Table 2.5: Effect of fertilisation on seedling emergence and the percentage of reproducing individuals [% of seeds sown] of cultivar Artus (means ± SE). Significant differences according to Mann-Whitney U-test are denoted by asterisks (* for p ≤ 0.05).

<table>
<thead>
<tr>
<th></th>
<th>seedling emergence [%]</th>
<th>flowering individuals [%]</th>
<th>fruiting individuals [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>unfertilised</td>
<td>24</td>
<td>10.5 ± 1.7</td>
<td>2.2 ± 0.6</td>
</tr>
<tr>
<td>fertilised</td>
<td>24</td>
<td>11.3 ± 2.5</td>
<td>3.3 ± 1.4</td>
</tr>
</tbody>
</table>

**Mortality factors**

Major mortality factors in the experiment were herbivory, competing vegetation and external disturbance by mowing or construction work (Fig. 2.5).

Fig. 2.5: Frequent causes of mortality for OSR plants in my experiment. Upper left to middle: Ruderal site was lost to a new tree planting. Upper right: Traces of slug herbivory. Central left to middle and lower left to middle: Previous sites with low vegetation cover were overgrown with vegetation within a few months. Central and lower right: Plants were lost to mowing and removal of the sward.
2.3.2 Seed density

A higher density of seeds led to a slight increase in the total number of seedlings, as shown by the positive highly significant relationship between the logarithms of seed density and the number of emerged seedlings (Fig. 2.6.a). The number of flowering and fruiting individuals was not related to seed density (Fig. 2.6.b, Appendix III.1 Fig. III.2).

![Fig. 2.6: Relationship between the density of seeds (cultivar Artus) sown per 0.05 m² and a) emerged seedlings (both variables log-transformed) and b) fruiting individuals. Each data point represents the mean of three plots on the respective site (RT = railway track, AF = agricultural field, RH = ruderal high-quality site, RL = ruderal low-quality site), n = 28. Results of a linear regression based on the data points from all four sites are shown in Figure a). Both intercept and slope of the regression line are significantly different from zero (p = 0.006 and p ≤ 0.001, respectively).](image)

2.3.4 Differences between sowing events

Variables of recruitment and establishment success partly varied between the three sowing events. Seedling emergence was significantly higher for spring than for fall 2007 (Tables 2.6 & 2.7), which was mainly visible on high-quality sites (Fig. III.3 in Appendix III.1). However, reproducing individuals showed no overall difference between spring and fall 2007. Comparing fall sowings of different years, I found that the sowing in 2007 yielded significantly more seedlings and flowering plants than the sowing in 2008 and also tended to show more fruiting individuals (Tables 2.6 & 2.7).
Table 2.6: Establishment and reproductive success of cultivar Artus are displayed for each sowing event. Sites which were not sown in each compared event were excluded, leaving 12 sites for contrasting the sowings of spring with fall 2007 and six sites for comparing 2007 with 2008. Both disturbed and undisturbed plots from each site were included. Means ± SE pooled over all sites (five blocks each) and disturbance treatments, n = number of replicates per sowing. See Appendix III.1 Fig. III.3-III.8 for means per treatment.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean ± SE</th>
<th>Mean ± SE</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>seedling emergence [%]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>spring</td>
<td>120</td>
<td>25.8 ± 1.7</td>
<td>0.64 ± 0.14</td>
<td>0.33 ± 0.09</td>
</tr>
<tr>
<td>fall</td>
<td>120</td>
<td>8.8 ± 1.0</td>
<td>0.83 ± 0.19</td>
<td>0.29 ± 0.11</td>
</tr>
<tr>
<td>fall 2007</td>
<td>60</td>
<td>24.7 ± 2.7</td>
<td>2.94 ± 0.76</td>
<td>1.31 ± 0.50</td>
</tr>
<tr>
<td>fall 2008</td>
<td>60</td>
<td>12.3 ± 1.7</td>
<td>0.81 ± 0.26</td>
<td>0.39 ± 0.17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean ± SE</th>
<th>Mean ± SE</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>flowering individuals [%]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>fruiting individuals [%]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.7: Effects of site environment and sowing event on variables of establishment and reproductive success of cultivar Artus. One-way analyses for the difference between ruderal and agricultural sites were carried out with means per site averaged over the two cultivars and disturbance treatments: Both sites with high and low vegetation cover were included. Sowing events were compared with the Friedman test. Significance levels (p), degrees of freedom (d.f.), sample size per factor level (n) and variance ratios (F) or χ²-values are displayed.

<table>
<thead>
<tr>
<th></th>
<th>d.f.</th>
<th>n</th>
<th>χ²</th>
<th>F</th>
<th>p</th>
<th>χ²</th>
<th>F</th>
<th>p</th>
<th>χ²</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>seedling emergence [%]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>flowering individuals [%]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>fruiting individuals [%]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

|                  |      |      |     |      |        |     |      |        |     |      |      |

| **ruuderal vs. agricultural** |      |      |     |      |        |     |      |        |     |      |      |
| all sites               | 1    | 10-14| 1.63| 0.215| —      | —    | —    | —      | 0.071| —    | —    |
| low vegetation cover   | 1    | 6-7  | 0.20| 0.661| —      | —    | —    | —      | 0.355| —    | —    |
| high vegetation cover  | 1    | 4-7  | 2.19| 0.173| —      | —    | —    | —      | 0.149| —    | —    |

| **sowing event** |      |      |     |      |        |     |      |        |     |      |      |
| spring vs. fall   | 1    | 120  | 47.21| <0.001| 0.00  | 1.000| 3.77 | 0.180|      |      |
| 2007 vs. 2008     | 1    | 60   | 16.67| <0.001| 10.71| 0.001| 2.67 | 0.134|      |      |

1 arcsine-square-root-transformed
a one way ANOVA    b Mann-Whitney U-test   c Friedman test
2.3.5 Ruderal vs. agricultural sites

Site environment showed no significant influence on seedling emergence (Table 2.7 and Figure 2.7), although there appeared to be more seedlings on ruderal than on agricultural sites if vegetation cover was high (U-test, p = 0.088, n = 4-6, when externally disturbed plots were excluded). Reproducing individuals were only found on ruderal and not on agricultural sites, regardless of initial site vegetation cover. The difference between the environments was significant for the flowering plants and indicated by a trend for the fruiting individuals if all sites were included in the analysis (Figure 2.7, Table 2.7).

2.4 Discussion

2.4.1 Factors influencing establishment success on ruderal sites

OSR plants sown into ruderal sites showed an overall poor establishment success. Mean seedling emergence amounted to at maximum 30.1%, and the mean percentage of flowering and fruiting individuals did not exceed 2.9% and 1.6% of seeds sown, respectively (means per treatment). Nevertheless, the maximum number of fruiting individuals recorded on a single plot amounted to as much as 25%. Sowing experiments in semi-natural and natural habitats found comparable establishment success after one year, which varied among habitats between 0 to 13% seedling emergence and 0-4.6% adult individuals (calculated from Table 1 in Crawley et al. 1993). Only on one site did I find a flowering F1 offspring of the spring sowing, and none were found for the following sowing events. For naturally occurring feral populations, as many as 12-80% can reoccur

Fig. 2.7: Seedling emergence and the percentage of reproducing individuals [% of seeds sown] in ruderal vs. agricultural environments with high or low vegetation cover. Data were averaged over both cultivars Artus and Smart and in case of sites with high vegetation cover also over disturbed & undisturbed plots (only disturbed plots were sown on sites with low vegetation cover). Mean ± SE, n = 4-7.
in the subsequent year (Dietz-Pfeilstetter *et al.* 2006, Menzel 2006). My findings are therefore surprising. I observed herbivory by slugs and rabbits, fast growth of competing vegetation and disturbance of sites (construction work, burrowing animals and mowing) to be frequent causes of mortality. It is possible that plants in my study suffered disproportionately from slug herbivory, as conditions were unusually favourable for slugs in 2007 and 2008 (Net tribune 2007, Sz-online 2008). The importance of herbivory by small mammals and molluscs on OSR plants has been observed previously in natural and agricultural habitats (Crawley *et al.* 1993, Dietz-Pfeilstetter *et al.* 2006, Frank 1998a, 1998b). In addition, regions with previous OSR sightings have partly become more urbanised, leading to higher levels of disturbance. In the first 2.5 to 5 weeks after sowing, there was almost no precipitation (spring 2007 and fall 2008) or reduced precipitation (fall 2007, Fig. II.13-II.15 in Appendix II.5), potentially increasing the risk of failed germination or seed predation. Moreover, several flowering plants withered during the particularly hot and dry months of May 2008 and April 2009 (Fig. II.11 and II.12 in Appendix II.5).

None of the site types chosen proved to be entirely unsuitable for establishment, but I could observe some differences in performance depending on site type and treatment. The most distinct and consistent observation was that establishment success was enhanced if the vegetation cover was disturbed in a 30*30 cm area. This effect was significant for emerging seedlings and reproducing individuals for nearly all sowing events, at least on sites with high vegetation cover. Seedling emergence was 1.9 to 3.3 times as high on disturbed as on undisturbed plots. The effect on fruiting individuals was strongest in fall 2007 when disturbed plots showed 0.8% fruiting individuals while undisturbed plots did not facilitate establishment of any reproducing individuals. Crawley *et al.* (1993) have made similar observations in natural and semi-natural habitats, where the rate of increase of OSR was negligible in undisturbed vegetation but could be greatly elevated by removing the vegetation in an area of 156 m$^2$. My results demonstrate that disturbance at a much smaller scale may enhance establishment success, which might explain why 27% of the feral OSR populations in Bremen occur on sites with more than 90% cover (Menzel 2006).

Even though the effects of disturbance stressed the requirement of gaps in the vegetation, the effect of initial site vegetation cover was not pronounced and unlike expected: Reproducing individuals tended to be more numerous on sites with high cover (disturbed plots). This effect may partly have been caused by soil conditions rather than by vegetation cover: Low-quality sites with low cover had a significantly lower water-holding capacity and organic matter
content (Table 2.1, U-tests, \( p < 0.05, n = 7 \)) and tended to have a lower content of \( \% \) C and \( \% \) N (U-tests, \( p < 0.1 \) \( n = 7 \)). Moreover, bare soil of high-quality sites was often recolonised so quickly that cover of competing vegetation reached levels similar to sites with high initial vegetation cover within few months (Fig. 2.5). However, positive effects of vegetation cover on survival are also possible: Competing vegetation could shield plants from wind and reduce loss of soil moisture. It might also shield plants from herbivore attack: The percentage of seedlings which had been defoliated through herbivory was considerably higher on sites with low cover than on sites with high cover (U-test, \( p = 0.004, n = 28 \), Table II.2 in Appendix II.4). Observations of naturally-occurring feral populations in Bremen are nevertheless found most frequently on sites with low vegetation cover (Menzel 2006), suggesting that competing vegetation usually shows negative effects in ruderal habitats.

Differences between cultivars were not apparent at the seedling stage but became evident for reproducing individuals. Cultivar Artus was more successful, showing a significantly higher number of flowering and fruiting individuals than Smart. Although part of this difference resulted from a higher level of external disturbance on plots with Smart, this observation remained as a statistical trend after exclusion of externally disturbed plots. Artus is one of the new hybrid lines which generally tend to show higher seed yield than non-hybrid lines like Smart. Artus also showed a higher seed yield than Smart in experiments in Saxony (Beese & Karalus 2005).

Site soil quality had limited consequences for establishment: In spite of a significantly higher seedling emergence on high-quality sites in the spring sowing of 2007, the number of reproducing individuals was not significantly affected. Nevertheless, soil quality mattered for reproductive success, as plants on high-quality sites produced substantially more pods. Agricultural experiments show that yield of OSR can be increased through nitrogen fertiliser (reviewed in Grant & Bailey 1993 and Rathke et al. 2006) and through the addition of phosphorus to soils with low or intermediate P content (Grant & Bailey 1993, Holmes & Ainsley 1978, Lickfett et al. 1999). In my experiment, soil quality was defined by high organic matter content, and sites classified as high-quality indeed showed a significantly higher content of N, P, K and C than low-quality sites (Table 2.1, U-tests, \( p \leq 0.001, n = 14 \)). However, I cannot exclude confounding with water-holding capacity, which was also higher on high-quality sites. Drought can reduce seed production per plant by up to 48\% (Champolivier & Merrien 1996, Richards & Thurling 1978). As my experimental fertilisation
on low-quality sites led to a nearly five-fold number of fruiting individuals in fall 2007, relevance of nutrient availability was nevertheless demonstrated. The influence of soil quality on seedling emergence could not be confirmed for the sowing of fall 2007 - neither by experimental fertilisation nor when comparing high-quality with low-quality sites. This may partly result from a greatly reduced number of replicates and partly from differences in environmental conditions. Seedling emergence was significantly lower in fall 2007 (Table 2.7), indicating a limitation by other factors than soil quality. Reduced precipitation (see also 2.4.3) is likely to have limited emergence on both low- and high-quality sites. Beneficial effects of fertiliser on OSR depend on adequate water status (Grant & Bailey 1993, Wright et al. 1988). Moreover, vegetation cover increased significantly from spring 2007 to fall 2007 on high-quality sites (Friedman test, $\chi^2 = 5.0$, $p = 0.025$, $n = 5$, Table II.1 in Appendix II.4) but remained similar on low-quality sites (Friedman test n.s.). This effect may have counter-balanced any positive effect of soil quality. While fertilisation has been shown to promote emergence of other crucifers (Goudey et al. 1988, Hilhorst & Karssen 1989), it is further possible that it does not affect emergence of OSR at all or that the fertiliser concentration chosen in this study was too high: Nitrogen fertiliser placed in direct contact with the seeds may greatly reduce seedling emergence of OSR (Grant et al. 2010, Johnston et al. 2002, Malhi & Gill 2004, Nyborg 1961) due to toxicity of compounds or metabolites (mainly ammonium) and the elevated osmotic pressure caused by soluble ions (Bremner 1995, Bremner & Krogmeier 1989, Dowling 1996, Dubetz et al. 1959). The detrimental effect of fertiliser becomes worse when soil moisture is low (Nyborg 1961), so it may have been relevant on the sandy soils used in my study.

**2.4.2 Ruderal vs. agricultural sites**

The performance of OSR varied with the environment it was sown into, showing significantly more flowering individuals in ruderal than in agricultural environments. This trend was visible regardless of initial site vegetation cover. Sites with high vegetation cover showed a significantly higher N content (Table 2.1, T-test, $p = 0.039$, $n = 4-7$), and trends for higher water-holding capacity and SOM, so one could have expected a better establishment in the agricultural plots. Possibly, the grassland soil in this experiment was too heavy for good establishment, as OSR is known to suffer from heavy soil with high clay content (Cramer 1990). In addition, plots were quickly overgrown with competing vegetation, the cover of which was significantly higher on agricultural than on ruderal plots (Table II.2 in Appen-
Establishment success of OSR under ruderal conditions

dix II.4), probably due to the overall higher soil quality. There was also a trend for higher seedling herbivory on the agricultural plots, where slugs were very numerous. On sites with initially low vegetation cover, soils of ruderal plots had a significantly higher soil pH and P content than agricultural fields (Table 2.1, T-tests, p ≤ 0.05, n = 6-7), but other soil parameters did not differ, nor did the extent of seedling herbivory or the cover of competing vegetation (Table II.2 in Appendix II.4). The trend for a higher percentage of flowering individuals in ruderal plots might therefore be a result of the higher phosphorus content which is known to enhance yield in OSR (Grant & Bailey 1993).

2.4.3 Differences between sowing events

I would have expected fall-sown plants of 2007 to show higher establishment success than those sown in spring 2007, as fall is the typical sowing season for winter OSR (Cramer 1990). In contrast, seedling emergence was significantly higher in spring, and reproducing individuals did not differ between the two sowings. The higher seedling emergence in spring may have been caused by precipitation, which was considerably higher in spring 2007 than in fall 2007 during the weeks following the first substantial rainfalls after sowing (Figures II.13 and II.14 in Appendix II.5). Reproducing individuals may have been limited in fall both by the lower number of seedlings and through changes in site conditions: Vegetation cover was significantly higher in fall than in spring (Table II.1 in Appendix II.4). Comparison of the two fall sowing events revealed higher establishment success for 2007 than for 2008. I attribute this to weather conditions, as neither vegetation cover, seedling herbivory nor disturbance showed differences between the two years.

2.4.4 Seed density

The density of sown seeds had only limited influence on the establishment success of OSR. While increasing seed density raised the total number of emerged seedlings in a log-log relationship, the percentage of emerging seedlings per seed sown decreased (implied by a slope smaller than one in a log-log relationship, Harms et al. 2000). In consequence, seedling emergence of OSR would be both seed- and microsite-limited, which is supported by similar results in agro-ecosystems (Boyd & van Acker 2004). More importantly, the number of reproducing individuals in my study was unaffected by seed density, suggesting that final establishment was more microsite-limited. There were indeed zero reproducing plants on all sites except the agricultural field, which demonstrates that feral plants are likely to be exposed to very stressful conditions. On my sites, even on the agricultural field, competing vegetation
emerged and grew rapidly. Dynamics of roadside populations suggest that feral OSR is seed-limited if the vegetation cover is disturbed, but not if cover by perennial grasses is high (Crawley & Brown 1995). My experiments give further evidence that feral plants will mostly be limited by other factors than seed density.

2.4.5 Conclusions

Overall, I showed that OSR is capable of reproducing even under stressful conditions such as low-quality sandy ruderal soils with competing vegetation. My results confirm the importance of vegetation cover for establishment but demonstrate that the immediate vicinity of the seeds is crucial: Sites with generally high vegetation cover may still facilitate establishment if gaps of 30*30 cm are present. Such small disturbances may well be caused by burrowing animals, e.g. moles. Some of these animals, e.g. rabbits, may however contribute to plant mortality through herbivory. I further found that high soil quality may have direct positive effects on establishment success, but that these can either be ineffectual if other factors become limiting or be overridden by indirect negative effects in the course of secondary succession, namely the simultaneous promotion of competing vegetation. These findings in combination with my observations for grassland soils indicate that an intermediate soil quality may be most suitable for OSR, as it is less easily outcompeted by other vegetation. Crawley et al. (1993) observed similarly that microsites for OSR establishment disappear more quickly with increasing soil fertility. Free microsites appear to be of greater importance than the amount of seeds dispersed.

Establishment success was mostly very low (treatment means between 0 and 1.6%), in spite of a substantial number of emerged seedlings, and cannot quite explain the great number of ruderal populations found in Bremen in a previous survey (Menzel 2006). On the one hand, my study likely underestimated success due to suboptimal conditions (see 2.4.1). On the other hand, I suspect that successful long-term establishment is limited to very specific conditions: bare sites with intermediate soil quality. The absence of slugs and rabbits also appears to be vital. This may be the reason why OSR survived well on railway tracks with gravel beds in another study (Menzel 2006). My chosen sites only partly fulfilled the requirements for OSR establishment. Randomly spilled seeds seem to have limited chances of hitting an appropriate habitat. So where do all the feral OSR plants come from? All things considered, I think it most likely that a majority of OSR populations establishes from seeds imported with topsoil from former agricultural areas. This method of dispersal is known to generate OSR roadside populations (Crawley & Brown 1995). In this case, seeds are automatically brought to an
appropriate habitat: bare soil in larger areas which vegetation and herbivores usually do not immediately recolonise. Furthermore, OSR seeds can persist in the seed bank for up to 11 years (Lutman et al. 2003) and thus outlast periods with unsuitable conditions. My study further showed that establishment success of OSR ferals can be higher in the ruderal than in the agricultural environment, stressing the importance of including ruderal populations in GMP risk assessment.

References


Establishment success of OSR under ruderal conditions


3. Establishment and reproductive success of oilseed rape (*Brassica napus* L.) and weedy relatives on poor-quality ruderal soils

**Abstract**

Adequate risk assessment of genetically modified (GM) oilseed rape (OSR, *Brassica napus*) needs to include all avenues of potential transgene escape. Therefore, its ability to grow in feral populations on disturbed ruderal sites is of particular concern, since these populations may serve as stepping-stones to fields with conventional cultivars or to wild hybridisation partners. Yet little is known about the fitness of OSR on low-quality ruderal soils and about how this compares to the performance of weedy relatives. I therefore determined short-term establishment success and reproductive potential of OSR, *B. rapa*, *B. nigra* and *Raphanus raphanistrum* on four ruderal substrates. The experiment was set up as a fully two-factorial split-plot design on a former dump site in Bremen in Northern Germany. Low soil quality (sand & shallow soil) reduced the reproductive potential (seeds produced per seed sown) of the cultivated species OSR and *B. rapa* significantly, while the weedy relatives were less (*B. nigra*) or even positively affected (*R. raphanistrum*). Nevertheless, the cultivated species produced substantially more seeds per seed sown than *R. raphanistrum* on all substrates, and nearly always more than *B. nigra*. The poor performance of the weedy species was mainly due to a reduced seedling emergence and a higher mortality, resulting in a substantially lower percentage of fruiting individuals than in the cultivated species. Mean seed production was highest for *B. rapa* but did not differ between the other three species. These findings raise concern about the uncontrolled spread of transgenes via ruderal OSR populations, for which I demonstrated a high potential weediness and the potential for population increase on all substrates. I further conclude that ruderal sites are potential refuges for hybridisation with weedy relatives.

**3.1 Introduction**

The introduction of genetically engineered crops has raised concern about potential risks for the environment, including the development of problematic weeds: Crops with transgenes conferring a fitness advantage may show a greater persistence in feral populations or increase the invasiveness of existing weeds by passing on beneficial traits through crop-wild hybridisation (Ellstrand 2001, Hails 2000, Pilson & Prendeville 2004, Snow & Morán Palma 1997). Hybridisation between conventional crops and wild relatives is indeed common and has
already led to aggressive weedy hybrid lineages and increased invasiveness of wild taxa in several cases (Ellstrand 2003, Ellstrand et al. 1999).

OSR is a widespread crop with transgenic herbicide-resistant lines already being cultivated in the USA, Canada, Australia and Chile (TransGen 2013) but not yet in the EU. In the context of GM risk assessment, it is a crop plant of particular concern as it can successfully hybridise with several weedy relatives (OECD 1997, Scheffler & Dale 1994) and may cause trouble as a weed itself, growing as volunteer populations in following crops (Gressel 2005). Furthermore, feral populations are frequently observed on disturbed sites in agricultural and ruderal areas (Crawley & Brown 1995, Elling et al. 2009, Luijten & de Jong 2010, Menzel 2006, Pessel et al. 2001). In case of GM oilseed rape cultivation, these populations might create refuges for long-term persistence of transgenes outside cultivated areas and for the evolution of new genotypes (Breckling & Menzel 2004, Elling et al. 2009, Squire et al. 2010). Feral populations are mostly of moderate size but may nevertheless serve as stepping-stones for uncontrolled transgene spread (Aono et al. 2006, Schafer et al. 2011, Schoenenberger & D'Andrea 2012) to conventional fields and to hybridisation partners and make containment impossible. Feral populations are more variable in their flowering time than cultivated OSR plants, thus showing an extended overlap with the flowering period of wild relatives and increasing the likelihood for hybridisation (Menzel 2006). Yet, population dynamics of feral populations are still poorly understood and the question whether they are self-sustaining remains contentious (Bond et al. 2004, Crawley & Brown 1995, 2004, Elling et al. 2009, Pessel et al. 2001). Although most populations are short-lived (Crawley & Brown 1995, Dietz-Pfeilstetter et al. 2006, Menzel 2006), cases of long-term persistence for 6-8 years have been documented (Bond et al. 2004, Menzel 2006, Pessel et al. 2001).

OSR has retained many of the common characteristics of weedy species, such as rapid growth and resource capture, a high reproductive capacity, and the ability to self-pollinate. Recent breeding objectives have aimed at higher seed number and seed weight, enhanced winter hardiness and disease resistance, all of which could potentially increase reproductive success of volunteer plants (Gressel 2005). Seed production under agricultural conditions can be very high: a well-developed OSR plant produces 2000-3000 seeds (Cramer 1990). But how high is the reproductive potential under stressful abiotic conditions? Rates of population increase in semi-natural and natural habitats are on average more than 40 times smaller than the mean performance of OSR in cultivation (Crawley et al. 1993, Crawley & Brown 1995). Similarly, average seed production by ruderal plants is lower than under
agricultural conditions, though some well-developed stands can show very high reproductive outputs (Dietz-Pfeilstetter et al. 2006, Menzel 2006). While perennial vegetation and herbivory are known to be partly responsible for this poor performance (Crawley et al. 1993), effects of soil conditions have so far been merely observational: Crawley & Brown (2004) noted feral populations along motorways to show low densities on calcareous substrates, and (Menzel 2006) found feral populations on soils not deemed suitable for OSR cultivation. Soil conditions may greatly differ between ruderal and agricultural sites. We can expect negative effects on the yield of OSR on ruderal sites as a consequence of drought (Champolivier & Merrien 1996, Clarke & Simpson 1978) and the absence of fertilisation (Diepenbrock 2000, Miller et al. 2003, Rathke et al. 2005, Sieling & Christen 1997). The fact that dump sites and areas of sand mining have been identified as centres for potential hybridisation between OSR and relatives (Menzel 2006) stresses the need for a better understanding of their population dynamics on low-quality ruderal soils.

I tested the performance of OSR in a ruderal environment and included three potential hybridisation partners which occur on the same ruderal sites (Menzel 2006): \textit{Brassica rapa}, \textit{Brassica nigra} and \textit{Raphanus raphanistrum}. \textit{B. rapa} is the most frequent and successful hybridisation partner of OSR (Scheffler & Dale 1994), occurs in cultivated and weedy varieties and shows a similar degree of domestication as oilseed rape (Gressel 2005). Stable introgression of the glyphosate resistance gene from OSR into weedy \textit{B. rapa} under commercial field conditions has already been reported (Warwick et al. 2008). In addition, I chose two weedy relatives OSR can produce fertile F1 hybrids with (Rieger et al. 2001, Scheffler & Dale 1994) and which also occur on disturbed ruderal and agricultural sites (Rich 1991), \textit{Brassica nigra} and \textit{Raphanus raphanistrum}. Such weeds can be expected to tolerate a wide amplitude of ecological conditions (Holzner & Numata 1982). Both are widespread and troublesome: \textit{R. raphanistrum} causes problems in agriculture in Europe, Africa, North America and Australia and actually ranks among the world’s worst weeds (Cheam 1995, Holm et al. 1977, Warwick & Francis 2005), while \textit{B. nigra} has become invasive in Californian grasslands (Bell & Muller 1973, White & Holt 2005).

The aim of my study was to investigate the effect of low soil quality as a stressor on many ruderal sites on the fitness of OSR and \textit{B. rapa} and to compare their performance to the success of the two weedy relatives \textit{B. nigra} and \textit{R. raphanistrum}. I thereby provide further insight into the degree of weediness of OSR and its implications for the survival of feral populations and hybridisation aspects. To address this question, I assessed the establishment
success and reproductive potential of all four species on four different substrates representative of ruderal soils in a two-factorial split-plot design on a dump site in Northern Germany. I tested whether
1) low-quality soils have negative effects on the reproductive potential of OSR and relatives;
2) reproductive potential of the cultivated plants OSR and B. rapa is reduced compared with the weedy relatives; and
3) reproductive potential of OSR facilitates potential population increase even on low-quality soils.

3.2 Methods

3.2.1 Site description

Location and general characteristics
The experiment took place on a former dump site for non-hazardous building rubble, the Siedenburg dump site in Bremen, Northern Germany, N 53° 7’ 21” and E 8° 47’ 31” (see Appendix II.8 for a detailed description). In 1977-1978, the rubble was covered with a layer of loamy sand and sandy loam and subsequent site succession was documented (Koehler, H. pers. comm., Koehler & Müller 2003). The site was freshly bulldozed prior to my experiment in September 2007, and was quickly recolonised by Rubus armeniacus and species such as Carduus crispus, Holcus lanatus, Reseda luteola, Arctium minus, Rumex obtusifolius and Poa trivialis (frequent species in 2008, Müller, J., pers. comm.). Bremen is characterised by a North Atlantic climate with a mean annual precipitation of 694 mm and a mean temperature of 8.8 °C (DWD 2010, see Appendix II.5.2 for climate data). On this dump site, I set up an experimental area with plastic containers filled with different ruderal substrates.

Substrates
Four substrates were filled into the containers to represent conditions ranging from areas of sand mining to dump sites with moderately rich soils: sand from a ruderal site next to the Centre of Environmental Research and Technology of the University of Bremen, humous soil (sandy loam) from the bulldozed Siedenburg dump site, a 1:1 mixture of the two (= mixed), and a shallow humous soil consisting of a 9 cm layer of Siedenburg humous soil over red building rubble provided by the Umweltbetrieb Bremen. Soil properties were analysed according to Appendix II.2.2. The soils had a very low to medium humus content and water-holding capacity varied from 20.6 to 36.6 at a neutral to acidic pH (Table 3.1).
Table 3.1: Properties of the four ruderal substrates. WHC$_{\text{max}}$ = maximum water-holding capacity (% dry mass); SOM = soil organic matter content (% dry mass).

<table>
<thead>
<tr>
<th>substrate</th>
<th>grain size</th>
<th>SOM</th>
<th>WHC$_{\text{max}}$</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>sand</td>
<td>sand</td>
<td>0.2 ± 0.0</td>
<td>20.6 ± 0.4</td>
<td>4.1 ± 0.0</td>
</tr>
<tr>
<td>mixed</td>
<td>slightly loamy</td>
<td>1.8 ± 0.1</td>
<td>25.2 ± 0.2</td>
<td>6.6 ± 0.0</td>
</tr>
<tr>
<td>humous soil</td>
<td>loamy sand</td>
<td>3.8 ± 0.0</td>
<td>36.6 ± 0.7</td>
<td>6.7 ± 0.0</td>
</tr>
<tr>
<td>shallow humous soil</td>
<td>loamy sand, 9 cm</td>
<td>3.8 ± 0.0</td>
<td>36.6 ± 0.7</td>
<td>6.7 ± 0.0</td>
</tr>
</tbody>
</table>

3.2.2 Design

The experiment was set up as a fully randomised two-factorial design. For each of 20 blocks, four plastic containers (Ø 53 cm, 65 L volume, open bottom) were dug in and filled to a level of 34 cm with one of the four substrates in random allocation (Fig. 3.1). The substrate surface was level with the ground (Fig. 3.2). Each plant species was randomly assigned to one quarter of each container. Thus, substrate was the between-plots factor, varying between containers, and plant species the within-plot factor, varying within containers. 20 replicates were initially set up for each species/substrate combination (but see 3.2.6). The area was protected against

Fig. 3.1: Set-up of the experimental site in twenty blocks of four containers (filled circles) each. The four substrates sand, mixed soil, humous soil and shallow humous soil were randomly arranged per block. In each container, each plant species was sown in one randomly assigned quarter. B.na. = B. napus (OSR), B.r. = B. rapa, B.ni. = B. nigra, R.r. = R raphanistrum.
larger herbivores with an IRKA slug fence by R+M Gartenbedarf, Rehling-Unterach, Germany (height: 15 cm, Fig. 3.3), and a rabbit-proof wire fence by Drahtwaren Driller, Freiburg, Germany (reaching from 20 cm below to 50 cm above ground level). A bird net was put up one week after sowing until spring to prevent birds from taking sown seeds or damaging the seedlings. Competing vegetation was not removed from the containers themselves but from the pathways between the blocks. Due to the open bottom, the tap roots of all plants could have reached the layer of humous dump site soil underneath. However, this layer was freshly bulldozed and very condensed and plant roots did not enter it, as could be seen when some of the containers were emptied after the experiment.

3.2.3 Seed origin and sowing

Seeds of OSR and *B. rapa* originated from cultivated varieties provided unimbibed by RAPOOL-RING GmbH, Isernhagen, Germany, and Wildacker.de, Wolmersdorf, Germany. The winter oilseed rape cultivar Artus was chosen for OSR because it is non-GM but near-isogenic to the GM cultivar Avalon\(^{\text{LL}}\) (Gruber *et al.* 2004). It is also one of the varieties occurring in feral populations in Northwest Germany (Elling *et al.* 2009). *B. rapa ssp. oleifera* (Buko) is a cultivar used as green manure and forage crop (Claußen 2007). Seeds of the weedy relatives were taken from ruderal populations. *B. nigra* seeds were personally collected from plants growing on the neighbouring dumpsite (Blockland dump site) in fall 2007. WeberSeeds, Simpelveld, Netherlands, supplied pods of *Raphanus raphanistrum* from wild populations in Hesse, Germany. As the indehiscent pods increase dormancy in *R. raphanistrum* seeds (Cheam 1986), they were removed to ensure a sufficient number of germinating seeds. Visually damaged, moldy or peculiar-shaped seeds were sorted out for all plants and seeds were stored at room temperature until sowing.
Sowing took place from 24-26 October 2007, which was 1.5 months later than the latest date recommended for cultivation of OSR in Northern Germany (9 September, Rapool Ring GmbH 2007). This delay could not be prevented as site preparation (bulldozing and subsequent set-up) began later and took longer than expected. 90 seeds for each replicate were mixed with 100 ml of the respective substrate and sprinkled evenly on top of the assigned quarter of the container, resulting in a random distribution of seeds. Seeds were then covered with a 1 cm loose layer of the respective substrate to minimize seed predation while still ensuring a suitable sowing depth for all species. Shallow burial of at maximum 1 cm enhances germination of *R. raphanistrum* compared with surface-sown seeds (Cheam 1986). For *B. nigra*, sowing at 1-2 cm (Brinton 1989 cited in Cramer 1990) is recommended, whereas OSR and *B. rapa* establish best if sown at 1-3 cm (Brouwer 1976, Cramer 1990).

### 3.2.4 Estimates of establishment success and reproductive potential

Several variables were measured over the course of the experiment to determine establishment success and reproductive potential of each plant species. Seedling emergence for each replicate was assessed as the maximum number of vital seedlings/plants found over the course of three censuses (Appendix II.9). I thus wished to ensure that nearly all emerging seedlings were counted, regardless of the time of emergence. As estimates for the reproductive potential, I assessed the percentage of flowering and fruiting individuals (% of seeds sown) as well as the mean pod production of up to four randomly chosen plants per replicate for each species. Seed production per plant was estimated for each replicate by multiplying mean pod production per plant with the mean number of fully developed seeds per pod, which were counted for up to four randomly chosen pods per replicate (= up to 80 pods per treatment). Ultimate reproductive output was calculated as the number of seeds produced per seed sown (seeds per plant*number of fruiting individuals/90).

### 3.2.5 Seed viability

The seeds collected from the fruiting plants (four pods per replicate) were tested for seed viability after several months of storage at room temperature. All seeds of the respective substrate/species combination were mixed and five to six replicates of 50 seeds each were drawn. A viability assessment of seeds from *R. raphanistrum* was not possible, as only approximately 100 seeds could be collected in total. Seeds were first tested for germination in plastic pots (Ø and height = 7 cm) filled with the respective defaunated\(^1\) substrate and watered

---

\(^1\) See defaunation treatment described in Appendix II.11
every second day to 70% WHC\textsubscript{max}, thus keeping conditions close to the field. Pots were incubated for 29 days, at a diurnal temperature cycle known to stimulate germination (Thompson & Grime 1983) (15 h of light at 25 °C and 9 h of darkness at 15 °C with a constant humidity of 80%), except for days 9 to 15 when more extreme shifts in temperature (25 °C / 5 °C) were applied since stratification has proven effective for breaking dormancy in OSR (Gruber \textit{et al.} 2004, Schlink 1994). Seedlings were counted and removed every second day. Seeds left at the end of the test were either considered germinated if the radicle protruded the testa, dead if seeds were soft, or tested for viability with tetrazolium chloride (TZ) following Duffy \textit{et al.} (2007) (see Appendix II.10). The proportion of viable seeds was calculated in % of seeds recovered from the experiment, as seeds of \textit{B. nigra} were very difficult to find if they hadn’t germinated. In addition, vital seedlings were also counted to estimate expected F1 seedling recruitment. All seedlings from the germination test were considered vital if at least one healthy cotyledon had developed. Vital seedlings were calculated as the proportion of all 50 seeds as germinated seedlings would not have been missed. The mean percentage of vital seedlings per substrate/plant species combination was then used to calculate the expected F1 seedlings per seed sown for each replicate.

\subsection*{3.2.6 Data analysis}

Loss of replicates occurred for the percentage of fruiting plants as an unauthorised hemp plantation had been set up in some containers in summer 2008. The two blocks concerned were left out of analysis. Several replicates, especially for the weedy relatives, did not give rise to reproducing individuals at all, so sample sizes of variables of seeds produced per plant varied greatly from 6 (\textit{R. raphanistrum}, humous soil) to 20 (\textit{Brassica rapa}). Analyses were carried out in GenStat, 8.1 (VSN Int. Ltd., Hemel Hempstead, Hertfordshire, UK), except for Welch tests which were done in SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Results were considered significant at p ≤ 0.05. Data were transformed prior to analysis to improve homogeneity of variances and normal distribution. Proportional data were always arcsine-square-root-transformed, and other variables based on count data (all variables of reproductive output) were log- or rank-transformed. A small value of ≤ 0.5 was added prior to transformation to all values in the dataset to avoid zero values. Data were back-transformed for presentation unless otherwise indicated and 95% confidence intervals (CI) computed after (Sokal & Rohlf 1969). As the CI are calculated on the transformed scale, these intervals are asymmetrical when back-transformed.
Block effects were only included if they were significant. A two-way ANOVA with Tukey post-hoc test was carried out whenever assumptions were met to test for differences between plant species and for overall substrate effects. Whenever this was not possible, or if the two-way analysis indicated an interaction between the two factors, one-factorial analyses were carried out for both factors. One-way ANOVA was chosen if data were normally distributed and with homogenous variances, Welch’s ANOVA and Tamhane post-hoc test for heterogenous variances and normal distributions, and Kruskal-Wallis H-test for skewed distributions. In the latter case, differences between factor levels were tested with U-tests, applying the Bonferroni correction. Moderate deviations from normality were tolerated because of ANOVA’s robustness in situations with equal sample sizes (Quinn & Keough 2007). In case of unequal sample sizes, the Welch test was preferred if distributions were not strictly normal, as it is both more robust to non-normality and robust against unequal sample sizes unless distributions are extremely skewed (Zijlstra 2004). Generalized linear models were not suitable due to variance heterogeneity whenever transformation proved to be ineffective in stabilising variance, which is why they were not used.

---

2 The two-factorial analysis was first carried out as a split-plot ANOVA. This analysis takes variation from blocks and containers into account (See Appendix II.7 and II.7.2 for a description of split-plot designs). As block effects were not significant, I display the results of simple two-way ANOVAs.
3.3 Results

3.3.1 Plant species differences

All variables of plant establishment and reproductive output showed significant or highly significant differences between plant species (Table 3.2, Table 3.3).

Cultivated vs. weedy species

The reproductive potential in terms of seeds produced per seed sown averaged over all substrates was substantially higher for both cultivated species (OSR and B. rapa) than for the two weedy species (B. nigra and R. raphanistrum) (Fig. 3.4.c, Table 3.3). OSR produced 3.4 times as many seeds per seed sown as B. nigra and 35.4 times as many as R. raphanistrum. This difference was even more pronounced for B. rapa which produced 13.7 and 141.5 times as many seeds per seed sown as B. nigra and R. raphanistrum, respectively. The high seed output of the cultivated species was mainly due to a significantly higher percentage of fruiting plants for both OSR and B. rapa (Fig. 3.4.a, Table 3.2), rather than to seed production of individual plants: OSR plants did not develop more seeds than individuals of the weedy species (Fig. 3.4.b), and although B. rapa plants produced significantly more seeds than B. nigra and R. raphanistrum (Fig. 3.4.b, Table 3.3), these differences were less pronounced. The viability of produced seeds was similar for B. nigra compared to the cultivated plants (Table 3.3, Table 3.4), so the expected number of viable F1 seedlings per seed sown reflected the findings for the seed production per plant (Table 3.4).

Better plant establishment of the cultivated species was already observed at the seedling stage, where more seedlings emerged for OSR (82%) and B. rapa (66%) than for B. nigra (28%) and R. raphanistrum (16%) (Table 3.2, Table 3.4). These differences became more pronounced for the numbers of flowering and fruiting individuals (Table 3.2, Fig. 3.4, Table 3.4), since less seedlings of the weedy species developed into fruiting plants than for the cultivated species (OSR: 41.1%, B. rapa: 83.4%, B. nigra: 8.7%, R. raphanistrum: 2.9%, all significantly different according to Kruskal-Wallis H-test, p < 0.001, and Bonferroni-corrected U-tests, p < 0.05).
Establishment success of OSR and weedy relatives

Table 3.2: Results of two-way ANOVAs performed on arcsine-square-root-transformed variables of plant establishment, with substrate and plant species as factors. Significant effects (p ≤ 0.05) are shown in bold type. n = 20 for each plant/substrate combination (except n = 18 for fruiting individuals). Degrees of freedom (d.f.), variance ratios (F) and significance levels (p) are displayed.

<table>
<thead>
<tr>
<th>effect</th>
<th>seedling emergence [%]</th>
<th>flowering individuals [%]</th>
<th>fruiting individuals [%]*</th>
</tr>
</thead>
<tbody>
<tr>
<td>substrate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12.38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>plant species</td>
<td>3</td>
<td>1092.99</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>species × substrate</td>
<td>9</td>
<td>9.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>error</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>304</td>
<td>304</td>
<td>204</td>
</tr>
</tbody>
</table>

† without R. raphanistrum because ANOVA assumptions would have been violated. Kruskal-Wallis H-test was performed additionally including all species and found plant effects to be significant (p < 0.001)

Table 3.3: Plant species effects on variables of reproductive potential tested with one-way analyses over all substrates. Viability of produced seeds was calculated as % retrieved from the germination test. Viable F1 seedlings were estimated from the no. of seeds produced per plant and the proportion of viable seedlings produced in the germination test. Significant effects (p ≤ 0.05) are shown in bold type. Degrees of freedom (d.f.), sample size per factor level (n), variance ratios (F) and significance levels (p) are displayed.

<table>
<thead>
<tr>
<th>effect of plant species</th>
<th>d.f.</th>
<th>n</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>seeds per plant</td>
<td>3</td>
<td>35-72</td>
<td>20.106</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>seeds per seed sown</td>
<td>3</td>
<td>72</td>
<td>310.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>viability of produced seeds</td>
<td>2</td>
<td>23-24</td>
<td>5.47</td>
<td>0.008</td>
</tr>
<tr>
<td>F1 seedlings per seed sown</td>
<td>2</td>
<td>72</td>
<td>—</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1 log-transformed for analysis
2 arcsine-square-root-transformed for analysis
3 rank-transformed for analysis

62
Table 3.4: Additional variables of establishment success and reproductive potential for OSR (B. napus) and relatives. Viability of produced seeds was calculated as % retrieved from the germination test. Viable F1 seedlings were estimated from the no. of seeds produced per plant and the proportion of viable seedlings produced in the germination test. Different letters indicate significant differences between plant species at p ≤ 0.05 (Table 3.2 and Table 3.3, Tukey or Tamhane post-hoc test or Bonferroni-corrected U-tests). Back-transformed means in bold and 95% confidence intervals (CI) in brackets. F1 seedlings are shown as original means ± SE since no transformation was used for analysis. n = 80 (seedling emergence & flowering individuals); n = 23-24 (seed viability); n = 72 (F1 seedlings).

<table>
<thead>
<tr>
<th></th>
<th>B. napus</th>
<th>B. rapa</th>
<th>B. nigra</th>
<th>R. raph.</th>
</tr>
</thead>
<tbody>
<tr>
<td>seedling emergence [%]</td>
<td>82.0(^a)</td>
<td>65.6(^b)</td>
<td>27.6(^c)</td>
<td>15.5(^d)</td>
</tr>
<tr>
<td>(80.7-83.4)</td>
<td>(63.4-67.8)</td>
<td>(24.6-30.7)</td>
<td>(14.5-16.4)</td>
<td></td>
</tr>
<tr>
<td>flowering individuals [%]</td>
<td>64.5(^a)</td>
<td>53.4(^b)</td>
<td>4.7(^c)</td>
<td>4.2(^c)</td>
</tr>
<tr>
<td>(62.6-66.4)</td>
<td>(51.3-55.5)</td>
<td>(3.8-5.6)</td>
<td>(3.6-4.8)</td>
<td></td>
</tr>
<tr>
<td>viability of produced seeds [%]</td>
<td>97.2(^a)</td>
<td>92.2(^b)</td>
<td>93.56(^{ab})</td>
<td>—</td>
</tr>
<tr>
<td>(95.6-98.4)</td>
<td>(88.6-95.2)</td>
<td>(88.4-97.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1 seedlings per seed sown</td>
<td>51.8(^a)</td>
<td>193.9(^b)</td>
<td>13.6(^c)</td>
<td>—</td>
</tr>
<tr>
<td>± 6.4</td>
<td>± 18.2</td>
<td>± 3.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 3.4**: Variables of reproductive potential averaged over the four substrates for the two cultivated species OSR (B. napus) & B. rapa vs. the two weedy relatives B. nigra & R. raphanistrum. Different letters above the bars indicate significant differences between plant species (Table 3.2 and Table 3.3, Tukey or Tamhane post-hoc test or Bonferroni-corrected U-tests). a) fruiting individuals in % of seed sown, back-transformed mean ± 95% confidence intervals (CI), n = 72; b) estimate for the seeds produced per plant, back-transformed mean ± 95% CI, n = 35-72; c) estimate for the seeds produced per seed sown, original mean ± SE, n = 72.
3.3.2 Substrate effects

A significant interaction between substrate and plant effects was found for all variables which could be analysed with two-factorial tests (Table 3.2). Therefore, substrate effects were analysed separately for each plant species. The most meaningful parameter for assessing plant invasiveness is the rate of increase (Crawley et al. 1993, Parker & Kareiva 1996), which in my study is simplified to the number of seeds produced per seed sown. For the following comparisons, I therefore only present this variable and the two variables it has been calculated from: the percentage of fruiting plants [% of seeds sown] and the number of seeds produced per plant. Other variables (see Appendix III.2 Tables III.6-III.8 and Figures III.9-III.12) did not necessarily show the same pattern and will only be mentioned if they were considered important for the evaluation of the reproductive potential. A summary of effects associated to my hypotheses is displayed in Appendix III.2.3, Tables III.9-III.11.

Table 3.5: Results of one-way analyses testing for the overall substrate effects on the reproductive potential separately for each plant species (blocked ANOVA, Welch’s ANOVA or Kruskal-Wallis H-test, d.f. = 3). Significant effects (p ≤ 0.05) are shown in bold type. n = 18 (except for seeds per plant: B. nigra (n = 13-16) and R. raph. (n = 6-12)). Degrees of freedom (d.f.), sample size per factor level, variance ratios (F) and significance levels (p) are displayed.

<table>
<thead>
<tr>
<th></th>
<th>d.f.</th>
<th>n</th>
<th>p</th>
<th>F</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>B. napus</td>
<td>B. rapa</td>
</tr>
<tr>
<td>fruiting individuals</td>
<td>3</td>
<td>18</td>
<td>0.092</td>
<td>2.24</td>
<td>0.002 5.34</td>
<td>2.c</td>
</tr>
<tr>
<td>seeds per plant</td>
<td>3</td>
<td>6-18</td>
<td>0.025</td>
<td>3.30</td>
<td>0.00114.08</td>
<td>1.c</td>
</tr>
<tr>
<td>seeds per seed sown</td>
<td>3</td>
<td>18</td>
<td>0.003</td>
<td>5.27</td>
<td>&lt;0.00115.37</td>
<td>1.a</td>
</tr>
</tbody>
</table>

1 log-transformed for analysis
2 arcsine-square-root transformed for analysis
3 rank-transformed for analysis
a Welch's ANOVA
b Kruskal-Wallis H-test
c unblocked ANOVA

Cultivated species

Substrate properties significantly affected the reproductive potential in terms of seeds produced per seed sown for both cultivated species (Table 3.5). OSR produced significantly less seeds per seed sown both on shallow soil (vs. humous and mixed soil) and on sand (vs. mixed) (Fig. 3.5, Table 3.5). These effects were visible but not significant in the percentage of fruiting plants and the seed production per individual plant (Table 3.5, Table 3.6). The
viability of produced seeds was not substrate-dependent and ranged between 96.1 and 98.5% (see Appendix III.2.2 Tables III.7 and III.8).

*B. rapa* was most clearly influenced by substrate type. The production of seeds per seed sown was significantly reduced on sand (vs. humous soil, Fig. 3.5, Table 3.5). This was due both to a significantly lower number of fruiting plants (Table 3.5, Table 3.6) and to a non-significant reduction in the number of seeds produced per individual plant (Table 3.5, Table 3.6). Even the viability of the produced seeds was lower on sand (79.7%) than on other substrates (92.7-98.1%) (see Appendix III.2.2 Table III.7 and III.8). Shallow soil also led to a substantial reduction in the seeds produced per seed sown, caused by a strong decrease in the number of seeds produced per individual plant (Table 3.5, Table 3.6).

![Fig. 3.5: Substrate effects on the no. of seeds produced per seed sown by the two cultivated species *B. napus* (OSR) & *B. rapa* vs. the two weedy relatives *B. nigra* & *R. raphanistrum*. Results are shown for the four different substrates. Different letters indicate significant differences between substrates but do not signify plant species differences (Table 3.5, Tukey or Tamhane post-hoc or Bonferroni-corrected U-tests). Original values are shown rather than back-transformed ones as different transformations were used for the analysis of different species. Mean ± SE, n = 18](image)

**Weedy species**

*B. nigra* produced significantly less seeds per seed sown on shallow soil than on sand (Fig. 3.5, Table 3.5, Table 3.6). These effects were visible but not significant in the percentage of fruiting plants and the seed production per individual plant (Table 3.5, Table 3.6). The viability of produced seeds was significantly lower on shallow soil (70.5%) compared with all other soils (96.0-98.9%) (Appendix III.2.2 Table III.7 and III.8). *R. raphanistrum* showed no negative response to low-quality soils but actually produced more seeds per seed sown on sand than on humous soil (Fig. 3.5, Table 3.5). This was a combined
effect of the non-significant but visible differences both in the percentage of fruiting plants and the seed production per individual plant (Table 3.5, Table 3.6).

Table 3.6: Effects of substrate type on the percentage of fruiting individuals and seed production per plant for the four different species. Substrates are ordered so that the substrate with the highest value is topmost. For the actual mean values of the variables see Table 3.9. Different letters indicate significant differences between substrates for the respective plant species (Table 3.5, Tukey or Tamhane post-hoc test or Bonferroni-corrected U-tests, p ≤ 0.05) but do not signify plant species effects.

<table>
<thead>
<tr>
<th>Fruiting Individuals [%]</th>
<th>B. napus</th>
<th>B. rapa</th>
<th>B. nigra</th>
<th>R. raph.</th>
</tr>
</thead>
<tbody>
<tr>
<td>mixed a</td>
<td>humous a</td>
<td>sand a</td>
<td>sand a</td>
<td></td>
</tr>
<tr>
<td>humous a</td>
<td>shallow a</td>
<td>mixed a</td>
<td>mixed a</td>
<td></td>
</tr>
<tr>
<td>sand a</td>
<td>mixed a</td>
<td>humous a</td>
<td>shallow a</td>
<td></td>
</tr>
<tr>
<td>shallow a</td>
<td>sand a</td>
<td>shallow a</td>
<td>humous a</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Seeds per Plant</th>
<th>B. napus</th>
<th>B. rapa</th>
<th>B. nigra</th>
<th>R. raph.</th>
</tr>
</thead>
<tbody>
<tr>
<td>mixed a</td>
<td>humous a</td>
<td>sand a</td>
<td>shallow a</td>
<td></td>
</tr>
<tr>
<td>humous a</td>
<td>mixed a</td>
<td>humous a</td>
<td>sand a</td>
<td></td>
</tr>
<tr>
<td>sand a</td>
<td>sand a</td>
<td>mixed a</td>
<td>mixed a</td>
<td></td>
</tr>
<tr>
<td>shallow a</td>
<td>shallow a</td>
<td>shallow a</td>
<td>humous a</td>
<td></td>
</tr>
</tbody>
</table>

3.3.3 Substrate effects on plant species differences

Cultivated vs. weedy

As described above, soil quality affected the plant species differently in quality or quantity. Sand greatly reduced the reproductive output of the cultivated species but had no negative effect on the weedy species. Shallow soil was not problematic for R. raphanistrum but for the two cultivated species and (less clearly) for B. nigra. However, these effects were mostly small compared to overall differences between plants. Accordingly, they rarely changed the ranking of plant species performance. The higher production of seeds per seed sown of the cultivated species vs. the weedy species was thus found to be consistently significant on almost all substrates, with the only exception of sand where OSR produced only as many seeds as B. nigra but still more than R. raphanistrum (Fig. 3.5, Table 3.7 & 3.8). This consistency was mainly due to the percentage of fruiting individuals, as the production of seeds per plant showed stronger substrate dependencies (Table 3.9). The magnitude of the superiority of the cultivated plants, however, clearly differed with substrate, as can be seen in the number of seeds per seed sown produced by B. rapa, which was only six times as high as that of B. nigra on sand but 17 to 25 times as high on the other substrates (Fig. 3.5).
The only variable in which a weedy species was actually significantly more successful than a cultivated species on a single substrate (but not overall) was the viability of produced seeds, which was higher for \textit{B. nigra} on sand than for \textit{B. rapa} (see Appendix III.2.1 Fig. III.11), but the number of F1 seedlings was still higher for \textit{B. rapa} even on this substrate (see Appendix III.2.1 Fig. III.12). In contrast, viability of \textit{B. nigra} seeds was lower than for \textit{B. rapa} and OSR seeds on shallow soil (see Appendix III.2.1 Fig. III.11).

**Table 3.7**: Plant species effects on the reproductive potential, tested with one-way analyses for each substrate separately (blocked ANOVA, Welch's ANOVA or Kruskal-Wallis H-test, d.f. = 3). Significant effects (p ≤ 0.05) are shown in bold type. n = 18 (except for seeds per plant sown: shallow (n = 6-18), humous (n = 11-18), mixed (n = 12-18) and sand (n = 7-18)).

<table>
<thead>
<tr>
<th>Plant effect for each substrate</th>
<th>shallow</th>
<th>humous</th>
<th>mixed</th>
<th>sand</th>
</tr>
</thead>
<tbody>
<tr>
<td>d.f. n p F</td>
<td>d.f. n p F</td>
<td>d.f. n p F</td>
<td>d.f. n p F</td>
<td></td>
</tr>
<tr>
<td>fruiting individuals [%]</td>
<td>3 18 &lt;0.001 — 2b</td>
<td>&lt;0.001 509.87 2a &lt;0.001</td>
<td>— 2b &lt;0.001 232.17 2c</td>
<td></td>
</tr>
<tr>
<td>seeds per plant</td>
<td>3 6-18 0.325 1.24 1a</td>
<td>&lt;0.001 14.57 1a 0.001</td>
<td>7.99 1a &lt;0.001 9.17 1a</td>
<td></td>
</tr>
<tr>
<td>seeds per seed sown</td>
<td>3 18 &lt;0.001 — b</td>
<td>&lt;0.001 410.14 3a &lt;0.001</td>
<td>113.76 1a &lt;0.001 52.46 1a</td>
<td></td>
</tr>
</tbody>
</table>

1 log-transformed for analysis 2 arcsine-square-root transformed for analysis 3 rank-transformed for analysis

**Table 3.8**: Statistical significance of differences between plant species in the number of seeds produced per seed sown (displayed in Fig. 3.5). **p ≤ 0.001; * p ≤ 0.01; * p ≤ 0.05; n.s. = not significant according to Tamhane post-hoc tests or Bonferroni-corrected U-tests, n = 18.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>B. rapa</th>
<th>B. rapa</th>
<th>B. nigra</th>
</tr>
</thead>
<tbody>
<tr>
<td>shallow</td>
<td>**</td>
<td>**</td>
<td>n.s.</td>
</tr>
<tr>
<td>B. nigra</td>
<td>**</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>R. raph.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>humous</td>
<td>***</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>B. nigra</td>
<td>***</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>R. raph.</td>
<td>***</td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td>mixed</td>
<td>***</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>B. nigra</td>
<td>***</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>R. raph.</td>
<td>***</td>
<td>***</td>
<td>n.s.</td>
</tr>
<tr>
<td>sand</td>
<td>***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. nigra</td>
<td>n.s.</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>R. raph.</td>
<td>***</td>
<td>***</td>
<td>*</td>
</tr>
</tbody>
</table>
3.4 Discussion

3.4.1 Performance of the cultivated species on ruderal substrates

Soil conditions had a significant impact on plant reproductive potential. According to expectations, sand and shallow soil had marked negative effects on the number of seeds produced per seed sown for both cultivated species, amounting to a reduction by up to 60% in OSR. Besides a low nutrient availability, drought effects are likely to have been severe, as precipitation in May was unusually low (Appendix II.5 Fig. II.11), in a phase when OSR is most sensitive to water stress (from anthesis to maturity, Champolivier & Merrien 1996). Additional factors likely depressed plant development, explaining why OSR plants achieved only approximately 4-6% of the reproductive potential of a well-developed individual. Intra- and interspecific competition will have been fierce, as plant densities in the containers (254-503 plants*\(m^{-2}\)) greatly exceeded densities in OSR cultivation of 40-80 plants*\(m^{-2}\) (Cramer 1990). High planting density can reduce yield or yield components of OSR and weedy B. rapa by over 60% per plant (Geisler & Stoy 1987, Hauser et al. 2003, Leach et al. 1999).

Finally, the sowing date in late fall may have reduced seed yield for OSR (Brouwer 1976, (reviewed in) Diepenbrock 2000, Mendham et al. 1981, Schulz & Troll) but less so for B. rapa which is sown 2-3 weeks later in the season than OSR (Brouwer 1976, Schulz & Troll). In spite of these drawbacks, OSR was capable of producing enough seeds to facilitate potential population growth, even at very low substrate quality. Other studies
confirm that seed production per plant on some ruderal sites may be substantial and can exceed my findings (Dietz-Pfeilstetter et al. 2006, Menzel 2006).

3.4.2 Comparison of cultivated and weedy species

Contrary to expectations, the reproductive potential of both cultivated species was higher than that of the related weedy species *B. nigra* and *R. raphanistrum*. Although the weedy species were less (*B. nigra*) or not at all (*R. raphanistrum*) impeded by low-quality soils, OSR produced significantly more seed per seeds sown on all substrates, except on sand where its seed production only slightly and non-significantly exceeded that of *B. nigra*. *B. rapa* outperformed the weedy species even more strongly. This was partly due to the significantly lower levels of seedling emergence shown by the weedy relatives (*B. nigra* 28%, *R. raphanistrum* 16% vs. OSR 82% and *B. rapa* 66%).

Field seedling emergence data reported for both weedy species are highly variable, ranging from 10 to 77.5% for *R. raphanistrum* if sown at shallow depths in undisturbed agricultural or garden soils (Cheam 1984, 1986, Reeves & Code 1981, Roberts & Boddrell 1983, Stanton 1984) and from 0 to 98% for *B. nigra* (Roberts 1986, Virtue & Thomas 1999, Walker et al. 2004). My substrate comparisons show that emergence of *B. nigra* may have been low due to low soil quality, which was not true for *R. raphanistrum*.

Low emergence rates of both species, especially *R. raphanistrum*, are partly attributed to a high potential for dormancy (Cheam 1986, Mekenian & Willemsen 1975, Reeves & Code 1981, Roberts 1986, Roberts & Boddrell 1983), which enables substantial proportions of seeds to persist ungerminated and viable for several years (Kivilaan & Bandurski 1973, Roberts & Boddrell 1983, Toole 1946). Even though dry storage and removal of the indehiscent seed pod, which occurred in my study, reduce dormancy levels in *R. raphanistrum*, some potential for dormancy remains and can be enhanced by stratification (Cheam 1984, Mekenian & Willemsen 1975), e.g. through my late sowing. For both species, I can assume that part of the seeds which had not germinated had the potential to produce seedlings in later years. However, substantial proportions of *R. raphanistrum* seeds exhumed from burial in another study may also be or have become non-viable (Reeves & Code 1981), a fate which likely applied to a large proportion of the ungerminated seeds in my study. I cannot rule out the possibility that the seeds of the weedy species, collected from ruderal populations, were less viable than the seeds of the cultivated species ordered from agricultural seed supply and probably grown under more favourable conditions. The better performance of the cultivated species in terms of seedling emergence would then only be representative for
agricultural seeds lost in transport and not for seeds produced under ruderal conditions. However, my seed viability tests show that seeds collected from ruderal OSR and *B. rapa* almost show no reduction in seed viability compared to commercial seeds.

The cultivated species displayed high seed viability and low levels of dormancy. In addition, they clearly exhibited greater plant fitness, as the proportion of seedlings which grew into reproducing individuals was also significantly and greatly higher compared with the weedy species, with 41.1 and 83.4% for OSR and *B. rapa*, compared to 8.7% for *B. nigra* and 2.9% for *R. raphanistrum*. While *B. rapa* even produced more seeds per plant than the weedy relatives, OSR produced at least equal amounts. In both weedy species most individuals developed poorly, and only few well-developed individuals produced large amounts of seeds. This L-shaped distribution in seed reproductive output has previously been described for populations of *R. raphanistrum* (Stanton 1985). Furthermore, interspecific competition within the containers will have favoured fast-developing species. *B. rapa* was observed to be the first to flower in early May, followed by OSR (mid- to late May). *R. raphanistrum* and *B. nigra* flowered considerably later in early June and mid-July, respectively. *B. nigra* was at a particular disadvantage as the only species with a considerable emergence in spring 2008, while seedlings of all other species emerged almost exclusively in fall 2007. In addition, *B. nigra* has much smaller seeds and consequently smaller seedlings than the other species, which can be disadvantageous in a competitive environment (Stanton 1985). Larger seedlings are more resistant to environmental hazards, have more reserves and gain better access to resources (Leishman *et al.* 2000). Thus, the cultivated species may have largely depleted resources such as light and nutrients, resulting in a poor development of *B. nigra* and *R. raphanistrum*.

Other studies confirm superior establishment success of OSR over *B. nigra* in competition with grassland vegetation under field conditions (Walker *et al.* 2004) and a tendency for higher seed production by *B. rapa* vs. *B. nigra* in non-competitive greenhouse experiments (Feldheim & Conner 1996).

### 3.4.3 Implications for transgene escape

The superior performance of the cultivated species on low-quality ruderal substrates indicates a higher potential weediﬁness for OSR and *B. rapa* than expected. In spite of low substrate quality and a stressful environment in terms of resource competition, the cultivated species were “weedier” than *B. nigra* and *R. raphanistrum* in some aspects which are typically attributed to successful weeds (Ammann *et al.* 2000): the capacity to germinate and produce
seeds in a wide range of environments, to produce a very large number of seeds under favourable conditions, as well as a rapid growth through the vegetative phase to flowering. Given that both OSR and *B. rapa* successfully established on ruderal soils and showed a high reproductive capacity, hybridisation of these species, which is frequent and may lead to transgene introgression in agricultural settings (see 1.2.1), may well occur in feral populations.

While it has been demonstrated that some domestication traits, such as a dwarfing gene (Rose *et al.* 2009), can reduce fitness, my findings suggest that crop genes in general are not disadvantageous in non-agicultural environments. Studies with crop-wild hybrids mostly show lower fitness for the hybrid than for the wild parent (reviewed in Campbell 2007, Ellstrand 2003 and Hails & Morley 2005), including hybrids of OSR and wild relatives (but see Hauser *et al.* 2003). However, these studies are mostly restricted to early-generation hybrids whose fitness may be influenced by effects such as outbreeding depression and heterosis (Burke & Arnold 2001, Ellstrand 2003), which may over- or underestimate the probability of crop genes to persist within weed populations (Campbell 2007).

Some studies investigating the consequences of specific domestication traits indeed confirm that crop traits can be beneficial to plant fitness in stressful or non-crop environments, for example large size of seedlings and plants, rapid growth, early flowering and large flowering disk diameter in sunflowers (Baack *et al.* 2008, Mercer *et al.* 2007). In contrast, random crop alleles in crop-wild hybrids of OSR and weedy *B. rapa* only showed a positive effect in the absence of competition (Rose *et al.* 2009). In several cases, conventional crop genes managed to persist in weeds for several years under natural conditions (genes from OSR persisting in weedy *B. rapa* (Hansen *et al.* 2001), or genes from *R. sativus* persisting in *R. raphanistrum* (Snow *et al.* 2001)), giving further indications that crop genes do not generally confer a selective disadvantage (Campbell 2007). More significantly, an invasive hybrid line of *R. sativus* and *R. raphanistrum* has completely replaced the wild progenitor in California and apparently led to its local extinction (Hegde *et al.* 2006).

Similarly, my study indicates that *R. raphanistrum* and *B. nigra* could potentially benefit from crop traits conferred by OSR. One has to keep in mind, though, that my results are restricted to first-year successional stages in the absence of vertebrate and slug herbivory where competition by perennial plants was minimal. All three factors can heavily influence long-term survival of feral OSR populations (Crawley *et al.* 1993). While my results hint at a lower competitive ability of the weedy relatives, further research is needed on resistance to
herbivores or to fungal attack and other mortality factors. Another, possibly more important factor, is long-term survival in the seed bank, which does occur in OSR (Lutman et al. 2003) but at generally lower rates than those observed in weedy relatives (Hails et al. 1997, Lutman et al. 2002). It is an important characteristic of annual weeds and a factor for feral population persistence (Ammann et al. 2000, Cheam 1995, Gressel 2005). For a full assessment of OSR weediness, the benefits and drawbacks of high dormancy levels versus high reproductive output in the first year need to be assessed in the view of long-term establishment success (see 6.1.5).

In conclusion, my findings do not support the common assumption that crop traits would necessarily be disadvantageous in a wild environment, especially under stressful conditions, and that selection against crop alleles would prevent or reduce the introgression of transgenes into wild relatives (Squire et al. 2010, Stewart et al. 2003). My findings imply that ruderal environments may well facilitate uncontrolled spread of transgenes from OSR. Even though it is a cultivated plant, OSR has proven successful under suboptimal soil conditions, displayed the potential for self-sustained population increase and even outperformed weedy relatives. Despite the fact that feral populations are mostly short-lived, their potential for weediness should thus not be overlooked or minimised, especially if further fitness advantages, such as herbicide resistance, are conferred through a transgene. Apart from the dangers this poses through uncontrolled transgene spread via feral OSR populations, it also raises concern regarding transgene spread via weedy or cultivated relatives, which does not seem to be limited by negative fitness effects of conventional domestication traits. Ruderal sites may thus serve as suitable refuges facilitating transgene escape. Given that an intensive monitoring of these sites is very time-consuming, transgene spread via these sites may go largely unnoticed.

References


Establishment success of OSR and weedy relatives


4. Can dwarfed oilseed rape (*Brassica napus* L.) measure up to tall cultivars under stressors relevant for feral populations? - Implications for the success of transgenic mitigation

**Abstract**

Herbicide-resistant varieties of oilseed rape (*Brassica napus* L.) have proven difficult to contain and could potentially lead to increased weediness of the crop itself or of wild hybridisation partners. It has been proposed that unintended gene flow could be reduced by linking transgenes with a gene for dwarfing, which may reduce fitness under competitive conditions (transgenic mitigation). Yet, a full assessment of implications on fitness is still lacking, especially with regard to feral populations which mainly grow on disturbed sites in ruderal or rural areas. I hypothesised that a dwarfed cultivar has an advantage in ruderal environments, as small plants may be more likely to escape damage and to tolerate low soil quality. Therefore, I tested the effects of simulated mowing and soil quality on relative fitness of the semi-dwarf hybrid PR45D03 in comparison with the tall cultivar Artus. Two separate experiments were set up on a former dump site in Bremen, Northern Germany, in which I measured the reproductive potential of the two cultivars on 1) three substrates of different soil quality and 2) unmown control plots versus plots mown to 2.5 cm in fall and plots mown to 10 cm in spring. In the substrate comparison, PR45D03 produced significantly less seeds than Artus on all soils. Unlike expected, dwarfing can thus be disadvantageous on soils of low nutrient content and water-holding capacity. However, cultivars did not differ in reproductive potential on control plots (humous soil) in the mowing simulation. Artus plants were significantly taller than the semi-dwarf hybrids at both mowing events and lost more dry plant biomass and % leaf area than PR45D03. Yet, the tall cultivar produced a similar number of seeds as PR45D03 in fall-mown and significantly more seeds in spring-mown plots, indicating that tall plants may have a higher compensatory ability. Cultivars showed a converse reaction to mowing: While only spring mowing had negative effects on the reproductive output of the semi-dwarf hybrid, the tall cultivar produced significantly less seeds in fall-mown than in spring-mown plots. In summary, my results suggest that dwarfing may reduce the success of feral plants in some but not all situations, so transgenic mitigation might reduce gene flow via low-quality ruderal sites. Considering that semi-dwarf hybrids still set seed under all conditions and that success is likely to be higher for other dwarfed cultivars, lower mowing heights or sites with higher soil quality, I consider dwarfing to be an inadequate solution for controlling transgene escape.
4.1 Introduction

The development of herbicide-resistant oilseed rape (OSR) lines through genetic modification (GM) has raised a controversy concerning potential environmental and economical risks (Chapman & Burke 2006, Hails 2000, Legere 2005, Senior & Dale 2002). Mounting evidence suggests that unwanted gene flow will be extremely difficult to control and predict (Devos et al. 2004, Jørgensen et al. 2009). Long-term persistence of herbicide-resistant plants as volunteers in subsequent crops (Beckie & Warwick 2010, D'Hertefeldt et al. 2008), transgene introgression into a wild relative (Warwick et al. 2008), the development of multiple resistance (Hall et al. 2000) and transgene escape via feral populations (Kawata et al. 2009, Schafer et al. 2011, Schoenenberger & D'Andrea 2012) have already been shown to occur. One proposed strategy of limiting such uncontrolled gene flow is transgenic mitigation (TM), the concept of linking a transgene with a gene which does not reduce crop yield in cultivation but is disadvantageous for weeds, crop volunteers and feral plants. Fitness advantages conferred by the transgene (e.g. herbicide resistance) are thus supposed to be counterbalanced by deleterious traits (Gressel 1999, 2005). To this aim, Al-Ahmad et al. (2006) have produced a herbicide-resistant oilseed rape variety with a dwarfing mitigator transgene (gibberellic acid insensitive, ∆gai). TM dwarf plants showed a reduced height and produced more seeds than tall non-transgenic plants when grown alone, but they were unfit if grown in competition with tall OSR. These and other findings (Fargue et al. 2004) suggest that dwarf OSR volunteers in OSR crops may indeed be less successful than tall volunteers.

However, agricultural environments are not the only avenues of transgene escape: Several studies have demonstrated that feral OSR populations growing on disturbed sites outside cultivation may well serve as stepping-stones for intra- and interspecific gene flow (see chapter 3, e.g. Breckling & Menzel 2004, Elling et al. 2009, Menzel 2006, Squire et al. 2010). Concern has been raised by Reuter et al. (2008) that dwarfing might actually prove to be beneficial for OSR feral populations: Dwarfed cultivars can outyield tall varieties under non-competitive conditions, and feral populations of OSR most frequently occur on disturbed sites with little competing vegetation. Reuter et al. (2008) showed that mean height in feral plants is significantly lower than in cultivated plants and suggested a phenotypic adaptation to habitats in which plants with smaller size will more frequently escape from damage. Typical habitats for feral OSR populations include road verges and rail tracks (e.g. Crawley & Brown 1995, Menzel 2006, Reuter et al. 2008), where damage may easily occur from mowing, passing trains or vehicles. More than 70% of roadside feral OSR populations can be destroyed.
Can dwarfed OSR measure up to tall cultivars?

by mowing before seed maturity (Pessel et al. 2001) – if dwarfed cultivars are indeed less susceptible to mowing, their survival as feral plants might be substantially increased. Mowing can benefit small species, allowing them to coexist with tall competitive species in grasslands by removing proportionally more of the leaf canopy of tall species (Armesto & Pickett 1985, Huhta 2001, Klimešová et al. 2010, Zobel 1992). Yet, this effect may not be based on plant size alone but could result from other factors, such as nutrient dynamics or species-specific tolerance. The question whether tall plants are truly more sensitive to mowing than small plants is complicated by the aspect of compensatory growth. Plants can compensate for biomass loss to a certain extent (Crawley 1983). Since tall individuals retain more absolute biomass at the same damage level, they can be more resilient than small plants (Ricciardi & Stelluti 1995). However, modelling and empirical evidence show that the effects of a high intrinsic or realised growth rate on compensation for biomass loss may be highly context-dependent (Hilbert et al. 1981, Hochwender et al. 2000, Klimešová et al. 2010, Stowe et al. 2000, Weis et al. 2000). As nearly all of these studies have assumed large and small plants to lose the same proportion of biomass, their predictive power concerning the effects of mowing is limited: Tall plants may be better at compensating, and yet still suffer more from mowing due to the larger proportion of biomass lost.

Another factor which might influence the relative performance of dwarfed and tall cultivars in feral populations may be soil quality. Feral oilseed rape plants can frequently be found on soil types which are not suited for cultivation (Menzel 2006) and are usually unfertilised. Such areas, e.g. areas for sand mining and dump sites, may be centres for potential hybridisation between OSR and wild relatives (Menzel 2006) and may thus be of great importance for transgene escape. Dwarfing may well be advantageous on soils with low nutrient content and low water-holding capacity. Dwarf or semi-dwarf hybrid lines of OSR tend to build up less vegetative biomass than tall cultivars while showing similar yield, resulting in a higher harvest index\(^1\) (Al-Ahmad et al. 2006, Wang et al. 2004). Nutrient requirements and water consumption might therefore be reduced compared to tall cultivars. Dwarfed wheat (*Triticum aestivum* L.) varieties indeed show a higher nitrogen-use efficiency for grain production (Singh & Arora 2001). Higher drought tolerance of dwarfed cultivars has been reported for sunflowers (Angadi & Entz 2002) and several wheat varieties (Blum & Sullivan 1997, Kirkham & Smith 1978, but see Ricciardi & Stelluti 1995).

---

\(^1\) seed mass per total plant biomass
My study addressed the question whether semi-dwarf hybrids show a greater tolerance of stressors relevant for feral populations than tall varieties. If semi-dwarf hybrids consequently reach a higher reproductive potential than tall cultivars, dwarfing as a proposed strategy for transgenic mitigation may indeed increase the risk for transgene spread under these conditions. I therefore carried out two separate field experiments to compare the performance of a semi-dwarf hybrid and a tall cultivar, depending on 1) substrate quality and 2) mowing regime. The number of fruiting individuals and produced seeds was assessed to test the following hypotheses: The semi-dwarf hybrid PR45D03 shows a higher reproductive potential than the tall cultivar Artus 1) on low-quality soils (shallow soil, mixed soil) and 2) when cultivars are cut to 2.5 cm in fall or to 10 cm in spring. I expected both cultivars to show the same reproductive potential on control plots (humous soil/unmown).

4.2 Methods

4.2.1 Experimental site

The experiments were set up on the Siedenburg dump site in Bremen, Northern Germany, a former dump site for non-hazardous building rubble with a cover layer of sandy loam, which had been bulldozed in September 2007 (see Appendix II.5.3 and II.8 for details and climate data). I used an experimental site from a previous study, made up of containers dug in at ground level and filled with different substrates (humous soil, mixed soil, shallow soil, see 3.2.1 and 3.2.2). Prior to the present study, several containers were cleared of all aboveground vegetation and larger roots, so that they could be used for the substrate comparison experiment. The site was further extended to include a 2.4 by 7 m area of dump site soil for the simulated mowing experiment. It was weeded, raked and loosened with a spade to a depth of approximately 25 cm. Fences excluded small mammals and slugs from the complete experimental area (see 3.2.2 for details). Competing vegetation was regularly removed from the experimental plots throughout the study.

4.2.2 Cultivars and sowing details

Two winter oilseed rape cultivars were compared in both experiments: the MAXIMUS® semi-dwarf hybrid variety PR45D03 from Pioneer Hi-Bred, Buxtehude, Germany, and the tall cultivar Artus (see 3.2.3 for details). I used unimbibed seeds which were sown on 3 October 2008 at a depth of 1 cm and covered with loose soil according to the following designs. In OSR cultivation, the latest recommended sowing date is 9 September (Rapool Ring GmbH
Can dwarfed OSR measure up to tall cultivars?

I was delayed in sowing due to other experiments which were also conducted in fall 2008.

4.2.3 Design

Substrate comparison

I compared the growth of the two cultivars on three ruderal substrates: 1) humous soil from the dump site, 2) shallow humous soil of 9 cm depth over building rubble and 3) mixed soil consisting of humous soil and sand in a 1:1 mixture (for details, see 3.2.1). Plants were grown in containers (Ø 53 cm, open bottom) filled to a depth of 34 cm with one of the respective substrates. Containers were set up in six blocks so that each substrate was present in each block in random arrangement (Fig. 4.1). I randomly assigned half of each container to one of the two cultivars and divided it into two replicate plots, resulting in a total of twelve replicate plots per substrate/cultivar combination. The set-up thus represented a fully randomised two-factorial split-plot design\(^2\) with substrate as between-plots and plant species as within-plots factor. Replicate plots consisted of one pie-shaped quarter of a container in which

Fig. 4.1: Set-up of the experimental site for the substrate comparison in six blocks of three containers (filled circles) each. Three substrates, mixed soil, humous soil and shallow humous soil, were randomly arranged per block. Half of each container was assigned randomly to the tall cultivar Artus (A) or the semi-dwarf hybrid PR45D03 (P) and divided into two replicate plots with four plants each.

\(^2\) see Appendix II.7 and II.7.3 for an introduction to split-plot designs
four plants were grown at a distance of 10 cm from each other and from plants in neighbouring plots. To ensure sufficient seedling emergence, ten seeds were sown at each spot where a plant was supposed to grow. One seedling per spot was randomly chosen to survive while the other seedlings were removed on 24 October 2008. Since winter mortality among plant rosettes was unexpectedly high, I attempted to replace dead plants in spring and therefore irrigated containers with 2 L of water every two to three days from 24 March to 15 April 2009 (see Appendix II.12 for details). However, these attempts failed and resown plants were left out of the analysis. Plant heights of flowering individuals were assessed to test if semi-dwarf-hybrids were truly smaller. Height was measured from soil level to the tip of the highest inflorescence.

**Simulated mowing**

Plants for the mowing simulation were grown in a full two-factorial randomised block design on the bare dump site soil, with cultivar and mowing regime as factors. Three different mowing regimes were applied: an unmown control, mowing in fall and mowing in spring. The resulting six treatments were replicated in eight blocks, each consisting of one replicate plot per treatment arranged randomly in two adjacent rows (see Fig 4.2).

![Figure 4.2](image_url)

*Fig. 4.2:* Experimental site for the mowing simulation with eight blocks consisting of four plots each for the cultivar Artus (A) and the semi-dwarf hybrid PR45D03 (P) in random arrangement. Each plot was randomly assigned to one of the four mowing treatments: unmown control, fall-mown plots, spring-mown plots, and summer-mown plots. Summer-mown plots were discarded in the course of the experiment due to time constraints. Each replicate plot held four plants each.

---

3 The design initially included a summer mowing as an additional treatment, but this had to be abandoned due to time constraints.
Replicate plots were 40*40 cm in size and included four plants arranged in a square so that neighbouring plants within plots stood 15 cm apart and the minimum distance between plants from different plots was 25 cm. As in the substrate comparison, ten seeds were sown at each spot where a plant was supposed to grow and seedlings were thinned to four plants per plot.

Plants from the fall-mown treatment were subjected to a simulated mowing on 18 December 2008: all leaf and stem area growing above 2.5 cm from ground level was removed with scissors (Fig. 4.3). This procedure was repeated with spring-mown treatments on 18 April 2009, except that the cutting height was raised to 10 cm. Cutting heights were chosen in such a way that only slight damage was caused to the semi-dwarf hybrids. I observed in chapter 2 that both heights were realistic for mowings of roadside vegetation in Bremen, though approximately 2-5 cm appear to be more common than cuttings at higher levels. Plant biomass from both mowing events was collected per replicate plot and dried at 60 °C for four days to determine the dry weight of removed biomass. The percentage of leaf area removed out of total plant leaf area before mowing was estimated in intervals of 10% for each individual plant and analysed as the median for each replicate plot.

![Cultivar PR45D03 (a) and Artus (b) after mowing in fall. PR45D03 (c) and Artus (d) before mowing in spring.](image-url)
I further measured plant height on five censuses (see Table II.6 in Appendix II.13) to determine if semi-dwarf hybrids were truly smaller. Plant height of all plants in the control plots was measured from the soil surface to the tip of the highest plant organ (leaf or apex in seedlings and bolting plants (i.e. plants in the phase of rapid stem elongation, developing the inflorescence), inflorescence/infructescence in flowering/fruiting plants) in the plant’s natural position, as my main concern was the height below which any part of the plant would be damaged by mowing.

4.2.4 *Estimates of establishment success and reproductive potential*

I measured several variables to compare establishment success and reproductive potential of the two cultivars (see Table II.6 in Appendix II.13 for dates of censuses). In both experiments, I estimated the reproductive potential via the number of fruiting individuals, the mean pod production per fruiting plant for each replicate plot and the number of fully developed seeds per pod for up to ten randomly chosen pods per replicate. From these parameters, I estimated the mean seed production per fruiting plant (mean pod number*mean number of seeds per pod) and the seed production per plot (seeds per plant*% fruiting individuals/100) for each replicate.

4.2.5 *Seed viability*

Seeds harvested from plants grown in the substrate comparison and mowing experiment were tested for viability after five months of storage at room temperature. I combined seeds collected from ten pods per plot for each treatment and tested five to six replicates of 100 seeds for germination. The seeds were germinated as described in chapter 3.2.5 on the substrate they had been grown on with the following alterations: Pots were watered to 60% maximum water-holding capacity and the incubation period (25 °C / 15 °C at light / darkness for 15 / 9 h) was shortened to four weeks. The stratification treatment (25 °C / 5 °C) occurred for the full duration of week 2. At the end of the test, remaining seeds were tested for viability with tetrazolium chloride (according to Duffy *et al.* 2007, see Appendix II.10 for further details).
4.2.6 Data analysis

Several replicates did not give rise to reproducing individuals. For these replicates, the seed production per fruiting plant could not be calculated, so that sample sizes for this variable varied from 7 to 11 in the substrate comparison and from 5 to 8 in the mowing simulation. For one replicate of spring-mown plots, I could not determine the seeds per plot or seed viability as pods had already been shed at the time of collection.

All data analyses were carried out in GenStat, 8.1 (VSN Int. Ltd., Hemel Hempstead, Hertfordshire, UK), except for Welch tests and Repeated Measures ANOVA which were done in SPSS 15.0. (SPSS Inc., Chicago, IL, USA). Results were considered significant at $p \leq 0.05$. Most variables were transformed prior to analysis as denoted in the results section to meet the requirements of homogenous variances and normal distribution. A small value of 0.5 was added prior to transformation to all values in the dataset whenever zero values had to be avoided. Data were back-transformed for presentation with 95% confidence intervals (CI) computed after Sokal & Rohlf (1969). As the CI are calculated on the transformed scale, these intervals are asymmetrical when back-transformed.

Two-factorial analyses were carried out whenever assumptions were met to test for the effects of cultivar and treatment (substrate or mowing) on variables of plant reproductive potential. Inclusion of the appropriate random blocking factor resulted in two-way split-plot ANOVAs for the substrate comparison and two-way ANOVAs for the mowing experiment, each followed by Tukey post-hoc tests. Block effects were only included if they were significant. Moderate deviations from normality were tolerated if sample sizes were equal owing to ANOVA’s robustness (Quinn & Keough 2007). For the analysis of fruiting individuals, generalized linear mixed models (GLMM) with quasi-binomial distribution and logit link function were carried out with the corresponding factors and blocking terms. In the GLMM, data were analysed as the proportion of fruiting individuals out of four plants. However, I used total numbers of fruiting individuals for data display, as a presentation of percentages did not seem appropriate for such a small number of total individuals tested. Wald F statistics were used to assess the significance of terms.

If the two-factorial analysis indicated an interaction between factors, one-factorial analyses were conducted to test for cultivar effects per treatment (i.e. per substrate or per mowing treatment) or for treatment effects per cultivar, applying blocked ANOVAs, Welch’s ANOVA$^4$ or GLMMs depending on data distribution. If more than two treatments were

---

$^4$ For normal distributions with heterogenous variances
compared with a GLMM, I performed multiple pairwise comparisons by calculating a Bonferroni-corrected confidence interval\(^5\) from the estimated means and standard errors. Means were considered significantly different if these confidence intervals did not overlap. Cultivar differences in plant heights in the mowing experiment were analysed over all censuses with a repeated measures ANOVA, for which I applied the Greenhouse-Geisser correction\(^6\) to account for violations of sphericity\(^7\) indicated by Mauchley’s test. The covariance matrix was homogenous according to the Box-M-test\(^8\). In addition, I performed separate one-way blocked ANOVAs for each census to test for cultivar effects. Dry plant biomass removed and the percentage of leaf area removed were tested for differences between cultivars for each mowing separately, as differences between spring and fall mowing were of no interest. The percentage of leaf area removed, an interval-scaled variable, was analysed with Mann-Whitney U-tests while I preferred blocked ANOVA for testing cultivar effects on the amount of dry plant biomass removed if assumptions were fulfilled.

---

\(^5\) The significance level alpha was divided by the number of pairwise comparisons and the corresponding quantile of the normal distribution was multiplied with the standard error. Example: The CI for a single comparison at \(p \leq 0.05\) equals 1.96*SE. For six comparisons, the Bonferroni-corrected \(p\) equals 0.05/6 = 0.008, corresponding to a quantile of the normal distribution of 2.64. The CI then equals 2.64*SE (2.64 is the quantile of the normal distribution for \(p = 0.008\)).

\(^6\) Adjustment of the d.f. based on the Greenhouse-Geisser epsilon (Quinn & Keough 2007)

\(^7\) “The sphericity condition is that the variances of the differences between values of the response variable are the same for all pairs of treatments” (Quinn & Keough 2007).

\(^8\) The Box-M-test tests the assumption “that the vectors of the dependent variables follow a multivariate normal distribution, and the variance-covariance matrices are equal across the cells formed by the between-subjects effects.” Its test statistic is transformed to an F statistic IBM (2013).
4.3 Results

4.3.1 Substrate comparison

For both cultivars, low-quality substrates seemed to reduce reproductive potential, as observed means of seed production (per plant and plot) were lower on mixed and shallow soil than on humous soil (Fig. 4.4.b and 4.4.c). Yet, the only significant effect was that individual plants produced less seeds on mixed than on humous soil (Fig. 4.4.b, Table 4.1, Tukey p ≤ 0.05). Seed viability was also significantly influenced by substrate type and was highest on humous soil, but the Tukey test did not indicate a difference between individual substrates (see Appendix III.3 Tables III.12 and III.13).

Semi-dwarf hybrids showed a significantly lower reproductive potential than the tall cultivar Artus (Fig. 4.4, Table 4.1): Averaged over all substrates, PR45D03 produced only 170 seeds per replicate plot compared to 1459 seeds produced by Artus. This was a result of a lower number of fruiting individuals (1.1 vs. 2.1, Fig. 4.4.a, Table 4.1) and a reduced seed production per plant (286 vs. 865, Fig. 4.4.b, Table 4.1). These differences were present irrespective of substrate type, as shown by the non-significant interaction term for all three variables (Table 4.1). The lower number of fruiting individuals in PR45D03 partly resulted from a higher winter mortality (see Appendix III.3.1 Fig. III.14 and Table III.14). Viability of produced seeds did not differ between the cultivars (see Appendix III.3.1 Tables III.12 and III.13).
Table 4.1: Effects of cultivar and substrate on variables of reproductive potential analysed in split-plot analyses with substrate varying between plots (containers), cultivar varying within plots, and block as blocking factor. Significance levels (p), variance ratios (F) and degrees of freedom (d.f.) are displayed. A split-plot ANOVA was carried out on the fourth-root-transformed seeds per plot. Log-transformed seeds per plant were analysed with an unblocked two-factorial ANOVA. Fruiting individuals were analysed as the number of fruiting individuals out of four plants in a generalized linear mixed model with quasi-binomial distribution and logit link function (dispersion parameter estimated). Wald F (F*), degrees of freedom (d_n) and denominator degrees of freedom (d_d) are displayed. n = 12 (fruited individuals; seeds per plot) or n = 7-11 (seeds per plant). Significant effects (p ≤ 0.05) are shown in bold type.

<table>
<thead>
<tr>
<th>effect</th>
<th>fruiting individuals</th>
<th>seeds per plant¹</th>
<th>seeds per plot²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d_n  d_d  F*  p</td>
<td>d.f.  F  p</td>
<td>d.f.  F  p</td>
</tr>
<tr>
<td>block</td>
<td></td>
<td>5 3.89</td>
<td></td>
</tr>
<tr>
<td>substrate</td>
<td>2 9.9 2.05 0.180</td>
<td>2 5.13 0.009</td>
<td>2 1.14 0.358</td>
</tr>
<tr>
<td>main plot error</td>
<td></td>
<td>10 1.09</td>
<td></td>
</tr>
<tr>
<td>cultivar</td>
<td>1 51.4 17.45 &lt;0.001</td>
<td>1 19.34 &lt;0.001</td>
<td>1 24.70 &lt;0.001</td>
</tr>
<tr>
<td>cultivar x substrate</td>
<td>2 51.8 0.60 0.552</td>
<td>2 0.07 0.929</td>
<td>2 0.52 0.597</td>
</tr>
<tr>
<td>subplot error</td>
<td></td>
<td>51</td>
<td>51</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td>56 71</td>
<td></td>
</tr>
</tbody>
</table>

¹ log-transformed for analysis  ² fourth-root-transformed for analysis
*results from generalized linear mixed model with quasi-binomial distribution and logit link function, dispersion parameter estimated (1.022), n = 12

4.3.2 Mowing effects

Plant heights and biomass removed by mowing

Repeated measures ANOVA showed significant effects of sampling date (F$_{2,391,75} = 1080.9$, p < 0.001), cultivar (F$_{1,78} = 13.1$, p = 0.003) and the interaction term (F$_{2,391,75} = 0.011$) on plant height. During early life stages, plants of semi-dwarf hybrids were constantly smaller than Artus plants (Fig. 4.5): Significantly lower values were found for log-transformed plant height of seedlings (19.11.08, blocked ANOVA, F$_{1,14} = 68.7$, p <0.001), plant rosettes (18.12.08, blocked ANOVA, F$_{1,14} = 185.9$, p <0.001) and bolting plants (18.04.09, unblocked ANOVA, F$_{1,14} = 6.2$, p = 0.026). At both mowing events, mean plant heights of Artus exceeded the heights of PR45D03 plants (3.3 vs. 2.1 cm in fall, 15.6 vs. 10.7 cm in spring) as well as the mowing heights of 2.5 cm (fall) and 10 cm (spring). However, no difference in plant height was detected for the flowering and fruiting plants (Fig. 4.5, unblocked ANOVA n.s.). At both mowing events, plant dry biomass removed was 16 to 19 times higher for Artus plants than for semi-dwarf hybrids (Table 4.2). Yet, the percentage of leaf area lost out of total leaf area was only 2 to 3.5 times as high for Artus as for the semi-dwarf hybrid (Table 4.2).
Can dwarfed OSR measure up to tall cultivars?

Fig. 4.5: Plant height of the tall cultivar (Artus) and the semi-dwarf hybrid (PR45D03) on control plots at the seedling stage (19.11.08), before fall (18.12.08) and spring (18.04.08) mowing, at flowering (26.05.09) and at the fruiting stage (18.07.09). Means ± 95% confidence intervals (CI) were back-transformed from analysis of log-transformed heights (n = 8). Significant differences between cultivars at each date according to one-way blocked ANOVAs are indicated by asterisks: *** p ≤ 0.001; * p ≤ 0.05; n.s. = not significant. Data from the first two censuses are displayed enlarged in the box.

Table 4.2: Biomass removed per plot in fall and spring mowing expressed as total dry plant biomass removed and as the percentage of leaf area removed out of total plant leaf area (analysed as percentage interval: 1 = 0-9%, 2 = 10-19%, 3 = 20-29%, 4 = 30-39%, 5 = 40-49%, 6 = 50-59%, 7 = 60-69%, 8 = 70-79%, 9 = 80-89%, 10 = 90-100%). Back-transformed means and ± 95% confidence intervals (CI) are displayed for the dry plant biomass (n = 8). Leaf area removed is shown as the median with the lower (LQ) to upper (UQ) quartile (n = 8). Results of one-way analyses testing for differences between cultivars (d.f. = 1, blocked ANOVA or Mann-Whitney U-test) are presented with variance ratios (F) and significance levels (p). Significant effects (p ≤ 0.05) and mean/median values are shown in bold type.

<table>
<thead>
<tr>
<th>variable</th>
<th>mowing</th>
<th>Artus</th>
<th>PR45D03</th>
<th>cultivar effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>CI</td>
<td>mean</td>
<td>CI</td>
</tr>
<tr>
<td>dry plant biomass removed [g]</td>
<td>fall</td>
<td>0.19 (0.13-0.26)</td>
<td>0.01 (0.01-0.02)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>spring</td>
<td>1.11 (0.72-1.59)</td>
<td>0.07 (0.00-0.27)</td>
<td>32.92</td>
</tr>
<tr>
<td>leaf area removed [percentage interval]</td>
<td>median</td>
<td>LQ-UQ</td>
<td>median</td>
<td>LQ-UQ</td>
</tr>
<tr>
<td></td>
<td>fall</td>
<td>7 (7-8)</td>
<td>2 (1-2)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>spring</td>
<td>2 (2-3)</td>
<td>1 (1-1)</td>
<td>—</td>
</tr>
</tbody>
</table>

1 square-root transformed for analysis a unblocked ANOVA b Mann-Whitney U-test

Effects of mowing on plant performance

Mowing had no significant effect on the reproductive potential pooled over both cultivars (Table 4.3). However, two-factorial analyses yielded a significant interaction between cultivar and mowing for the fruiting individuals and for the seeds per plot (Table 4.3). Seed viability was not affected by cultivar identity or mowing (see Appendix III.3 Tables III.12 and III.13). Analysis of mowing effects separately for each cultivar showed that for PR45D03, spring mowing significantly reduced the number of seeds per plot compared to the unmown control.
Table 4.3: Effects of mowing and cultivar on variables of reproductive potential analysed in blocked two-factorial analyses. Seeds per plant (original data) and seeds per plot (square-root-transformed) were analysed in blocked ANOVAs. Significance levels (p), variance ratios (F) and degrees of freedom (d.f.) are displayed. A generalized linear mixed model with quasi-binomial distribution and logit link function (dispersion parameter estimated) was run for the number of fruiting individuals as a proportion out of four plants. Wald F (F*), degrees of freedom (d_n) and denominator degrees of freedom (d_d) are displayed. n = 8 (fruiting individuals); n = 5-8 (seeds per plant); n = 7-8 (seeds per plot). Significant effects (p ≤ 0.05) are shown in bold type.

<table>
<thead>
<tr>
<th>effect</th>
<th>d_n</th>
<th>d_d</th>
<th>F*</th>
<th>p</th>
<th>d.f.</th>
<th>F</th>
<th>p</th>
<th>d.f.</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>mowing</td>
<td>2</td>
<td>42</td>
<td>1.68</td>
<td>0.199</td>
<td>2</td>
<td>0.26</td>
<td>0.769</td>
<td>2</td>
<td>1.51</td>
<td>0.233</td>
</tr>
<tr>
<td>cultivar</td>
<td>1</td>
<td>42</td>
<td>0.52</td>
<td>0.475</td>
<td>1</td>
<td>4.71</td>
<td>0.036</td>
<td>1</td>
<td>7.65</td>
<td>0.008</td>
</tr>
<tr>
<td>cultivar*mowing</td>
<td>2</td>
<td>42</td>
<td>3.22</td>
<td>0.050</td>
<td>2</td>
<td>3.11</td>
<td>0.056</td>
<td>2</td>
<td>7.33</td>
<td>0.002</td>
</tr>
<tr>
<td>error</td>
<td>38</td>
<td></td>
<td></td>
<td></td>
<td>34</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
<td>46</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 square-root-transformed for analysis

(Fig. 4.6.c, unblocked ANOVA of square-root transformed data, $F_{2,20} = 3.72$, p = 0.042, Tukey post-hoc test), a pattern which was mirrored but not significant in the number of seeds per plant (unblocked ANOVA n.s., see Table III.15 in Appendix III.3.2). Mowing in fall, on the other hand, did not affect the reproductive potential of the semi-dwarf hybrid (Appendix III.3.2 Table III.16). For Artus, in contrast, mean seed production per plot was significantly lower in fall-mown than in spring-mown plots (unblocked ANOVA of square-root-transformed data, $F_{2,21} = 5.17$, p = 0.015, Tukey post-hoc test), although differences between mown and control plots were non-significant. The number of fruiting plants was also lower in fall-mown than in spring-mown plots (GLMM, Wald $F_{2,14} = 7.56$, p = 0.006 and Bonferroni-corrected multiple comparisons, see Appendix III.3.2 Table III.16). This resulted partly from an increased winter mortality in fall-mown plots (see Appendix III.3.2 Fig. III.16).

The opposite responses of the cultivars to the two mowing events influenced their relative performance compared to each other. While they did not differ in the number of fruiting plants, Artus and PR45D03 showed highly significant differences in seed production (Table 4.3). Separate analysis per mowing treatment showed that spring-mown plants of Artus yielded 9.4 times as many seeds per plot as semi-dwarf hybrids (Fig. 4.6.c, unblocked ANOVA, $F_{1,13} = 19.57$, p ≤ 0.001), and also more seeds per plant (Fig. 4.6.b, Welch’s ANOVA, $F_{1,9.23} = 9.094$, p = 0.014). In contrast, the cultivars did not differ in their reproductive output on fall-mown and on control plots (Fig. 4.6., GLMM and unblocked ANOVA n.s., see Appendix III.3.2 Table III.17). A summary of cultivar differences with reference to my hypotheses is given in Appendix III.3.3, Table III.18.
Can dwarfed OSR measure up to tall cultivars?

Fig. 4.6: Reproductive potential of the tall cultivar Artus and the semi-dwarf hybrid PR45D03 on unmown control plots vs. plots mown in fall or spring. Means ± 95% confidence intervals (CI) were back-transformed from analysis (Table 4.3) for the fruiting individuals and the seeds per plot. Original means ± SE are shown for the seeds per plant. n = 8 (fruiting individuals) ; n = 5-8 (seeds per plant) ; n = 7-8 (seeds per plot). Significant differences between cultivars at each mowing treatment according to one-way analysis (Appendix III.3.2 Table III.16) are indicated by asterisks: * p ≤ 0.05; n.s. = not significant. Significant differences between mowing treatments for each cultivar are displayed in Tables 4.3 and III.16 (Appendix III.3.2).
4.4 Discussion

4.4.1 Substrate comparison

Contrary to expectations, the tall cultivar Artus showed a significantly higher reproductive potential (fruiting individuals, seeds per plant and seeds per plot) than the semi-dwarf hybrid, irrespective of substrate type. I thus found no evidence that dwarfing is advantageous on low-quality substrates or on humous soil under ruderal conditions. The pronounced superiority of the tall cultivar Artus is surprising: Dwarfed genotypes of OSR have repeatedly shown a higher or equal seed yield per area compared to tall varieties under non-competitive or cultivation conditions (Al-Ahmad et al. 2006, Muangprom et al. 2006, Rose et al. 2009, Wang et al. 2004), which also applies for the genotypes PR45D01 and PR45D03 (Klüßendorf-Feiffer 2009, LWK Niedersachsen, Sieling & Kage 2008). Densities in the containers amounted to at maximum 54 plants*m⁻², while 40-80 plants*m⁻² are recommended for OSR cultivation (Cramer 1990). Competition in my study will therefore have been comparably low. Contrary to my assumptions, dwarfed plants seem to experience a higher fitness loss through low soil quality than tall plants. While a lower amount of vegetative biomass requires less nutrients and water, it may also provide less stored assimilates to overcome periods of stress. In some dwarfed crops, root biomass may be reduced (Arora & Mohan 2001), which would decrease the uptake of nutrients and water. Moreover, Sieling & Kage (2008) recently found that semi-dwarf hybrids do not require less nitrogen than tall cultivars for maximum yield and show a similar nitrogen harvest index.

Perhaps the most important aspect limiting the relative success of dwarfed cultivars on low-quality soils is that they are less likely to benefit from their higher resistance to lodging. Lodging refers to the collapse or strong inclination of the plant and can result in severe harvest losses through a decreased seed production and a less efficient harvest (Islam & Evans 1994). Lodging is known to increase with higher nitrogen supply (Sheppard & Bates 1980, Wang et al. 2004). Tall plants are particularly susceptible, and the dwarfing gene has been introduced to mitigate this problem (Gressel 1999). Some studies indicate that the high yield per area of dwarfed varieties can indeed be attributed to an increased lodging resistance (Klüßendorf-Feiffer 2009, Muangprom et al. 2006, Wang et al. 2004). In my study, flowering plants only reached a mean height of 62 cm, with no significant difference between cultivars (see Appendix III.3.1 Figure III.15). Final plant height was apparently limited by other factors than genetic disposition, for example low water availability and nutrient supply. Accordingly, lodging could not be observed in my substrate comparison and will hardly have diminished
Can dwarfed OSR measure up to tall cultivars?

seed yield in the tall cultivar. The semi-dwarf hybrid PR45D03 can apparently only measure up to the tall cultivar in terms of seed yield when the higher lodging resistance confers a fitness advantage. Climatic factors may have further contributed to this outcome. Plants in my study suffered from a high winter mortality, probably due to a period of severe frost in January (see Appendix II.5.3 Fig. II.16). The tall cultivar Artus showed improved winter hardiness, probably due to a quick initial growth in fall (see plant heights in the mowing experiment).

All in all, these findings suggest that semi-dwarf hybrids may indeed be less fit than tall cultivars on low-quality substrates. However, it would be premature to conclude from my findings that dwarfed cultivars per se perform worse on low-quality soils. The cultivars used in my study were not near-isogenic lines, so the observed differences may be due to genetic differences besides dwarfing. Studies with wheat and rice have shown that drought tolerance may vary more strongly with genetic background than with plant size (Lafitte et al. 2006, Nagarajan et al. 1999). Dwarfing reduced root length or biomass in some wheat cultivars (Arora & Mohan 2001) but not in others (Blum & Sullivan 1997, Siddique et al. 1990), even though all cultivars carried the rht dwarfing gene conferring insensitivity to gibberellins. In OSR, effects of dwarfing on plant growth also vary: Some cultivars can show a reduction in aboveground plant biomass (Wang et al. 2004), while others do not (Rose et al. 2009, Sieling & Kage 2008). Dwarfing in OSR is achieved through the dwarfing genes ndf-1 (Wang et al. 2004) and Δgai (Rose et al. 2009) and, in my cultivar PR45D03, through the bzh dwarfing gene (Koch & Bruins 2008). All of these genes confer insensitivity to gibberellins by similar but not equal modes of action (Huapeng et al. 2011, Renard et al. 2010, Rose et al. 2009). Both differences between the modification and the parental lines might explain differences between cultivars.

4.4.2 Mowing simulation

As expected, Artus plants were significantly taller than the semi-dwarf hybrids during early growth stages from seedling emergence to bolting stage. However, this difference disappeared for the flowering and fruiting individuals. Like in the substrate comparison, final height of the tall cultivar was likely limited by abiotic stressors. I conclude that tall cultivars on low-quality substrates are indeed more likely to be damaged by mowing, but only during early growth stages. Accordingly, mowing both in fall and spring resulted in a substantially higher loss of dry plant biomass for the tall cultivar. Effects on reproductive potential were, however, not as expected: Although Artus lost a higher percentage of leaf area, plants did not necessarily
suffer the greatest reduction in reproductive output. Instead, the tall cultivar suffered relatively more from fall than from spring mowing, while only spring mowing decreased the seed production per plot of semi-dwarf hybrids. Observations in fall met my expectations more closely, yet they were not truly confirmed. Seed production per plant seemed to be less reduced for the slightly damaged PR45D03 plants (32%) than for the heavily-damaged Artus plants (50%). Yet, the difference compared to control plots was significant for neither cultivar, and semi-dwarf hybrids did not outyield Artus. Mowing in spring had remarkably converse effects, as seed production per plot was significantly reduced by nearly 80% for the semi-dwarf hybrids while the tall cultivar more than fully compensated. At this mowing, Artus lost only slightly less leaf area than PR45D03 and substantially less than in the fall mowing, but the apex of the main shoot was also lost in approximately two out of three plants. In contrast, very few shoots of the semi-dwarf hybrids were decapitated.

Artus plants thus exhibited a strong ability to compensate for biomass loss at both mowing events. Under cultivation or greenhouse conditions, OSR is capable of compensating for artificial defoliation of seedlings of up to 75%-100% (Gavloski & Lamb 2000, Nowatzki & Weiss 1997, Sutherland et al. 2006), though only 50% leaf loss can be tolerated without yield penalties if the apical meristem is also damaged (Gavloski & Lamb 2000) or if plants are defoliated at rosette stage (Susko & Superfisky 2009). In my study, compensation under more stressful conditions was sufficiently high in fall for Artus rosettes not to suffer significantly from 60-69% reduction in leaf area and occasional damage to the apical meristem.

Yet why did the tall cultivar successfully compensate for mowing in spring when the semi-dwarf hybrid suffered less damage and could not compensate? I can consider two possible explanations. On the one hand, a higher damage level may not automatically translate into a higher reduction in yield. McNaughton (1983) proposed that the effect of grazing on plant fitness may depend on grazing intensity in the form of an optimum curve – moderate levels of damage may increase plant fitness (overcompensation), while very high levels have detrimental effects. The phenomenon of overcompensation in cases of moderate damage has been reported in several cases (Lennartsson et al. 1998, Oesterheld & McNaughton 1988, Paige & Whitham 1987). In my study, Artus plants produced significantly more seeds per spring-mown than per fall-mown plot and also, though not significantly, more than in control plots. I suspect that another compensation mechanism, initiated by loss of apical dominance, played a major role in spring. When the apical meristem is damaged, plants sometimes compensate by releasing lower-order meristems from dormancy (Hendrix 1979, Huhta et al.
Can dwarfed OSR measure up to tall cultivars?

2000, Paige & Whitham 1987, Pilson & Decker 2002), an effect which has indeed been observed in OSR (Tatchell 1983). However, compensation may fail at high levels of damage: Topping of OSR at bud and flowering stage can significantly reduce seed yield (Khan et al. 2007). With the mowing height of 10 cm in spring, I found that the tall cultivar reached a significantly higher reproductive potential than the semi-dwarf hybrid. However, according to the concept of overcompensation, mowing at a lower height would likely have shifted the damage levels so that the tall cultivar would have been negatively affected, while the dwarfed cultivar might indeed have received the “optimal” amount of damage necessary for overcompensation.

On the other hand, semi-dwarf hybrids may simply have been more sensitive to mowing due to a generally weaker growth. Not only were they less tall, but they also had a reduced total leaf area, according to visual impressions. This is supported by the fact that in fall, a loss of 0.01 g dry biomass corresponded to a reduction in total leaf area of 3.2 to 3.6% in Artus versus 10 to 19% in semi-dwarf hybrids (see Table 4.2). Both my results and evidence from the literature suggest that large plants may compensate for a higher biomass loss through a higher compensatory ability (Byington et al. 1994, Hendrix 1979, Marquis 1984). Accordingly, Ricciardi & Stelluti (1995) showed that tall cultivars of durum wheat suffered less reduction in grain yield from 100% defoliation than semi-dwarf isolines. It is possible that the mechanism leading to dwarfing in my OSR cultivar, namely insensitivity to gibberellins, inhibited compensatory growth. The latter is partly achieved through redistribution of plant hormones which promote cell division and elongation or activate remaining meristems (McNaughton 1979). Gibberellins can indeed promote axillary bud elongation (Philipps 1971) and could thus be involved in compensatory growth. In this case, gibberellin-insensitive cultivars would be at a disadvantage.

A higher intrinsic compensatory ability may be the reason why the tall cultivar reached a significantly higher seed yield than the semi-dwarf hybrid in my spring mowing. However, as indicated by my fall mowing, this compensation may get weaker with increasing damage level. Several factors can further limit compensatory growth, such as low nutrient availability, drought, developmental stage, competing vegetation and time for regrowth (Boege 2005, Hochwender et al. 2000, Klimešová et al. 2010, Maschinski & Whitham 1989, Weis et al. 2000). Such limitations may prevent tall plants from catching up with plants which lost less biomass (Hochwender et al. 2000, Weis et al. 2000).

---

9 Differences in spring were even more pronounced but are less meaningful as dry plant biomass removed included a substantial amount of shoot biomass.
4.4.3 Implications for transgenic mitigation

The substrate comparison showed that the semi-dwarf hybrid PR45D03 was less fit than the tall cultivar Artus under stressful conditions, such as low soil quality combined with a severe winter. It is likely that these stressors limited the plant height of the tall cultivar, so that the dwarfed cultivar could not profit from a higher resistance to lodging. Transgenic mitigation through dwarfining might therefore reduce (but not prevent!) gene flow in a ruderal environment on low-quality substrates. However, the literature suggests great variation in stress response of dwarfed crop plants and cultivars, so these results would need to be verified for individual cultivars chosen for transgenic mitigation. Other cultivars, e.g. the one developed by Al-Ahmad et al. (2006), might not depend on higher lodging resistance to outyield tall varieties. Nevertheless, my results suggest that potential fitness advantages of dwarfing are likely restricted to disturbed ruderal or rural sites with higher soil quality. These sites would resemble cultivation conditions, under which dwarfed cultivars may show higher yield than tall varieties (Al-Ahmad et al. 2006, Rose et al. 2009), more closely. Control plants in my mowing simulation indicate that slightly more favourable conditions can already allow the semi-dwarf hybrid to catch up: The reproductive potential of PR45D03 was not significantly different from Artus on humous soil. Plants in this experiment were approximately 10 cm taller at flowering than those on humous soil in the substrate comparison, possibly because the soil had been loosened and provided better conditions for root growth than the containers used in the substrate comparison.

The mowing simulation showed that the dwarfed cultivar lost less biomass but failed to compensate for this loss in spring. In contrast, the tall cultivar lost more biomass through mowing but seemed capable of compensating for this loss. However, compensation was significantly less efficient for the fall mowing when loss of leaf area was high than for spring mowing when loss of leaf area was lower and decapitation of the main shoot potentially boosted compensation through loss of apical dominance. I conclude that the effect of mowing on the relative performance of the two cultivars may be context-dependent and suggest further studies to test for the effects of different mowing heights and stressors on compensatory ability. I also suggest tests with near-isogenic lines to investigate the effects of dwarfining per se, as other genetic differences between the two cultivars may have confounded this effect in my study. Under the conditions presented here, I found no evidence that a dwarfed cultivar would outyield a tall cultivar on mown sites.
In conclusion, semi-dwarf hybrids did not clearly profit from a fitness advantage when confronted with stressors important for feral plants on low-quality ruderal soils. As the tall cultivar showed a superior performance in some situations, transgenic mitigation through dwarfing may reduce gene flow via ruderal populations. Yet, it needs to be stressed that semi-dwarfed hybrids still produced seeds under all conditions, and sometimes matched the yield of the tall cultivar. Even dwarfed varieties will thus contribute to gene flow via ruderal sites, especially those of high soil quality, enabling transgene escape to conventional fields and hybridisation partners. I thus consider the method of transgenic mitigation through dwarfing as insufficient for an effective limitation of transgene escape.

References


Can dwarfed OSR measure up to tall cultivars?


Can dwarfed OSR measure up to tall cultivars?


5. Impact of soil properties, fungi and mesofauna on the persistence of oilseed rape (*Brassica napus*) seeds

Abstract

Potential weediness of genetically modified (GM) oilseed rape (OSR) cultivars is a higher risk under conditions which favour seed bank survival. Yet, there is only little knowledge on the influence of soil properties and none concerning the role of soil biota. I therefore buried OSR seeds on nine sites covering a wide range of soil properties and applied several treatments to exclude fungi or the meso- and small macrofauna. The seed burial was conducted in two years with different climate which differentially affected the outcome: At dry conditions, seed persistence was high and fungi only had a small negative impact. At more humid conditions, seed persistence was low, but the level of fungal-induced seed decay could not be determined as the fungicide on the control treatment was ineffective. The meso- and small macrofauna increased seed persistence at humid conditions and low nutrient availability via unresolved mechanisms. Seed persistence significantly decreased with increasing soil pH within the range of pH 5.1–7.3. Soil water-holding capacity (WHC) and organic matter content (SOM) had no impact on persistence. My findings indentify environmental conditions conducive to OSR seed persistence, which can improve predictability of potential transgene spread. Mechanisms underlying the observed effects merit further attention.

5.1 Introduction

The invention of genetically modified (GM) oilseed rape (OSR) plants with herbicide tolerance has raised concerns about potential weediness and unintended spread of transgenes in the environment (Beckie & Warwick 2010, Devos et al. 2004, D'Hertefeldt et al. 2008). Accidental dispersal of seeds occurs regularly by harvest losses of up to 10,000 seeds*m\(^{-2}\) (Lutman et al. 2005) and spillage in transport (Crawley & Brown 1995). Potential weediness of the resulting feral populations greatly depends on their ability to develop a persistent seed bank (Gressel 2005). While ripe OSR seeds germinate readily given sufficient moisture (Schlink 1994), water stress and darkness can induce secondary dormancy (Pekrun et al. 1997) and facilitate persistence in the soil for up to 11 years (Lutman et al. 2003). Studies on field seed persistence show high variability (Hails et al. 1997, Lutman et al. 2003) and substantial differences among sites and habitats. Yet, we have only scarce knowledge concerning the influence of the burial environment. Soil properties, for instance, are likely to influence dormancy levels of OSR. Pekrun et al. (1998) observed higher seed bank
The persistence of OSR seeds in the soil is also influenced by their survival. A study on the seed bank of OSR indicated losses of up to 75% to seed predation, diseases or fatal germination (Gruber et al. 2005). Yet, I know of no study dealing with the influence of biota causing the death of buried OSR seeds. Besides seed ageing and seed predation, soil microorganisms are frequently suspected as a major mortality factor of buried seeds (Baskin & Baskin 1998). Fungicide addition has repeatedly been shown to increase the number of viable seeds or seedlings retrieved from burial (Dostál 2010, Wagner & Mitschunas 2008). While meal of OSR seeds can suppress certain soil-borne fungal diseases in other plants (Mazzola 2007), OSR seeds can still be colonised and deteriorated by fungi during storage (Pronyk et al. 2006, Tańska et al. 2011). Fungi might thus cause mortality in OSR seeds buried in the seed bank. In this case soil conditions may have indirect effects on seed mortality by influencing fungal growth (see review by Wagner & Mitschunas 2008). Mortality of buried seeds often increases with elevated soil moisture, partly due to a higher level of fungal-induced seed decay (Wagner & Mitschunas 2008). The role of soil organic matter is not clear, but there are indications for a positive impact on seed-infecting fungi (Wagner & Mitschunas 2008). Soil pH is an important factor for fungal growth, with increasing fungal growth observed at decreasing pH (Rousk & Bååth 2011), but hardly anything is known concerning the effect on seed mortality except indications that seeds of some species deteriorate more rapidly in acid peat than in loam soil (Lewis 1973).

Buried seeds appear to be relatively safe from predation, but can still be ingested by rodents (Hulme 1994) or earthworms (Eisenhauer et al. 2009). Also, isopods and granivorous carabid beetles prey on OSR seeds (Koprdova et al. 2008, Saska 2008) and might reach buried seeds through soil cracks. Buried OSR seeds appeared to be influenced by soil fauna in one study (Gruber et al. 2004).

On the other hand, recent studies show that fungivorous soil mesofauna may increase seed persistence: More seeds survived in or emerged from the soil if Collembola were present both in the laboratory (Mitschunas et al. 2006, Nietschke et al. 2011) and in the field (Mitschunas et al. 2008). There is no evidence yet for an influence of soil fauna on buried OSR seeds. However, damping-off disease caused by Rhizoctonia solani in other Brassica or cruciferous species was significantly suppressed by Collembola (Shiraishi et al. 2003), nematodes (Lagerlöf et al. 2011) and oribatid mites (Enami & Nakamura 1996). Numerous other studies
show that plant diseases caused by soil-borne pathogenic fungi can be suppressed by fungivorous soil fauna (reviewed e.g. in Friberg et al. 2005, McGonigle & Hyakumachi 2001).

The present study aims at identifying environmental factors relevant for the persistence of OSR in the soil seed bank. Ruderal sites were included in the survey, as they provide potentially important refuges for feral populations (e.g. Breckling & Menzel 2004, Squire 1999) and may support OSR seed banks (Dietz-Pfeilstetter et al. 2006), and as they encompass a wide range of soil conditions. A higher accuracy of predictions for the potential spread of GM rape could thus be achieved. I conducted a field experiment on nine sites to investigate the persistence of seeds buried in soils with varying properties. Soil pH, organic matter content (SOM) and water-holding capacity (WHC) were examined as potential explanatory variables. The effects of soil biota on seed persistence were assessed on each site through the exclusion of fungi and/or soil meso- and macrofauna in some treatments. I expected the impact of soil biota, especially fungi, to depend on soil properties. The main hypotheses tested were that the proportion of seeds persisting in the soil is:

1) reduced by seed-infecting fungi
2) higher in soils with low water-holding capacity
3) influenced by the presence of meso- and small macrofauna

5.2 METHODS

5.2.1 Experimental sites

Site characteristics

Seeds were buried on sites in and near Bremen, Northern Germany, with a mean annual precipitation of 725 mm and a mean temperature of 10.2 °C in the period of 2007-2009 (DWD 2010, see Appendix II.5.4). I chose three agricultural fields (AF) with high water-holding capacity (WHC) and high soil organic matter content (SOM) and six ruderal sites with either high soil quality (RH) or low WHC and low SOM (RL). Soil samples were

<table>
<thead>
<tr>
<th>site</th>
<th>pH</th>
<th>SOM</th>
<th>WHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF1</td>
<td>5.9</td>
<td>3.5</td>
<td>39.2</td>
</tr>
<tr>
<td>AF2</td>
<td>5.4</td>
<td>5.6</td>
<td>53.9</td>
</tr>
<tr>
<td>AF3</td>
<td>4.3</td>
<td>18.0</td>
<td>86.2</td>
</tr>
<tr>
<td>RH1</td>
<td>6.0</td>
<td>4.7</td>
<td>45.7</td>
</tr>
<tr>
<td>RH2</td>
<td>5.1</td>
<td>6.0</td>
<td>47.0</td>
</tr>
<tr>
<td>RH3</td>
<td>6.6</td>
<td>6.2</td>
<td>48.3</td>
</tr>
<tr>
<td>RL1</td>
<td>7.0</td>
<td>1.4</td>
<td>22.9</td>
</tr>
<tr>
<td>RL2</td>
<td>7.3</td>
<td>2.8</td>
<td>34.2</td>
</tr>
<tr>
<td>RL3</td>
<td>6.3</td>
<td>0.8</td>
<td>25.1</td>
</tr>
</tbody>
</table>

Table 5.1: Soil properties of the nine burial sites. AF 1-3 = agricultural fields, RH 1-3 = ruderal sites with high soil quality, RL 1-3 = ruderal sites with low soil quality; WHC = maximum water-holding capacity; SOM = soil organic matter content.
taken and analysed as described in Appendix II.2.4. SOM ranged from 0.8 to 18.2% dry mass (DM), WHC from 22.9 to 86.2% DM, and pH reached values between 4.3 and 7.3 (Table 5.1). The location of the experimental sites is displayed in Fig. II.21 in Appendix II.14.

**Mesofauna**

Faunal densities were assessed with soil samples taken from each block with a soil corer (Ø = 5 cm) from a depth of 2.5 to 7.5 cm and of the upper 2.5 cm, close to the buried minicontainer bars (see 5.2.2). Soil samples were taken on all nine sites on 21-22 August 2008 and 23 October 2008, but extraction by heat failed1 (modified after Macfadyen 1953, see Appendix II.15). Samples were taken again on 18-19 May 2009 on the three sites which had shown mesofauna effects in the first burial, and soil biota were successfully extracted by water flotation according to Hopkin (1997): Each soil core was subdivided into three parts which were spread in a plastic container and doused with 100 ml of water. After 30 s of stirring, the supernatant was passed through a sieve of 50 µm mesh size. This procedure was repeated three times. Soil biota caught in the sieve were conserved in 70% ethanol until identification. The extracted meso- and small macrofauna was overall dominated by Collembola (approximately 75% of extracted individuals) – other frequent taxa were Acari (mainly Gamasina, few Oribatida), larvae of Coleoptera (mainly Staphylinidae), and Formicidae. Collembola were suspected to play a role through fungal grazing and were further identified to order or, in case of Isotomidae, Entomobryidae and Onychiuridae, to family level.

**5.2.2 Design**

Seeds were buried in a minicontainer system developed for litter decomposition studies (Eisenbeis et al. 1995). 75 seeds and 0.5 ml of dried soil from the respective experimental site were filled into small tube-shaped PE-minicontainers (height: 16 mm, Ø: 11 mm). The openings were sealed with gauze differing in mesh size according to three treatments which were prepared to allow access of different organisms: 20 µm mesh size penetrable only by microfauna; 20 µm + F treated with fungicide and ~2 mm accessible to microfauna, mesofauna and small macrofauna. Containers of the latter treatment were sealed with cuttings from a pantyhose with approximately 2 mm mesh size, which could be stretched to enable the passage of organisms with a diameter of up to 4 mm but prevented the escape of seeds. The

---

1 Only very few organisms were found in the extraction vessels. The second analysis revealed that many small Collembola had desiccated in the soil. I therefore chose extraction by water for further assessments.
containers were then placed into perforated PVC-bars which were pushed horizontally into the soil for burial at a depth of 4 cm (Fig. 5.1). Each bar was equipped with four minicontainers per treatment, later pooled to constitute one replicate of 300 seeds. One bar was buried in each of 6 blocks per site, resulting in six replicates per treatment and site. Further six bars were placed into site AF3 for early exhumation after six months for a preliminary check-up on seed persistence (data not shown).

![Fig. 5.1: Eisenbeis minicontainer bar buried horizontally 4 cm beneath soil level. Minicontainers filled with seeds (with or without fungicide) were sealed with gauze of different mesh size according to treatment (2 mm or 20 µm).](image)

Seed burial was repeated in 2008, on three sites which had shown treatment effects in the first burial and on one site with high Collembola density where mesofauna exclusion (see 5.2.3) had not been successful in the first year. I generally applied the same design and treatments, except for the agricultural field where treatment 20 µm + F was substituted by a new treatment with 500 µm mesh size, penetrable by microfauna and small mesofauna. This was done to single out the faunal fraction responsible for indicated seed predation. Furthermore, the number of replicates was raised to 10-12 (1-2 per block).

**5.2.3 Minicontainer preparation & fungicide application**

I chose seeds of the winter OSR cultivar Smart, which is found in feral populations in Northern Germany (Dietz-Pfeilstetter et al. 2006) and exhibits a high potential for dormancy (Gruber et al. 2004). Seeds harvested in the previous year (2006 for burial 1 and 2007 for burial 2)\(^2\) were provided by Syngenta Seeds (Bad Salzuflen, Germany) unimbibed, i.e. not coated with fungicides or other chemicals. Damaged or misshapen were excluded. The empty minicontainers and the gauze were sterilised under UV-light for 2 h prior to the experiment.

\(^2\) It was necessary to take different batches of seeds for the two burials as storage can greatly reduce the potential for dormancy (Schlink 1994).
Soil filled into the minicontainers was sieved to 0.63 mm and defaunated by repeated freezing (24 h at -20 °C / 24 h at room temperature / 48 h at -20 °C). For the second burial, I extended the duration of the freezing and thawing cycles (to 48 h / 72 h / 72 h, respectively), as some containers with 20 µm mesh size retrieved from the first burial were inexplicably inhabited by mesofauna.

I coated seeds of treatment **20 µm + F** with fungicide prior to burial. In 2007, seeds were soaked in an aqueous solution of chitosan (6 mg/ml; ChitoPlant, ChiPro GmbH, Bremen, Germany) for 10 s, passed through a sieve and left to air-dry. Chitosan is a natural compound obtained from chitin and is known to inhibit numerous soil- and seed-borne plant-pathogenic fungi (reviewed in Badawy & Rabea 2011). As a registered plant strengthener, chitosan can be applied in organic farming and hence in my agricultural fields. The conventional fungicide captan was used for the seed burial in 2008, as chitosan proved to be ineffective. Captan is a broad spectrum heterocyclic nitrogen fungicide commonly used as seed treatment against seedborne pathogens (Agarwal & Sinclair 1997, Torgeson 1969). It is attributed with a high efficacy against seed-rotting organisms (Neergaard 1979) and can indeed reduce the decay of seeds buried in soil (Mitschunas et al. 2009). Seeds were soaked in a 0.8% aqueous solution of captan (prepared from Merpan 80 WDG with 80% captan w/w; Feinchemie Schwebda GmbH, Eschwege, Germany) for five minutes. Dormancy status of seeds was not influenced by soaking seeds in water for the same period according to a test burial (Appendix II.16).

**5.2.4 Burial and exhumation**

I needed to induce secondary dormancy in seeds to prevent immediate germination. This can be achieved by incubation in relatively dry soil (Pekrun 1994). Hence, all prepared minicontainers were buried on a site with dry sandy soil near the University of Bremen 3-4 weeks before the experimental burial. The area was protected from rainfall, and minicontainer bars were wrapped in cloth to prevent access of seed predators. Burial on the experimental sites took place on 22-29 September 2007³ (burial 1) and on 13-14 November 2008 (burial 2) before dawn or after sunset. Dormancy of OSR seeds can be broken by light or sudden changes in temperature (Schlink 1994). Therefore, seeds were always exhumed from pre-incubation on the day they were re-buried and transported to their destination wrapped in light-blocking cloth and surrounded by soil. I recovered the minicontainer bars after nine

---

³ Only 1 to 2 sites could be processed per day in summer due to the fact that seeds needed to be buried in twilight before dawn and after sunset. The experiment-conductor did not wish to be too sleep-deprived when driving.
months of burial, in June 2008 (burial 1) and August 2009 (burial 2). One minicontainer bar could not be retrieved on one site.

5.2.5 Seed viability

Seeds were transferred onto dry soil one day after recovery and stored at 15 °C for up to five days, after which seeds which had recently germinated during burial were counted. Water was added to the soil (see below) and the remaining seeds were tested for viability with a germination test. Defaunated\(^4\) soil (loamy sand from a derelict site near the Centre of Environmental Research and Technology, University Bremen, Germany) sieved to 2 mm was used as test substrate to avoid spread of fungi observed in previous germination tests on filter paper\(^5\). All seeds from the respective replicate were spread onto the soil in the test vessel. Test vessels for the first burial were petri dishes (Ø = 9 cm) sealed with parafilm and filled to a height of 3 mm with soil at 80% WHC. Another 3 mm of soil were added after five days to reduce fungal spread in the dishes. As this did not solve the problem, other test vessels were used for the second burial: plastic planting pots (Ø = 7 cm) filled with 179 g of soil and watered every two days to 70% WHC (See Appendix II.17, Fig. II.22, for a summary of differences in test conditions between the first and second burial).

The seeds were incubated in a climate chamber (Sanyo MLR-350H) for seven days at a diurnal temperature cycle which stimulates germination (Thompson & Grime 1983) (12 h of light at 25 °C and 12 h of darkness at 15 °C with a constant humidity of 80%). Temperatures were then changed to 25 °C / 3 °C for the ensuing seven days, as stratification is known to break dormancy in OSR (Gruber \textit{et al.} 2004), and then set back to 25 °C / 15 °C for the remaining test period of 3-4 weeks. Seedlings and germinated seeds (radicle protruded the testa) were removed and counted every 1-2 days. Soft seeds were considered as dead and were removed, and seeds remaining ungerminated at the end of the experiment were tested for viability with tetrazolium chloride (TZ) according to Duffy \textit{et al.} (2007) (see Appendix II.10). No TZ test was done for the first burial, as no intact seeds were retrieved from the soil. Seed persistence was calculated for each replicate as the percentage of viable seeds retrieved out of the number of buried seeds. I could not prevent that, for the first burial, a substantial number of seeds was affected by fungi spreading from adjacent seeds. This rarely seemed to prevent germination, but development of a healthy seedling in the petri dish was unlikely. I noted whether seedlings decayed in the germination test in this way, but counted them as viable

\(^4\) See Appendix II.11

\(^5\) Despite the fact that seeds were surface-sterilised with 1.25% sodium hypochloride before the germination test.
seeds in the data analysis as the observed fungi appeared to have spread mostly due to the artificial conditions of the petri dishes.

5.2.6 Data analysis

Data were analysed with SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Proportions were arcsine-square-root-transformed prior to analysis to meet the requirements of parametric analyses. I displayed original values unless otherwise noted. Results are reported as significant at $p \leq 0.05$. Replicates were treated as lost when the gauze had loosened, and containers colonised with Collembola despite being sealed with gauze of 20 µm mesh size were omitted from the analysis of treatment effects. For sites AF2 and RH3, this meant a severe reduction in the number of replicates, and results from these sites should be interpreted with caution (burial 2007/2008). I tested for treatment effects with one-way ANOVAs performed for each site, followed by Tukey post-hoc tests. Blocks within the sites were included as a random factor if the block effect was significant. Moderate deviations from normality were tolerated if sample sizes were equal, counting on ANOVA’s robustness (Quinn & Keough 2007). In cases of skewed distributions or heterogenous variances, a Kruskal-Wallis H-test was performed instead.

All intact replicates, regardless of unintended entry of mesofauna, were used to test for differences between sites and for the influence of environmental variables. A one-way ANOVA was conducted to analyse the effect of burial site, pooled over treatment, followed by Tukey post-hoc tests. I further tested in single regression analyses whether the environmental variables WHC, SOM and pH showed an effect on the proportion of seeds remaining viable after the burial of 2007/2008. For this, I calculated the mean proportion of viable seeds per site, averaged over all replicates for all treatments from the respective site. A quadratic regression was performed to relate log-transformed pH with the arcsine-square-root-transformed proportion of viable seeds. The analysis was repeated without site AF3, a site with marsh soil which showed extreme values of pH, WHC and SOM. Regressions performed between seed persistence and WHC or SOM were non-significant, both for linear and quadratic regression models and regardless of whether the outlier site was included.
5.3 Results

5.3.1 Treatment effects

Fig. 5.2: Percentage of viable seeds retrieved from 9-month burial periods in a) 2007/2008 (nine sites) and b) 2008/2009 (four sites). Different letters denote significant differences between treatments (mesh sizes of 20 µm, 500 µm or 2 mm and fungicide addition (+ F)) according to one-way blocked or unblocked ANOVA and Tukey post-hoc tests performed on arcsine-square-root-transformed values (Table 5.2, p ≤ 0.05). AF1, AF2, AF3 = agricultural fields; RH1, RH2, RH3 = ruderal sites with high soil quality; RL1, RL2, RL3 = ruderal sites with low soil quality. Mean ± SE, n = 5-6 for 2007/2008 (except for AF2 and RH3 with n = 1-6) and n = 8-12 for 2008/2009.


**Fungicide**

Coating of seeds with the fungicide chitosan did not affect seed persistence in the burial of 2007/2008 on any site (Fig. 5.2.a, 20 µm *versus* 20 µm + F). However, the fungicide captan, applied for the second burial, significantly raised the proportion of viable seeds retrieved on site RL2, albeit merely from 92.3 to 96.4% (Fig. 5.2.b, Table 5.2, Tukey post-hoc test, p = 0.010, n = 9-12). While fungicide treatments also showed the highest seed persistence on site RH3, this effect was only significant when contrasted with 2 mm mesh size (Tukey post-hoc test, p = 0.011). No fungicide effect could be observed on site RL1.

<table>
<thead>
<tr>
<th>Table 5.2: Effects of treatment (d.f. = 2; mesh sizes of 20 µm, 500 µm (2008/2009) or 2 mm and fungicide addition (+ F)) on the arcsine-square-root-transformed percentage of viable seeds remaining after the two burials of 2007/2008 and 2008/2009. Separate analyses were made for each of the nine sites: one-way ANOVA with or without blocking factor or Kruskal-Wallis-H-test. Significance levels (p), total degrees of freedom (d.f.) and either variance ratios (F) or chi-square values (χ²) are displayed. Significant effects (p ≤ 0.05) are shown in bold type. AF1-AF3 = agricultural fields; RH1-RH3 = ruderal sites with high soil quality; RL1-RL3 = ruderal sites with low soil quality.</th>
</tr>
</thead>
<tbody>
<tr>
<td>---</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>AF1</td>
</tr>
<tr>
<td>AF2</td>
</tr>
<tr>
<td>AF3</td>
</tr>
<tr>
<td>RH1</td>
</tr>
<tr>
<td>RH2</td>
</tr>
<tr>
<td>RH3</td>
</tr>
<tr>
<td>RL1</td>
</tr>
<tr>
<td>RL2</td>
</tr>
<tr>
<td>RL3</td>
</tr>
</tbody>
</table>

\(^a\) unblocked one-way ANOVA  
\(^b\) blocked one-way ANOVA  
\(^c\) Kruskal-Wallis H-test

**Mesofauna exclusion**

I found no consistent treatment effect on seed persistence for the burial of 2007/2008. Looking at individual sites, only two out of nine sites revealed significant differences between treatments (RL1 and RL2, Fig. 5.2.a, Table 5.2). Both were ruderal sites with low soil quality and showed a similar pattern: The proportion of persisting seeds was 1.3 to 1.8 times as high in containers with 2 mm mesh size as in containers with 20 µm mesh size (Table 5.2, Tukey post-hoc tests, p = 0.016 for RL1 and p = 0.003 for RL2, n = 6). A wide mesh size thus had a significant positive influence on seed persistence. A contrasting effect was indicated in the...
agricultural field AF1, where less seeds seemed to persist in containers with wide than with small mesh size (weak statistical tendency, Table 5.2, Tukey post-hoc test, p = 0.120 for 20 µm versus 2 mm).

The second burial did not confirm any of these effects. The proportion of viable seeds was similar for 20 µm (no fungicide) and 2 mm mesh size (Fig. 5.2.b) on all four sites. On site RH3, only treatments 20 µm + F and ~2 mm differed significantly, so that effects of fungicide and mesh size cannot be differentiated. Only on site AF1 did a weak statistical tendency suggest that more seeds remained viable in containers with 500 µm mesh size than in those with 2 mm mesh size (Table 5.2, Tukey post-hoc test, p = 0.121), indicating a positive effect of small mesofauna which was counteracted by larger fauna. Extraction of the soil fauna in 2009 showed that Isotomidae and Onychiuridae were abundant on all of the three sites which showed mesofauna effects in the first burial (Table 5.3). However, Onychiuridae appeared to be less abundant on the sites with positive mesofauna effects (RL1 and RL2) than on site AF1 where mesofauna appeared to have slightly negative effects.

See Appendix III.4 Table III.19 for a summary of the results.

<table>
<thead>
<tr>
<th></th>
<th>RL1</th>
<th>RL2</th>
<th>AF1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-2,5 cm</td>
<td>2,5-7.5 cm</td>
<td>0-2,5 cm</td>
</tr>
<tr>
<td>Isotomidae</td>
<td>57.0 ± 34.7</td>
<td>8.8 ± 5.4</td>
<td>30.7 ± 12.2</td>
</tr>
<tr>
<td>Entomobryidae</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>2.0 ± 0.7</td>
</tr>
<tr>
<td>Onychiuridae</td>
<td>12.1 ± 5.1</td>
<td>5.4 ± 1.5</td>
<td>8.0 ± 4.7</td>
</tr>
<tr>
<td>Other Poduromorpha</td>
<td>1.0 ± 0.5</td>
<td>0.3 ± 0.2</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Symphypleona</td>
<td>3.1 ± 1.4</td>
<td>0.2 ± 0.2</td>
<td>5.8 ± 3.3</td>
</tr>
<tr>
<td>Total Collembola</td>
<td>73.2 ± 36.0</td>
<td>14.8 ± 6.1</td>
<td>46.5 ± 17.0</td>
</tr>
</tbody>
</table>

**5.3.2 Differences between sites**

Seed persistence varied significantly with burial site for the burial of 2007/2008 (one-way ANOVA, F$_{8,149} = 13.33$ p ≤ 0.001, n = 15-18). No consistent pattern could be observed when comparing agricultural and ruderal sites or high-quality with low-quality sites (Fig. 5.3). Particularly few viable seeds remained on sites RH3, RL1 and RL2 with a seed persistence
between 38.8 and 49.4%, which was significantly less than on the four sites with the highest persistence (67.6-82.5% on sites RH1, RH2, AF2 and RL3) according to Tukey post-hoc tests (p ≤ 0.05).

![Graph showing proportion of viable seeds persisting after 9-month burial in 2007/2008 on the nine different sites. AF1-AF3 = agricultural fields; RH1- RH3 = ruderal sites with high soil quality; RL1-RL3 = ruderal sites with low soil quality. Mean ± SE, n = 15-18 (pooled over treatments).]

**5.3.3 Persistence and soil properties**

Soil organic matter content (SOM) and water-holding capacity (WHC) did not significantly affect the proportion of viable seeds (Fig 5.4.b and 5.4.c). In contrast, seed persistence depended on log-transformed soil pH in a highly significant linear relationship with a negative slope (Fig. 5.4.a, linear regression, $r^2 = 0.791$, $p = 0.002$). Inclusion of the outlier site AF3 (pH 4.3) resulted in a unimodal relationship with maximum seed persistence at pH 5-5.4 (Fig. III.17 in Appendix III.4).
5.4 Discussion

Viable OSR seeds were retrieved at relatively high percentages (29-96%) after 9 months of burial. Gruber et al. (2004) reports comparable persistence levels for cultivar Smart, amounting to 67.5-83.5% for 6-7 months. Both studies purposely induced dormancy by incubating seeds in dry soil. My study is the first to demonstrate an effect of soil pH on OSR seed persistence, and the first to show a relevance of soil biota. Interpretation of my results needs to keep in mind that I could not differentiate between seed decay and fatal germination. Both seed mortality and release from dormancy can therefore be responsible if persistence levels were low. Furthermore, some seedlings in the germination test for the first burial may have been overlooked if they were killed by microorganisms before emergence.
5.4.1 **Fungicide effects on seed persistence**

Treatment of seeds with chitosan ( burial 2007/2008) did not show a significant influence on seed persistence on any site. However, many retrieved seeds were visibly colonised with fungi, regardless of chitosan coating. Chitosan thus seems to have been ineffective against the seed-attacking fungi in this study. Similar observations were made with seeds of grassland species which were probably attacked by zygomycetes of the order Mucorales (Mitschunas et al. 2006). Some Ascomycota and Zygomycota display little or no sensitivity towards chitosan (Allan & Hadwiger 1979, Hirano & Nagao 1989), including pathogenic and saprophytic genera. Furthermore, while the applied concentration of 6 mg·ml⁻¹ ought to be highly sufficient for short-term effects (Bautista-Baños et al. 2006), efficacy of low concentrations may decrease within a few days (Benhamou 1992) and can further depend on the type of chitosan (Badawy & Rabea 2011). In contrast, the fungicide captan significantly increased the persistence of OSR seeds in the burial of 2008/2009 on site RL2. On site RH3, a similar effect was only significant versus treatments with 2 mm mesh size, so that I cannot differentiate between effects of fungicide and effects of fauna exclusion. While it is possible that captan coating merely increased levels of dormancy in seeds, this appears to be a short-term effect (Mitschunas et al. 2009), so I can be reasonably confident that the higher seed persistence is indeed a result of reduced fungal attack. Other studies have shown that OSR can be attacked by various fungal diseases, some of which infect seeds or seedlings (Falloon 1980, Srivastava et al. 2011). My study is the first to show that fungi also affect OSR persistence in the soil seed bank. I cannot be absolutely sure that this was due to seed decay, as fungal attack may also release seeds from dormancy (Wagner & Mitschunas 2008), although this has only been shown for species with physical dormancy. However, several soil- and seedborne fungi reduce germinability in *Brassica* spp. (Rude et al. 1999, Shiraishi et al. 2003) or cause seed rot in other plant species (Agarwal & Sinclair 1997, Neergaard 1979).

Still, fungicide only increased the percentage of persisting seeds by 4.4%, and the overall percentage of viable seeds retrieved in the second burial was very high (75 to 96%). Unlike the first burial, which started in September, the second burial included merely two weeks of the fall period, so that seeds were only exposed to one season with maximum fungal attack: In temperate regions, seed-colonising fungi are most abundant in spring and fall (Kienwick 1964). In addition, climatic conditions were probably less favourable for fungal growth in the second than in the first burial: Mean monthly precipitation from November to April was considerably lower in 2008/2009 than in 2007/2008, as were winter temperatures (see Figures
II.17 and II.18 in Appendix II.5.4). It is further important to consider the possibility that the seed lot used in the second burial may have carried less seed-borne pathogens – besides climatic factors, this could also have led to reduced seed mortality. Alternatively, OSR might not be very susceptible to fungal attack, as it has relatively large seeds and this tends to reduce the impact of fungal seed decay (Christ & Friese 1993). However, I suspect that fungi might have played a more important role in the first burial, when low persistence levels coincided with the observation of many fungal hyphae on the exhumed seeds. I cannot be sure of this interpretation, as the low seed persistence could also have resulted from bacterial seed decay. Generally, little is known concerning the impact of bacteria on buried seeds (Wagner & Mitschunas 2008). They may have beneficial effects by antagonising seed-rotting fungi or may themselves inhibit seed germination (Kremer 1987). There are indeed bacteria which cause seed rot in Brassica spp. (Neergaard 1979).

Conclusions on the magnitude of microbial seed rot are complicated by the fact that I buried seeds at unnaturally high densities, potentially overestimating fungal attack (van Mourik et al. 2005), but used commercial (though unimbibed) seed lots, which tend to show low pathogen levels.

### 5.4.2 Influence of soil properties

Contrary to my expectations, seed persistence was not correlated with WHC or SOM of my sites. This finding stands in contrast to results of previous studies, which predict both higher seed mortality through fungal decay (Schafer & Kotanen 2003) and lower dormancy rates (Pekrun et al. 1997) at high moisture levels. Soil moisture content probably did not limit fungal growth in the burial of 2007/2008 due to overall high precipitation levels. Seed rot through fungi can occur under very dry conditions (Griffin 1966), and some plant-pathogenic fungi can even be more severe at low moisture levels (Lootsma & Scholte 1997). It is likely that moisture limited fungal growth only in the second burial (see 5.4.1) in which I did not assess the effect of soil properties. Furthermore, confounding factors may have obscured any effect of WHC, such as site differences in precipitation, fungal community composition and other soil properties.

Most importantly, any impact of WHC was obviously overridden by a strong effect of soil pH, which showed a significant linear relationship with seed persistence when log-transformed. From pH 5.1 to 7.3, persistence declined with higher pH. As discussed above (5.4.1), I consider fungal decay to be a likely factor responsible for low seed persistence, even if the chitosan treatment in the first burial could not provide back-up for this interpretation.
Therefore, the influence of pH on persistence may have resulted from an indirect effect on fungal growth. Yet, I would have expected the opposite relationship, as fungi are usually favoured by acidic conditions (Rousk & Bååth 2011). A possible explanation is that changes in pH can affect fungal community composition in the field, with some taxa benefiting from a high pH (Fritze et al. 1993). Certain saprophytic fungi and several isolates of the pathogenic and seed-rotting (Srivastava et al. 2011) fungus *Rhizoctonia solani* have growth optima under neutral to slightly alkaline conditions (Parmeter 1970, Yamanaka 2003). Stephens et al. (1994) indeed found disease severity of *R. solani* to be higher in calcaerous sandy loam (pH 8.3) than in red-brown earth (pH 5.0). It is thus possible that the fungal species involved in seed decay in the present study were favoured by increasing soil pH. Alternatively, seed-rotting bacteria may have been the dominant cause of high seed mortality in the first burial, and bacteria are usually favoured by increasing pH (Rousk & Bååth 2011). Apart from this, I cannot exclude the possibility that pH affected the dormancy status of OSR seeds. Although hydrogen ions themselves are not known to have such an effect, changes in pH may influence e.g. the availability of nutrients (Scheffer & Schachtschabel 2010) or ethylene production in the soil (Baskin & Baskin 1998), which could potentially influence dormancy levels. Yet, the only chemical factors known to alter OSR seed dormancy so far are soil moisture and oxygen levels (Pekrun et al. 1997, Schlink 1994).

**5.4.3 Mesofauna effects on seed persistence**

My data did not reveal any significant negative effect of the soil meso- and small macrofauna on seed persistence. While such an effect was indicated by a weak statistical tendency in one agricultural field in the first burial, it was not confirmed in the second burial in spite of an increased number of replicates. Blaney & Kotanen (2002) similarly found that exclusion of macroinvertebrates merely tended to increase the persistence of buried weed seeds. I thus did not find any evidence for seed predation. However, I did not assess the effects of larger seed predators, such as rodents which prey on buried plant seeds to some extent (Hulme 1994).

Instead, the burial of 2007/2008 demonstrated that the presence of soil mesofauna can significantly increase seed persistence in the soil (sites RL1 and RL2). Similar effects in grassland species have been attributed to a reduction in seed-rotting fungi by collembolan grazing (Mitschunas et al. 2006, 2008, Nietschke et al. 2011). My evidence for an involvement of fungi in seed persistence is limited to the second burial. It is not surprising that I found no mesofauna effect in this burial, as disease control by mesofauna is sometimes only apparent at high infection levels (Stephens et al. 1994). I can however not be certain that
the observed mesofauna effect in the first burial was connected to fungal-induced seed decay. It is very likely that the effect was a result of collembolan activity. The mesofauna effect was found on sites with dry sandy soil which are not very suitable for earthworms or enchytraeids. Collembola were present in many of the containers with 2 mm gauze and were also moderately abundant in the soil (compare Petersen & Luxton 1982), with 10083-11500 individuals*m$^{-2}$ on sites RL1 and RL2 (Table 5.3). Collembola often prefer saprophytic and pathogenic fungi to biocontrol species (Curl et al. 1988) or arbuscular mycorrhizal fungi (Gormsen et al. 2004, Klironomos & Kendrick 1996, Tiunov & Scheu 2005, but see Larsen et al. 2008). It it therefore likely, but not certain, that the observed mesofauna effect was due to collembolan grazing on seed-rotting microorganisms.

While this study demonstrates a beneficial effect of mesofauna on seed persistence, it also shows that this is restricted to specific situations. Environmental conditions may have affected the underlying interactions in various ways. The activity of Collembola (Frampton et al. 2000), and e.g. their ability to control plant-pathogenic fungi (Innocenti et al. 2011), can be limited by extreme drought stress. Precipitation in the first burial period was relatively high, but low precipitation may have contributed to the lack of a mesofauna effect in the second burial. In the first burial, I only found an effect on sites with low soil quality, namely sandy soil with a low WHC, a low SOM, and a high pH. These sites also showed the lowest seed persistence (Fig. 5.3), indicating that factors affecting mortality or seed dormancy were strong. If the mesofauna effect was due to collembolan grazing on fungi, its restriction to nutrient-poor sites is feasible: The ability of fungi to compensate for grazing seems to be higher in nutrient-rich than in nutrient-poor environments (Mitschunas 2008). Similarly, *Folsomia candida* enhanced germination rates of *Hypericum perforatum* in artificial soil with low SOM, but not in field soil with high SOM (Nietschke et al. 2011). I can also imagine that feeding pressure on seed-infecting fungi might simply be heavier on low-quality sites due to a lack of alternative food sources. It has to be kept in mind that my sites did not only differ in abiotic soil properties and that grazing effects may depend on fungal and Collembola species (Tordoff et al. 2008).

### 5.4.4 Conclusions

This study revealed that both abiotic and biotic factors can significantly influence the persistence of OSR seeds in the soil on ruderal sites. The percentage of viable seeds strongly decreased with increasing soil pH within the range of 5.1 to 7.3. Estimates for dynamics of OSR seed banks should therefore account for soil conditions. Further research is needed to
identify the responsible mechanisms behind the observed effect. I further showed that fungal attack can slightly reduce the persistence of OSR seeds in the soil. Estimates of maximum seed bank persistence should therefore be made with fungicide-coated seeds. My findings suggest that weediness of GM lines could be slightly increased if they acquire a resistance to certain fungal seed-rot pathogens. Such resistance could either be deliberately introduced by genetic modification or develop from hybridisation with resistant conventional lines. I also showed that mesofauna can significantly increase seed persistence, possibly due to fungal grazing by Collembola. This effect was, however, only seen on two sites with low WHC, low SOM and high pH. My findings contribute to a better understanding of the impact of environmental conditions on OSR seed bank dynamics, which can improve predictability of potential transgene spread.

References


Lutman, P. J. W., Berry, K., Payne, R. W., Simpson, E., Sweet, J. B., Champion, G.T., May, M.J., 
and herbicide tolerant oilseed rape (Brassica napus). Proceedings of the Royal Society B, 272, 
1909–1915.

rape (Brassica napus) in arable fields. The Journal of Agricultural Science, 141, 231–240.


meal-induced pathogen suppression differ in a Brassicaceae species and time-dependent manner. 
Phytopathology, 97, 454–460.

Comparison with Saprotophic and Mycorrhizal Systems. Biotic interactions in plant-pathogen 


studies. SSR, 19, 51.


densities of fungivorous soil mesofauna. The Journal of the Torrey Botanical Society, 135, 272– 
280.


Berkeley, California.

Pekrun, C. (1994) Untersuchungen zur sekundären Dormanz bei Raps (Brassica napus L.), 
Dissertation, University of Göttingen, Göttingen.


(Brasica napus L.) by prolonged imbibition under conditions of water stress or oxygen deficiency 

in Decomposition Processes. Oikos, 39, 288–388.


and Alternaria alternata on seed germination of Brassica rapa canola. Seed Science and 
Technology, 27, 795–798.


6. General Discussion

The first chapter of the discussion (6.1) provides a summary and consolidating discussion of the main results of my four experiments. Implications of these results for GMO risk assessment are considered in chapter 6.2, followed by the main conclusions (6.3).

6.1 Brassica napus – potential for weediness

6.1.1 Overview

The aim of this study was to quantify the establishment success of oilseed rape (OSR) on ruderal sites. Fitness assessments were generally made in field experiments with artificial sowings or seed burial. In chapter 2, I assessed the impact of several environmental factors on OSR establishment success under largely unmanipulated conditions (Table 6.1). Establishment success was overall low and was influenced, among other factors, by small-scale disturbance and soil quality.

Table 6.1: Overview of the four experimental chapters in which we investigated establishment success (chapters 2-4) of OSR on ruderal soils or the persistence of seeds buried in soil (chapter 5).

<table>
<thead>
<tr>
<th>variables</th>
<th>chapter 2</th>
<th>chapter 3</th>
<th>chapter 4</th>
<th>chapter 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>tested factors</td>
<td>establishment</td>
<td>establishment</td>
<td>establishment</td>
<td>seed persistence</td>
</tr>
<tr>
<td>soil quality</td>
<td>soil quality</td>
<td>soil quality</td>
<td>soil quality</td>
<td>soil quality</td>
</tr>
<tr>
<td>vegetation cover</td>
<td>comparison with related species</td>
<td>comparison with semi-dwarf hybrid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>disturbance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fertilisation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>seed density</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sowing environment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sowing event</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>herbivory</td>
<td>natural levels</td>
<td>reduced*</td>
<td>reduced*</td>
<td>excluded or included</td>
</tr>
<tr>
<td>competing vegetation</td>
<td>low to high cover</td>
<td>unmanipulated</td>
<td>removed</td>
<td>—</td>
</tr>
<tr>
<td>sowing/burial date</td>
<td>01.04. - 13.04.07</td>
<td>24.10. - 26.10.07</td>
<td>03.10.08</td>
<td>22.09. - 29.09.07</td>
</tr>
<tr>
<td></td>
<td>28.08. - 02.09.07</td>
<td></td>
<td></td>
<td>13.11. - 14.11.08</td>
</tr>
<tr>
<td></td>
<td>03.09. - 05.09.08</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* vertebrate and slug exclusion
The impact of soil quality was confirmed in chapter 3. In this chapter, I also put establishment success of OSR in perspective by comparing its performance with weedy relatives. OSR performed better on ruderal soils than *Brassica nigra* and *Raphanus raphanistrum*. Chapter 4 addressed the potential benefits or risks of dwarfing as a method of transgenic mitigation. A semi-dwarf hybrid cultivar performed worse than a tall cultivar on low-quality ruderal soils and was less tolerant to mowing. I also investigated the impact of environmental factors on seed persistence in the soil seed bank (chapter 5) and found that it was slightly negatively affected by fungi and increasing pH. The meso- and small macrofauna had a positive influence on seed persistence.

### 6.1.2 Establishment success

**Hypothesis:** Establishment success on ruderal sites is high enough to facilitate self-sustained population growth.

Establishment success was determined by assessing the percentage of fruiting individuals which developed per seed sown and their reproductive output (seeds or pods produced per plant). Whenever possible, the discussion of hypotheses is based on the composite variables seed production per seed sown or per plot, but these are not available for chapter 2. Establishment success differed considerably in magnitude between the different experiments. A comparably poor performance was observed under the full range of stressors, such as low soil quality, herbivory, rapid growth of competing vegetation and plot disturbance by construction work or mowing (chapter 2, Table 6.2). These data most closely resemble the chances of establishment to be expected for seeds randomly dispersed in the ruderal environment. Contrary to my expectations, establishment success with 0-1.6% fruiting individuals was not distinctly higher than in natural habitats in the UK, where on average 0.7% of sown seeds developed into mature individuals (maximum of 4.6%, Crawley *et al.* 1993). It is possible that establishment success in my study was unusually low. The experiment in chapter 2 certainly underestimated average establishment success considerably, due to very high levels of slug herbivory and intensified urban development in the harbour area (2.4.1). Exclusion of these major mortality factors raised the percentage of fruiting individuals to 20.0-62.5% (Tables 6.1 & 6.2, chapters 3 & 4). Reproductive output was high under favourable conditions, reaching averages of 182-2359 seeds for plants grown at low densities (Table 6.2, chapter 4). Similar data are reported from naturally-occurring ruderal

---

1 Calculated from Table 1 for the control line
OSR populations in Germany with averages of 158-678 seeds per plant and maxima of 5000 seeds for well-developed plants (Dietz-Pfeilstetter et al. 2006). Ruderal plants can thus match the average yield of OSR volunteers in winter wheat (<121 to 637 seeds per plant, Colbach et al. 2001a, Gruber & Claupein 2007). Chapter 2 (2.4.2) even suggests that establishment success of feral plants can be higher in urban than in abandoned agricultural areas. This is supported by results from Menzel (2006). Yet, like in natural habitats in the UK (Crawley & Brown 1995), successful establishment is only reached when slug and vertebrate herbivory are excluded and competition with other plant species is reduced. The latter implies a specialisation for gaps, which is in agreement with OSR showing a competitive-ruderal life strategy (BfN 2013). This strategy is typical of abundant weeds in Europe (Lososová et al. 2008).

I found a clear potential for self-perpetuating ruderal populations which are independent of renewed seed input (33-90 seeds per seed sown, Table 7.2, chapter 3), confirming my hypothesis. However, I observed only one F1 plant in chapter 2. In chapter 3, no F1 plants

<p>| Table 6.2: Establishment success of Brassica napus (all cultivars, Artus, Smart and PR45D03) determined in the four experimental chapters. Values represent the range of treatment means for the respective variable. Data from experimental manipulations (fertilisation, mown plots) were not included and seed persistence data refer to containers with 2 mm mesh size. In chapter 4, seedlings were thinned to four seedlings per replicate plot. See Table 6.1 for a summary of experimental conditions. “—” = not applicable/not assessed |
|---------------------------------|-----|-----|-----|-----|</p>
<table>
<thead>
<tr>
<th></th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>seeds*m(^{-2})</td>
<td>1200</td>
<td>1636</td>
<td>--</td>
<td>—</td>
</tr>
<tr>
<td>seedlings*m(^{-2})</td>
<td>60  - 360</td>
<td>1291 - 1382</td>
<td>25  - 73</td>
<td>—</td>
</tr>
<tr>
<td>fruiting plants*m(^{-2})</td>
<td>0   - 18</td>
<td>473  - 618</td>
<td>9    - 46</td>
<td>—</td>
</tr>
<tr>
<td>seedling emergence [%]</td>
<td>5.7 - 30.1</td>
<td>79.0 - 84.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>flowering individuals [%]</td>
<td>0.0 - 2.9</td>
<td>61.5 - 67.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>fruiting individuals [%]</td>
<td>0.0 - 1.6</td>
<td>29.2 - 37.7</td>
<td>20.0 - 62.5</td>
<td>—</td>
</tr>
<tr>
<td>pods produced per fruiting plant</td>
<td>3   - 713</td>
<td>7    - 16</td>
<td>22  - 147</td>
<td>—</td>
</tr>
<tr>
<td>seeds produced per fruiting plant</td>
<td>—</td>
<td>79  - 171</td>
<td>182 - 2359</td>
<td>—</td>
</tr>
<tr>
<td>seed production per seed sown</td>
<td>—</td>
<td>33  - 90</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>seed viability [%]</td>
<td>—</td>
<td>96.1 - 98.5</td>
<td>94.7 - 98.8</td>
<td>—</td>
</tr>
<tr>
<td>seed persistence [%]</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>40.9 - 94.7</td>
</tr>
</tbody>
</table>
developed in the four container blocks left undisturbed after the experiment, but a multitude of seedlings emerged when the remaining experimental site was cleared of all vegetation for the experiment described in chapter 4. In consequence, repeated, well-timed disturbance or remaining open soil is necessary for populations to self-reproduce and increase. Seeing that as many as 12-80% of feral populations may reoccur in the following year (Crawley & Brown 1995, Elling et al. 2009, Menzel 2006), these conditions are not as rare as suggested by chapter 2 and can apparently be found on motorway verges with continuing roadwork and along the gravel beds of railway tracks (and possibly on sites with burrowing animals such as moles, see 6.1.4). Despite my efforts to include such sites in this study, they were probably underrepresented due to restricted access. It is therefore likely that I underestimated establishment success. My findings can certainly not explain why feral OSR populations occur at densities as high as 0.9 to 2.8*km$^{-2}$ in and near Bremen and are frequently found on ruderal sites (Menzel 2006, Squire et al. 2010). There are several possible explanations for these high frequencies.

For one thing, feral populations might show adaptations to relevant stressors. Plants in a persistent OSR feral population in the UK show strong genetic deviations from common winter cultivars, possibly resulting from selection pressure for winter hardiness (Bond et al. 2004). Similarly, wild *B. rapa* may exhibit a substantially higher potential for seed persistence than crop *B. rapa* and responds differently to germination cues (Adler et al. 1993).

It is further possible that seed input through transport losses is massive, so that many feral populations establish despite small chances of success. So far, only two studies have quantified OSR seed dispersal through vehicles. They show that road verges in agricultural areas can receive an input of 404 OSR seeds*m$^{-2}$ through harvest losses from grain trailers (Bailleul et al. 2012), and that seed deposition in suburban tunnels amounts to 2 to 67 OSR seeds*m$^{-2}$*yr$^{-1}$ (von der Lippe & Kowarik 2007). Many roadside OSR populations originate from seed spillage from trucks which transport seeds to processing plants (Crawley & Brown 1995).

Finally, I suspect that the high frequency of feral populations is a result of systematic dispersal to appropriate sites, e.g. when construction work occurs along seed transportation routes (compare Crawley & Brown 1995). I further consider it highly likely that topsoil imported for landscaping and green areas is a major seed dispersal vector for OSR (see also Wilkinson et al. 1995), as it will often ensure dispersal to suitable habitats, especially if soil is imported to large, only recently developed areas with continuing construction work. Several OSR populations in Bremen may have originated from topsoil imported from former
agricultural areas (Menzel 2006), and seed movement with fill dirt was indicated in North Dakota (Schafer et al. 2011). Seed imports with topsoil have led to high initial densities of OSR on motorway verges (Crawley & Brown 1995).

Previous observations not only show high frequencies of feral OSR plants but also long-term persistence of single populations (Crawley & Brown 1995, Menzel 2006, Pessel et al. 2001). Models predict that local seed production and long-distance dispersal within meta-populations may prolong OSR population persistence (Claessen et al. 2005b, Garnier & Lecomte 2006). Yet, considering the high dependency of OSR on disturbance, long-term persistence clearly requires the development of a persistent seed bank to outlast periods with unsuitable conditions. Little is known about OSR occurrence in ruderal seed banks: Viable seeds were found in the soil at only 8-13 % of the ruderal OSR populations near Braunschweig, Germany (Dietz-Pfeilstetter et al. 2006). Nevertheless, Pivard et al. (2008) estimate that 35-40% of feral OSR populations recruit from persistent seed banks, and some form substantial seed banks (Wilkinson et al. 1995). I demonstrated that potential seed persistence in ruderal soils can be high (chapter 5, Table 6.2) and matches the persistence in agricultural environments (compare Gruber et al. 2004c). Soil quality does apparently not limit seed bank formation on ruderal sites. Likely limiting factors are plant mortality before reproduction and lack of soil disturbance: Dormancy levels in OSR are much higher for buried seeds than for seeds on the soil surface (Schlink 1994).

Overall, my results support previous observations that ruderal OSR populations are mostly transient. However, they also predict substantial replenishment of the seed bank by ruderal plants under favourable conditions, so that populations could persist under the appropriate disturbance regimes, build up a soil seed bank or disperse to other suitable sites, suggesting a potential for long-term survival on a metapopulation scale.

6.1.3 Effects of soil quality

A major focus of this study was to investigate the influence of soil quality on establishment success of feral OSR. Impressions reported from other studies were lower OSR densities on calcareous substrates (Crawley & Brown 2004), highest yield within a pH range of 5.5 to 8.3 and an overall broad tolerance of soil conditions (Cramer 1990, Gulden et al. 2008). To my knowledge, my study is the first to systematically investigate the influence of low soil quality on establishment success of feral OSR (Fig. 6.1). I focus on the effects of soil water-holding capacity (WHC) and soil organic matter content (SOM) and will also discuss effects of pH.
Fig. 6.1: a) Summary of significant effects of soil quality on variables of establishment success of *Brassica napus* (percentage of emerging seedlings and fruiting individuals, seed production per seed sown or per plot and seeds or pods per plant. Effects of soil variables were assessed in different chapters (2-5). For each chapter, I calculated a mean effect size (symbols) as well as minimum and maximum effects (lines). Effect size was calculated as % difference from control. Control conditions are described in boxes on the control line. Read as: A decrease in soil WHC and SOM in chapter 3 reduced the number of seeds per seed sown (blue triangles) on average by 19% compared to control conditions (WHC = 25.2 and SOM = 1.8).

b) Summary of effects of soil pH on seed persistence, assessed in chapter 5.

**High versus low substrate quality (decrease in SOM and WHC)**

**Hypothesis:** Soil quality (WHC and SOM) affects the establishment success of OSR.

It is noteworthy that OSR showed a potential for self-sustained population growth even on low-quality soils (chapter 3). Phenotypic plasticity was overall high, ranging from poorly developed individuals with a single pod to fruiting plants with several hundred pods. Low soil
quality (low SOM and WHC) significantly affected the reproductive output of fruiting plants: Seed production per seed sown was up to 52.7% lower on sand than on mixed soil (chapter 3, Fig. 6.1), but not lower on mixed than on humous soil (chapters 3 & 4). Produced seeds showed a high viability irrespective of soil quality. Both low SOM and lower WHC could be responsible for the observed effects of soil quality (chapter 2.4.1). SOM provides the largest pool of macronutrients in unfertilised soil (Baldock & Nelson 2000). SOM and nutrient content therefore tend to be correlated (as in 2.2.1), though other factors also play a role in determining nutrient content and availability. I found some evidence that nutrient content is relevant to OSR establishment success, as NPK compound fertiliser significantly increased the percentage of fruiting individuals by up to 300% (chapter 2). Yet, the net increase was moderate (0.3 versus 1.4% fruiting individuals). High-quality sites in my study showed both high SOM and high WHC. As soil WHC is increased by SOM (Baldock & Nelson 2000), these factors are related, but WHC strongly depends on grain size, too (Dunger & Fiedler 1997). Water availability is known to influence yield of OSR (Champolivier & Merrien 1996) and could therefore also be responsible for the observed effects of soil quality.

I found no effect of soil organic matter content on the persistence of buried seeds (chapter 5). Similar studies are lacking, but some insights can be gained from studies analysing the effects of nutrient content, which is often related to SOM (see above). In line with our results, dormancy rates of OSR seeds show only marginal responses to nutrient (NPK) content at field-relevant temperatures (Linder 1998). Debeljak et al. (2008) however found a positive correlation between the number of OSR seeds in the seed bank and soil carbon and nitrogen content. In view of my results, I suggest that this relationship is most likely the result of differences in seed input, rather than due to differences in seed persistence. In my study, seed persistence was also not related to soil WHC. As I artificially induced dormancy in the seeds, this mainly suggests that this factor is not relevant for the survival of dormant seeds. Yet, water stress is known to induce dormancy in OSR (Pekrun et al. 1997, Schlink 1994). The number of persisting seeds results from the number of dormant seeds minus seed mortality. The effect of water stress on the induction of dormancy may explain reports of higher seed persistence in sandy soil than in silty clay loam (Pekrun et al. 1998).

In general, my results demonstrate that soil quality (SOM and/or WHC) may certainly affect the reproductive output of feral OSR, although the impact was moderate compared to other factors (6.1.4). Can we then expect feral OSR to perform best on high-quality soils? Indirect effects of soil quality also need to be taken into account. Very high soil quality possibly had
an overall negative effect by promoting the growth of competing vegetation (chapter 2.4.1 and 2.4.2). I therefore expect the overall best performance on soils with intermediate quality. Microsites for OSR establishment tend to disappear more quickly on fertile soils (Crawley et al. 1993), and reproductive output on mixed soil is not necessarily reduced compared to humous soil (chapter 3). A further experiment on the effect of soil quality in the presence of competing vegetation failed due to a severe winter (Appendix I.1).

**Soil pH**

This study is the first to demonstrate that soil pH can have a strong impact on the persistence of buried seeds. Within a range of 5.1-7.3, an increase in pH led to a steep decline in OSR seed persistence (chapter 5.4.2). I suspect that a high pH favoured specific seed-rotting fungi or bacteria (see 5.4.2), but this could not be confirmed by my experiment. The negative correlation between pH and seed persistence does not necessarily apply at a more acidic pH range, as I found indications for an unimodal relationship (Appendix III.4 Fig. III.17). Possibly, seed persistence decreases in very acidic soils (pH 4.3). Similarly, the positive effect of soil acidity on fungal growth is reversed if pH drops below 4.5 in arable and grassland soils (Rousk et al. 2009, 2011). Increased aluminium levels (demonstrated by Aciego Pietri & Brookes 2008) and resulting toxicity to fungi or plants are possible mechanisms discussed by the authors. Soil acidity raises the availability of toxic metals (Baskin & Baskin 1998, Scheffer & Schachtschabel 2010), and seed viability and germination can be reduced by exposure to high metal concentrations (Kranmer & Colville 2011). For instance, high copper concentration inhibits seed germination of *Brassica pekinensis* (Xiong & Wang 2005). Seed persistence of OSR might thus suffer from elevated mortality caused by high metal concentrations at pH values below 5.

If the negative correlation of seed persistence with soil pH at pH 5.1-7.3 is indeed due to a pH effect on microbial seed decay, it should be most pronounced in years conducive for seed-deteriorating microbes. Seed decay can be limited to very low levels in some cases, possibly due to climatic conditions or low infestation levels of the investigated seed lot (chapter 5, burial 2). In these cases, the pH effect on seed persistence might be less pronounced or other factors might become more important.
6.1.4 Other factors limiting establishment

Fig. 6.2: a) Summary of effects of environmental conditions on variables of establishment success of *Brassica napus* (see Fig. 6.1 for explanations). I assessed various effects, e.g. mowing, increase in seed density from 4 to 200, the effects of an agricultural (vs. ruderal) environment and the influence of soil meso- and small macrofauna. Effect size was calculated as % difference from control.

Read as: Lack of small-scale disturbance in chapter 2 reduced the percentage of fruiting individuals (red squares) on average by 63% compared to disturbed control conditions.

b) Correlation between seedling emergence and seed density, assessed in chapter 2.
Besides soil quality, I assessed or observed the effects of several other environmental factors on OSR establishment success. An overview of experimentally tested factors and their respective impact is given in Fig. 6.2. In the following, I highlight selected aspects of particular interest.

**Microsite versus seed limitation**

Chapter 2 shows that microsite availability is a crucial factor for OSR establishment on ruderal sites. Small-scale disturbance consistently increased establishment success (Fig. 6.2). Lack of such vegetation gaps reduced the percentage of fruiting individuals by up to 100%. The seed density manipulation corroborates this interpretation (Fig. 6.2, chapter 2.4.4). Similar conclusions have been drawn for OSR populations along motorways and in natural habitats: Recruitment mostly requires major soil disturbance, so that OSR is deemed unlikely to invade existing plant communities (Crawley et al. 1993, Crawley & Brown 1995). However, disturbance through natural processes creates microsites for plant establishment in many natural habitats. One example is sea cliffs, where a parental species of OSR, *Brassica oleracea*, occurs naturally (Rich 1991).

The present study shows that suitable microsites for OSR may already be provided by small-scale disturbances, which might facilitate low-level persistence in established plant communities. Habitat invasion by alien plants can be favoured by disturbances (Daehler 2003, Thompson et al. 1995), e.g. by the activity of burrowing vertebrates (Torres-Díaz et al. 2011). OSR is hardly going to be favoured by digging activities of herbivores such as rabbits. Yet, feral OSR might establish on molehills, which provide microsites for less competitive species (Canals & Sebastià 2000, Seifan et al. 2010). However, the successful development of F1 individuals depends on the creation of new gaps. Model simulations of feral populations on road verges predict population growth only if the disturbance rate exceeds 13 or 28% (with or without seed dispersal, respectively, Claessen et al. 2005a, 2005b). The authors also deduced from population dynamics along motorways in the UK (Crawley & Brown 1995) that realistic disturbance rates amount to ~11-20%.

**Disturbance as mortality factor**

Major site disturbances often led to the loss of established plants under typical ruderal conditions (chapter 2): 0.1-1.7 plots per site (out of five) suffered disturbance on more than 30% of the plot area before the onset of reproduction. Construction work, mowing and development of planted areas were primary causes. Mowing is a factor known to eradicate
many feral OSR populations (chapter 2.4.1, Elling et al. 2009, Menzel 2006, Wilkinson et al. 1995). Simulated mowing may reduce reproductive output of OSR, but plants may show high compensatory ability in some cases (chapter 4). Other studies confirm that mowing is not necessarily fatal (Knispel & McLachlan 2010, Wilkinson et al. 1995) and might even benefit OSR by creating new microsites (Claessen et al. 2005a, 2005b). While my fall mowing reflects a standard mowing height (2.5 cm), the spring mowing at 10 cm height represented rarer cases, e.g. plants which grow in depressions. Mowing at lower height would likely have had a more negative effect on plant development. In addition, compensatory ability can be limited by a number of factors, including drought, nutrient shortage and competing vegetation (chapter 4.4.2).

Effects of the fauna

Herbivory by slugs and rabbits was obviously a major mortality factor, although it was difficult to quantify and I did not test the effect experimentally. The percentage of completely defoliated seedlings in chapter 2 ranged from 5-26% (treatment means), which is a conservative estimate as I may have overlooked some of the left-over grazed stalks. Damage at later developmental stages also led to plant death in many cases. The importance of herbivory is also indicated by chapter 3, in which slug and small mammal exclusion was one prominent factor enabling OSR to reach approximately 20 times more fruiting individuals than in chapter 2.

If seeds are incorporated into the soil and secondary dormancy is induced by reduced soil moisture, biotic factors may also influence OSR survival. I demonstrated that buried seeds may be lost to fungal decay, although I could only provide evidence for small rates (Fig. 6.2, chapter 5). Possibly, OSR is not very susceptible to fungal attack: Its seeds are relatively large, which tends to reduce the impact of fungal seed decay (Christ & Friese 1993). Alternatively, seed decay through fungi plays a greater role in OSR under different environmental conditions: Soil fungi may lead to up to 89% mortality in seeds of other species, and there is some evidence that fungal-induced seed decay is highest at intermediate soil moisture (Wagner & Mitschunas 2008). Precipitation levels were low during the burial period in which I effectively tested the impact of fungicide (chapter 5.4.1). I also observed that seeds collected from feral plants (chapters 3 & 4) were often visibly infected with fungi while still on the mother plant. Since I used commercially produced seeds for the burial study, infestation with seed-borne fungi was probably lower than for seeds which have not undergone quality control.
I further showed that soil meso- and small macrofauna can increase seed persistence by up to 78% in some cases (Fig. 6.2). These effects were most likely caused by the activity of Collembola and were only apparent on two sites with sandy soil which were low in organic matter content. Collembolan grazing on seed-rotting fungi may possibly explain the observed effects (chapter 5.4.3). It has previously been demonstrated that Collembola can reduce seed mortality of grassland species (Mitschunas et al. 2006). Unfortunately, a laboratory study to provide evidence for a comparable effect of Parisotoma notabilis on OSR seeds failed due to insufficient levels of fungal infection (Appendix I.2).

### 6.1.5 Differences between cultivars and related species

![Figure 6.3: Summary of effects of genotype on variables of establishment success of OSR (B. napus) and related crucifers (see Fig. 6.1 for explanations). Effect size was calculated as % difference from B. napus cultivar Artus (= control). I compared Artus with other cultivars (Smart, semi-dwarf hybrid PR45D03), a cultivated relative (B. rapa) and two weedy relatives (B. nigra and Raphanus raphanistrum). The comparison with the semi-dwarf hybrid was made both in a substrate comparison (s.c.) and a simulated mowing (s.m.) experiment. Read as: In chapter 2, B. napus cultivar Smart produced on average 69% less fruiting individuals (red squares) than the control (B. napus, Artus).](image-url)
Differences between cultivars of B. napus

OSR cultivars are often bred for individual specific properties (e.g. cultivars especially suited for late sowing or less susceptible to a certain disease) and may differ in seed yield by 0-32%, although the difference rarely exceeds 5% (Beese & Karalus 2005, Cramer 1990, LWK Niedersachsen 2011). I found that establishment success of fruiting individuals was 69.2% lower for cultivar Smart than for Artus (Fig. 6.3), but the net difference was low (0.05 versus 0.16 fruiting individuals) and other fitness variables did not differ. Short-term establishment success of feral plants of other conventional cultivars should therefore deviate only moderately from my results. Long-term establishment success, however, might vary more strongly due to pronounced differences in seed dormancy levels between cultivars (Gruber et al. 2004c).

Hypothesis: Establishment success on ruderal sites is higher for the semi-dwarf hybrid PR45D03 than for the tall cultivar Artus in the absence of competing vegetation.

Contrary to my expectations, semi-dwarf varieties may be considerably less fit than tall cultivars under stressors relevant on ruderal sites. I cannot deduce from my experiment whether this is true for dwarfed cultivars in general or only for the cultivar used in my study. The cultivar PR45D03 produced up to 88.4% less seeds per plot than the tall cultivar Artus in the substrate comparison (chapter 4, Fig. 6.3), so the semi-dwarf hybrid was clearly at a disadvantage on low-quality soils. The severe winter in combination with a late sowing may have amplified this effect: Semi-dwarf hybrids tend to be less tolerant to late sowing than tall varieties (Schulz 2009). Analysis of plant height showed that the tall cultivar Artus was less likely to escape damage through mowing during early growth stages. It consequently sustained larger biomass losses in the simulated mowing but displayed a higher compensatory ability than the semi-dwarf cultivar. Artus even outyielded PR45D03 in spring-mown plots, while yield was similar for both cultivars in control and fall-mown plots. It is possible that the semi-dwarf hybrid has an inherently lower compensatory ability or that my results were alternatively caused by a non-linear relationship between damage level and yield, leading to overcompensation at intermediate damage (chapter 4.4.2). In the latter case, different damage levels, e.g. at lower mowing heights, might have reversed the relative fitness of the two cultivars. In both cases, the comparably high fitness of the tall cultivar depends on a high compensatory ability. Yet, tall plants thus lose their fitness advantage under conditions which prevent effective compensation (6.1.4, Hochwender et al. 2000, Weis et al. 2000). OSR rarely survives mowing on ruderal sites with competing vegetation (6.1.4, chapter 2).
Comparison with relatives

Hypothesis: Establishment success is lower for crop Brassicaceae than for wild relatives.

One aim of this study was to set establishment success of OSR in relation to the performance of weedy relatives (chapter 3). OSR showed an overall higher fitness on low-quality ruderal substrates (Fig. 6.3), with *B. nigra* and *R. raphanistrum* yielding on average 71% and 97% less seeds per seed sown. The better performance of OSR can partly be explained by a higher seedling emergence, which could result both from higher seed viability and lower levels of dormancy (chapter 3.4.2). Seedling survival was also higher in OSR and led to a significantly higher percentage of fruiting individuals: Crop plants benefited from fast growth and development (chapter 3.4.2). Sowing late in fall possibly amplified this effect, as quick growth in fall should have increased winter hardiness. Under the conditions prevailing in this study, OSR may reach substantially higher short-term establishment success than weedy relatives. Similarly, volunteer OSR tends to produce a higher number of seeds than the weed *Sinapis arvensis* in agricultural fields (Soltani *et al.* 2011). *Brassica* crop plants do not *per se* suffer from reduced fitness in non-agricultural environments and could even benefit from increased vigour (chapter 3.4.3). However, relative fitness of OSR compared to weedy relatives might be reversed under feeding pressure, which may play a major role in OSR feral plant survival (chapter 2, Crawley *et al.* 1993), and *B. nigra* and *R. raphanistrum* might well show a higher resistance to herbivory (see 6.2.5).

Fecundity is not the only relevant measure of fitness. Microsite availability is more important for OSR recruitment than seed density (chapter 2, 6.1.4). In such cases, plant fecundity is not necessarily a reliable predictor for invasion success (Bergelson 1994). The success of *R. raphanistrum* and annual weeds in general is attributed both to a high seed production and a high potential for dormancy, enabling plants to survive periods with unsuitable conditions (Ammann *et al.* 2000, Baker 1965, Cheam 1995, Gressel 2005). Lessons from plant invasions confirm the importance of both traits (reviewed by Pyšek & Richardson 2007). Dormancy can be induced in *B. napus* under environmental stress (López-Granados & Lutman 1998, Pekrun 1994) and seeds may persist in the seed bank for over 10 years (Lutman *et al.* 2003). In some cases, this appears to facilitate long-term persistence of OSR feral populations (Pessel *et al.* 2001, Pivard *et al.* 2008). However, seed bank decline is high compared with weeds (Hails *et al.* 1997, Lutman *et al.* 2002), which is seen as a major factor limiting the persistence of OSR feral populations (Gressel 2005). Even so, recruitment from the seed bank was likely responsible for the persistence of GM OSR ten years after deliberate release in an experiment.
Seed bank persistence does not appear to be higher for *Brassica nigra* than for OSR (Walker *et al.* 2004). Direct comparisons with *R. raphanistrum* are lacking, but burial studies indicate a higher seed persistence for *R. raphanistrum* than for *Sinapis arvensis* (Roberts & Boddrell 1983), which in turn shows higher seed persistence than *B. napus* (Hails *et al.* 1997).

For a full comparison of weediness, we not only need to analyse dormancy and seed persistence levels, but also have to assess the relative importance for long-term establishment success of a) high short-term reproductive output *versus* b) a high proportion of dormant seeds. Models suggest that persistence of feral OSR in the seed bank is of minor importance if habitat conditions are uniformly suitable for establishment (Garnier *et al.* 2006, Garnier & Lecomte 2006), but becomes more important than plant fecundity when disturbance is assumed as a prerequisite for successful establishment (Claessen *et al.* 2005a, 2005b). Still, if short-term seed production is substantially higher for OSR than for wild relatives, the seed bank could be replenished in spite of a comparably low potential for dormancy.

Both *B. nigra* and *R. raphanistrum* are weeds of disturbed ruderal sites with similar distributional patterns as OSR (Menzel 2006, Rich 1991). They are therefore not likely to show a higher competitive ability, and this has indeed been suggested in comparisons with *B. nigra* (Walker *et al.* 2004). Nevertheless, *B. nigra* has become invasive in annual Californian grasslands formerly occupied by perennial grasses (Bell & Muller 1973). In this case, over-grazing facilitated the invasion of annual species and *B. nigra* appears to successfully prevent recolonisation through the production of allelopathic substances. It is therefore premature to conclude that species with ruderal life strategy cannot invade habitats with high vegetation cover. Successful invaders show no consistent pattern concerning Grime’s life strategy (Pyšek & Richardson 2007, Thompson *et al.* 1995) and studies indicate that invasion success can be reached, amongst other traits, by being either more pronounced K-strategists or more pronounced r-strategists than native plants (Crawley *et al.* 1996). Based on my results, OSR appears to be “weedier” in several traits attributed to successful weeds and invaders (Baker 1965, Crawley *et al.* 1996, Gressel 2005, Pyšek & Richardson 2007): 1) high fecundity under favourable environmental conditions, 2) rapid growth and 3) early flowering.
6.1.6 Summary and conceptual model

Establishment success

- Successful establishment on ruderal sites is rare and restricted to very specific conditions.
- Reproductive output may be high under favourable conditions, but F1 establishment requires renewed or maintained soil disturbance.
- High density of ruderal populations could e.g. be explained by high levels of seed input or seed dispersal patterns which favour appropriate sites.
- Comparisons with literature show that, despite its mostly transient character, ruderal OSR has the potential for long-term survival on a meta-population scale.

Factors determining establishment success

- Soil quality may have a significant impact on OSR reproductive output, with reductions in yield on soils with low WHC and low SOM.
- Yet, self-sustained population growth is possible even on low-quality substrates, and soil quality plays a secondary role compared to major mortality factors.
- Strong dependency on microsite availability suggests that indirect effects of soil quality on competing vegetation need to be taken into account, leading to an expected maximum performance on soils with intermediate quality (Fig. 6.4).
- Seed bank dynamics can be strongly affected by the influence of soil pH on seed persistence: Maximum persistence occurs at lowest pH within the range of 5.1-7.3.
- Micro- and mesofauna can also affect the persistence of buried OSR seeds.
- Major factors limiting establishment success are microsite availability, herbivory by slugs and rabbits and major disturbance such as mowing and construction.
- Disturbance both facilitates establishment and eradicates populations, depending on intensity and timing.
- A conceptual model for the main factors and important interactions is given in Fig. 6.5.

Differences between cultivars and related species

- Short-term establishment success in the absence of major herbivores is higher for OSR than for related species known as successful weeds of ruderal environments.
- OSR can therefore be expected to show similar success as a weed on ruderal sites.
- However, long-term establishment, the role of dormancy and herbivore pressure are potentially important factors for a full assessment of potential weediness.
- The dwarfed cultivar shows lower fitness on low-quality soils than the tall cultivar.
- The dwarfed cultivar suffers reduced biomass loss through mowing but does not necessarily have a fitness advantage over the tall cultivar which shows higher compensatory ability.
Fig. 6.4: Simplified conceptual model of the composite effect of soil quality defined by WHC and SOM on reproductive output of OSR plants on ruderal sites.

Fig. 6.5: Conceptual model of major factors influencing establishment success of OSR in ruderal environments. Climate is not included as a factor as its effects were not addressed in this study – multiple effects on OSR establishment and influential factors are however likely.
6.2 Implications for GMO Risk Assessment

6.2.1 Limitations of this study

Several general aspects should be considered when drawing conclusions from this study, including my choice of criteria to assess fitness and weediness. I generally evaluated the performance of OSR using variables connected with reproductive output (chapters 2-4, Table 6.2). While this approach is widely used in studies on relative fitness of OSR (Allainguillaume et al. 2006, Hauser et al. 2003, Snow et al. 1999), seed dormancy may also play a major role for invasion success (see chapter 6.1.5). Results of this study are clearly limited to the appraisal of short-term establishment. Predictions for long-term success would require sampling of the soil seed bank to determine the potential for re-establishment after renewed soil disturbance. Some experiments (chapters 2 & 3, see 6.1.2) provide insight into long-term dynamics, but only for sites with largely undisturbed vegetation.

An equally important point is that my results and conclusions may only be seen as representative for the specific conditions prevailing in this study. Any deviations could alter establishment success of the investigated species and cultivars both in magnitude and in relation to each other. OSR establishment success can depend on soil conditions (6.1.3) and probably also on the species composition of the competing vegetation. Climatic conditions may affect OSR development and yield (indicated in chapter 2.4.1 and 2.4.3, Peltonen-Sainio et al. 2011), the densities of feral populations (Knispel & McLachlan 2010) and the activity of herbivores (chapter 2.4.1, Kozłowski et al. 2011). The sowing date likewise affects establishment success and yield (chapter 2.4.3), so that results from sowings in late fall or spring do not necessarily represent the typical fate of transport losses during harvest-time. Nevertheless, they reflect realistic situations: Emergence of volunteers and feral plants can be quite variable in time, occurring also in late fall, winter and spring (Gruber et al. 2004a, Menzel 2006).

Different cultivars may deviate in their performance (chapter 6.1.5), and so could different populations of the same cultivar: Mineral nutrition of the mother plant affects the germination of produced seeds in several plant species (Baskin & Baskin 1998). I only used seeds from one seed lot for cultivar Artus in this study to avoid confounding of comparisons between different sowing dates or experiments. Dry OSR seeds can be stored for three years without loss of vitality (Ellis et al. 1996). For commercially produced seeds, I would expect moderate variations between seed lots due to quality standards such as 85% minimum germinability and
maximum limits for fungal infection (BMELV 2006). Seeds collected from feral populations might have differed more strongly in their performance, possibly due to adaptations which favour growth outside cultivation (see 6.1.2) or due to higher densities of pathogens.

### 6.2.2 Consequences for the fitness of transgenic lines

An aim of this study was to contribute to risk assessment for genetically modified (GM) OSR, yet all experiments were conducted with conventional (non-GM) cultivars. Can I predict the performance of GM cultivars from my data? This depends on the assumption that GM OSR would have similar characteristics, except for the introduced new genetic trait. This is a common assumption in risk analysis (Breckling, B., pers. comm.). Within limits, I can assume that herbicide-resistant (HR) GM cultivars would show similar population dynamics on ruderal sites. GMHR plants are expected to benefit from increased fitness in the presence but not in the absence of the target herbicide. When no herbicide is applied, transgenic plants are expected to show either equal fitness or to suffer a disadvantage from the ‘cost of resistance’ (Chapman & Burke 2006, Garnier & Lecomte 2006). The latter can be assumed to be low, as the yield potential in cultivation is apparently not reduced. Fitness comparisons between GMHR and conventional OSR cultivars found either no difference or a slightly worse performance of the GMHR cultivars in the absence of herbicides (reviewed by Devos et al. 2012). Intra- and interspecific competitiveness does not differ from conventional lines (Fredshavn et al. 1995), and presence of the transgene does not appear to increase weediness of volunteer OSR plants (Norris et al. 1999) or weedy relatives (Snow et al. 1999). Most relevantly for the present study, GMHR OSR showed similar or slightly reduced establishment success compared to a conventional line in natural habitats (Crawley et al. 1993, 2001). GMHR OSR seeds were also less persistent in the soil seed bank (0.25% vs. 2%) (Hails et al. 1997). Yet, most GMHR OSR lines display seed persistence levels similar to conventional lines (Gruber et al. 2004c, Lutman et al. 2005).

Overall, evidence suggests that GMHR OSR plants do not per se show a different potential for invasiveness than conventional OSR. Despite occasionally reported reductions in fitness, GMHR OSR plants are able to persist as volunteers for four to ten years after cultivation (Beckie & Warwick 2010, D'Hertefeldt et al. 2008, Roller 2005) and appear in feral populations on roadside verges and in harbour areas (Kawata et al. 2009, Nishizawa et al. 2009, Schafer et al. 2011, Yoshimura et al. 2006). Application of the target herbicides, however, would lead to a substantial fitness advantage of GMHR OSR, which would profit further from reduced competition. This also applies to nearby areas occasionally subjected to
herbicide drift, where transgene presence is beneficial for progeny from hybrids of OSR and *B. rapa* (Londo *et al.* 2010). Considering that competing vegetation is one of the major factors governing establishment success of OSR outside cultivation, weediness might improve considerably in these areas. This may well explain the relatively high frequency of roadside OSR in North Dakota (Schafer *et al.* 2011). Glyphosate-based herbicides are frequently used throughout Europe on ruderal sites such as railway tracks, industrial sites, pavements and road verges (Devos *et al.* 2004, Monsanto 2010, Schoenenberger & D'Andrea 2012). The first reported GMHR OSR plants in Europe were found along railway lines in Switzerland (Schoenenberger & D'Andrea 2012).

Similar comparisons in fitness would have to be made for any other GM cultivar released – my data should be seen as baseline data from which GM can potentially deviate. It is important to run such tests under a wide range of environmental conditions, as the fitness consequence of the transgene may vary between habitats and depend on site conditions (Crawley *et al.* 2001, Linder 1998).

6.2.3 Relevance of establishment on ruderal sites

Cross-pollination between neighbouring fields as well as the occurrence of OSR volunteers in subsequent crops are likely to cause problems in case of GM OSR cultivation (Messéan *et al.* 2009). In contrast, feral OSR populations are not likely to threaten impurity thresholds: Their contribution to crop impurities is negligible, as at maximum 0.002% flowering feral plants were found in relation to crop plant abundance in Europe, and seed output of ferals was less than 0.0001% of the seed output of crop plants in the same region (Squire *et al.* 2010). Still, ferals contribute to transgene spread to some extent: Models found that management of field borders (waysides, field margins) could reduce the transgenic seed bank (Colbach *et al.* 2001b). Feral plants on urban ruderal sites are even less likely to cause major crop impurities, as they often grow within a great distance to the next OSR field. Chapter 2 confirmed that feral plants encounter many obstacles on ruderal sites, which probably prevent invasive growth and major genetic exchange with cultivated OSR.

Why then should we worry about these remote OSR populations? Feral populations still increase the risks involved with GM OSR cultivation, as they facilitate the evolution of new, potentially more problematic genotypes (Squire *et al.* 2010). These could quickly re-enter cultivation areas and become relevant weeds and sources of impurity. Feral OSR populations may be composed of several different genotypes, suggesting repeated input from different sources and hybridisation between different cultivars or with wild relatives (Elling *et al.* 2009,
Chapter 6

Pascher et al. 2010, Pessel et al. 2001). We can therefore expect stacking of advantageous traits, originating from GM or conventional cultivars with different breeding goals. Moreover, ruderal OSR populations increase the likelihood of hybridisation with wild relatives: They are more variable in their flowering time than cultivated OSR, showing an extended overlap with the flowering period of wild relatives (Menzel 2006). Many closely related Brassicaceae occur mostly in urban areas, while their occurrence in agro-ecosystems has been reduced by herbicide application. Not only were they found on the same urban site types as OSR, but they also repeatedly co-occurred on the same sites in Bremen (Menzel 2006). Three sand mining areas, a dump site and two industrial areas were identified as hotspots for potential hybridisation. Similarly, feral OSR and B. rapa co-occur in the area of Osnabrück (Elling et al. 2009).

6.2.4 Conditions and traits which can increase weediness

My results suggest that soils with intermediate nutrient levels and water-holding capacity (WHC) provide optimal conditions for OSR growth in ruderal environments (6.1.3). Such soils were underrepresented in chapter 2, so that weediness under natural conditions would likely be higher in studies or regions with more favourable soil conditions. A soil pH of ~5 seems to favour persistence in the seed bank (6.1.3). Due to a number of mortality factors (6.1.4), increased weediness of OSR is only to be expected on sites where several conditions are combined:

1) Slug herbivory must be reduced, either through low to intermediate soil WHC or climatic conditions;
2) Sites must be secluded from rabbit foraging, e.g. through physical barriers along motorways, large paved areas, traffic or major disturbances such as construction work;
3) Gaps in the vegetation cover need to be created repeatedly, either through small-scale disturbance or through major disturbance after pod ripening.

Apart from this, massive seed input might lead to high rosette densities, which can suppress competing vegetation (Crawley & Brown 1995) and would likely protect central plants from herbivory. Weediness of herbicide-resistant OSR would further be increased on sites where application of the target herbicide reduces competing vegetation (6.2.2, e.g. along railways, roadsides and field margins, Garnier & Lecomte 2006, Londo et al. 2010) and possibly herbivore activity. As we found herbivores to cause considerable mortality in OSR, a reduction in herbivory through herbicide application would greatly improve the chances of OSR establishment.
These restrictions to OSR weediness could be reduced if OSR acquires certain traits through genetic modification, hybridisation or gene stacking. Regulation of GM plants will need to take great care regarding the introduction of traits which could increase the survival of feral plants. Even if fitness advantages conferred by a single trait might not increase weediness dramatically, gene stacking in feral OSR populations (see 6.2.3) could lead to problematic genotypes. Already we know of feral or volunteer OSR plants with multiple herbicide resistance resulting from hybridisation between different GM cultivars (Aono et al. 2006, Beckie et al. 2003, Hall et al. 2000, Schafer et al. 2011). Both conventional breeding programmes and genetic modification aspire to create cultivars with increased resistance to abiotic and biotic stressors. Present cultivars or wild relatives could also become sources of advantageous traits. Traits which could be obtained from B. rapa are of particular concern due to the high frequency and fitness of hybrids, but hybridisation with other relatives is also possible (chapter 1.2.1).

Resistance against herbivory would certainly improve establishment success in ruderal environments, especially if OSR becomes less palatable to slugs and rabbits. GM OSR with a Bacillus thuringiensis (Bt) transgene could cause problems in the environment due to higher resistance against insect herbivory (Stewart et al. 1997). OSR might also gain a higher content of glucosinolates through hybridisation with existing genotypes. Glucosinolates defend against herbivore attack by molluscs (Glen et al. 1990, Noret et al. 2005), generalist insects, mammals and birds (Halkier & Gershenzon 2006, Mithen 1992). Glucosinolate content of seeds is lower in present “00” than in former “0” OSR cultivars (Cramer 1990). This increases susceptibility to slug herbivory in seedlings (Glen et al. 1990). Old “0” varieties can still be found in feral OSR populations (Menzel 2006, Pessel et al. 2001), and their high frequency could result from improved defence against herbivory (Dietz-Pfeilstetter et al. 2006). Moreover, Brassica species differ in glucosinolate quality and quantity (Bellostas & Sørensen 2007, Kirkegaard & Sarwar 1998), and e.g. B. nigra tends to contain more isothiocyanate-releasing glucosinolates in shoot and root tissue than OSR. Wild relatives such as B. nigra, R. raphanistrum and some genotypes of B. rapa can further induce trichomes as a defence against herbivores (Agrawal 1999, Agren & Schemske 1993, Traw & Dawson 2002), which could deter slugs (Scheidel & Bruelheide 1999). Density of trichomes is low in OSR but can intentionally be increased by genetic modification (Gruber et al. 2006). There are therefore various pathways through which resistance against herbivory could be increased in OSR.
As pointed out in chapter 6.1.5, persistence in the seed bank may also be a crucial factor limiting weediness of OSR. Dormancy and seed persistence levels may be increased by genetic modification of OSR seed oil content (Linder 1998, Linder & Schmitt 1995). Chapter 3 gave no indications that OSR could receive higher potential for dormancy from crop *B. rapa*. Yet, weedy *B. rapa* (syn. *B. campestris*) has a higher potential for dormancy than OSR, and the same applies for hybrids between these two species (Landbo & Jørgensen 1997).

Any genetic transformation which enables OSR to outcompete surrounding vegetation could likewise lead to drastic increases in fitness. Competitive ability was higher in crop *B. rapa* than in OSR (chapter 3). Similar findings were made in other studies (Damgaard & Kjær 2009, Rose et al. 2009), but see Hauser et al. (2003). Competitive ability could also be raised by increasing plant tissue content of glucosinolates: Breakdown products of *Brassica* glucosinolates inhibit germination in many weed species (Petersen et al. 2001).

My results further suggest that increased drought tolerance or lower nutrient requirements would presumably lead to minor increases in weediness, as OSR can already grow and produce seeds under a wide range of soil conditions. However, these traits might enable OSR to invade more extreme habitats where low soil quality weakens competing vegetation. I likewise found only limited evidence (6.1.4) that weediness of OSR could be amplified by acquiring resistance to fungal seed-rot pathogens.

6.2.5 Mitigation approaches

Growing awareness of the problem of transgene escape has spurred the development of strategies to prevent or reduce gene flow from transgenic plants (Daniell 2002, Gressel 2005). One proposed method of transgenic mitigation is the insertion of a dwarfing gene (see chapter 1.3.3): Dwarfing reduces yield under competitive conditions and therefore lowers potential fitness of OSR volunteers and hybrids with wild relatives in subsequent crops (Al-Ahmad & Gressel 2006, Fargue et al. 2004). Yet, approximately one third of feral populations in urban areas grow on sites with vegetation cover below 10%, so that dwarfed cultivars should suffer only moderately from their lower competitive ability (Reuter et al. 2008). Chapter 2 confirms that successful establishment of ruderal OSR largely depends on competition-free microsites. I therefore investigated fitness of semi-dwarf hybrid OSR under non-competitive conditions with stressors which might favour plants with reduced height (chapter 4). Against my expectations, semi-dwarf hybrids did not show a higher tolerance of low soil quality (see 6.1.5), and any fitness advantage is more likely to occur on soils with higher quality than the
soils used for this study (chapter 4.4.1). As the cultivar developed for transgenic mitigation shows higher yield on agricultural soil with reduced competition, one can expect a fitness advantage on comparable ruderal sites. Many sites with high soil quality can be found in urban environments, particularly along transportation routes (see chapter 1.1.1).
In addition, effects of mowing as another stressor on ruderal sites need to be considered (chapter 4). Semi-dwarf hybrids are more likely to escape damage during early growth stages (see 6.1.5). If damage occurs, however, tall cultivars may display a higher compensatory ability. As discussed above (6.1.4), feral OSR usually does not survive mowing, so compensatory ability was clearly higher in this study than we can expect for most natural situations. It is unclear whether the fitness advantage of the tall cultivar still applies when additional stressors or lower mowing heights limit compensatory ability (see 6.1.5).

My results indicate that dwarfing might indeed reduce transgene flow in ruderal environments, if the dwarfed GM variety shows a similar response as the conventional cultivar used in this study. However, fitness of the semi-dwarf hybrid sometimes matched that of the tall cultivar. This questions the efficacy of dwarfing as a transgenic mitigation strategy. I found that relative fitness may be context-dependent. Other dwarfed cultivars may well show higher fitness, e.g. the cultivar developed for transgenic mitigation (Al-Ahmad et al. 2006). This could both arise from differences in the parental lines and from differences in the modification itself. Dwarfing in OSR cultivars is achieved by insensitivity to gibberellins, yet the responsible genes differ between cultivars (Al-Ahmad & Gressel 2006, Huapeng et al. 2011, Koch & Bruins 2008).

Transgenic mitigation is a tool which may assist in preventing transgene spread. Gressel (2005) argue that several mitigation transgenes could be combined to reach higher efficiency (e.g. dwarfing and non-shattering pods in OSR). Yet, the implications of introducing mitigation transgenes need to be examined just as carefully as for the target transgenes. Unforeseen fitness advantages in non-agricultural environments are a possible risk. On the other hand, “successful” mitigation genes might endanger wild relatives, as the presence of crop-wild hybrids with reduced fitness could lead to the shrinking of wild populations (demographic swamping) as predicted by Haygood et al. (2003).
6.2.6 Consequences for modelling, monitoring and management

If GM OSR is cultivated, we can expect ruderal populations of transgenic feral plants in agricultural and urban areas. My results provide supporting evidence that self-sustained population growth is possible under certain conditions, confirming that urban populations are vectors for uncontrolled transgene spread with possible negative effects. Some models predicting transgene spread already include feral OSR populations (Colbach 2009, Colbach et al. 2005b, Middelhoff et al. 2011, Reuter et al. 2011). Yet, population dynamics are difficult to predict and the driving forces are not fully identified – leading e.g. to an overestimation of populations on road verges (Colbach 2009, Colbach et al. 2005a). I observed a high variability in feral plant survival and multiple mortality factors. Nevertheless, my results may help to obtain more realistic estimates and to identify conditions with increased survival rates.

Complex models already integrate regional environmental data and empirical small-scale data for a large-scale evaluation of GM OSR dispersal (Breckling et al. 2011, Middelhoff et al. 2011, Reuter et al. 2011). They can identify regions with high potential for interactions between transgenic and conventional crops. Other spatial environmental data could be added to improve estimates and predict the frequency of feral populations. Regions with high risk of transgene spread could then be declared as GMO-free zones – an approach presently discussed as a solution to the problem of coexistence in Germany (Winter & Stoppe-Ramadan 2011). Alternatively, these regions need to be subjected to an intensive and well-targeted monitoring and management plan in case of GM OSR cultivation to comply with EU legislation (see chapter 1.1.1). A feasible inclusion of urban populations necessitates the identification of local hotspots. According to my results, this will require the inclusion of several factors and their interactions. The high dependency of OSR on microsite availability (6.1.4) shows that models need to account for disturbance intensity and timing, e.g. as in Claessen et al. (2005a, 2005b). Possibly, land-use plans, CORINE land cover data and information on maintenance of green areas and construction sites can be used to identify local hotspots with appropriate disturbance levels. Records of slug and rabbit activity are equally important but would require extra monitoring. Soil quality appears to be a factor of secondary importance, both according to my results and previous observations (Menzel 2006). Yet, inclusion of this factor may improve estimates of fecundity and seed persistence and will be relevant for determining the necessary disturbance rate (6.1.3). Digital soil maps such as BÜK 200 and the “Bodenschätzungskarte” (LBEG 2012) could provide large-scale information on soil quality, although local conditions will be more variable.
Identification of potential hotspots for OSR monitoring should particularly focus on hybridisation with wild relatives or between cultivars with different GM traits. Wilkinson (2003) estimated nationwide hybridisation frequency with weedy *B. rapa* for the UK, using data from surveys, floras and databases. A spatially explicit model by Middelhoff *et al.* (2011) includes individual feral plants and allows for the representation of multiple transgenes. Possibly, similar methods can be used to identify hybridisation centres in urban areas. Several studies are currently concerned with establishing concepts for a network for GMO monitoring and possibilities to connect it with existing databases (Graef *et al.* 2012, Mönkemeyer *et al.* 2006, Pascher *et al.* 2011, Reuter *et al.* 2007, Züghart *et al.* 2008).

Identification of hotspots should be updated regularly, as changes in urban landscape planning may alter the conditions for OSR (6.1.2). Large construction areas in particular should be inspected for their potential to become a hotspot. Likely hotspots could then be monitored intensively, including the screening of GMOs, hybrids and stacking of GM traits in feral populations (as suggested by Breckling 2007 and Züghart & Breckling 2003). Problematic populations should probably be eradicated manually: Application of herbicides could have more adverse effects on the environment than the ferals themselves, and increase selection pressure for herbicide tolerance (Devos *et al.* 2012). Mowing is also not a safe management option (Devos *et al.* 2012), as it might create new vegetation gaps and thereby favour OSR survival or re-establishment. My results support that mowing does not necessarily eradicate OSR populations (6.1.4), and that successful management must minimize soil disturbance, especially any disturbances which could lead to the formation of a ruderal seed bank.

Transportation vectors should be key elements of monitoring and management: I suspect that transport losses of high magnitude result in large seed input into ruderal areas which might be directed specifically to suitable sites (see 6.1.2). In case of GM cultivation, a reduction of seed input and specific monitoring of transportation vectors (e.g. Bailleul *et al.* 2012) could make an important contribution to reducing uncontrolled transgene spread. Most importantly, export of topsoil from GM cultivation areas to construction sites should be prevented or followed by monitoring. Roadwork in fall and spring along known OSR transportation routes should likewise be accompanied by monitoring.
6.3 Conclusions and Outlook

6.3.1 Main findings
- Actual establishment success of OSR on ruderal sites under natural conditions was limited due to a variety of mortality factors.
- Feral establishment success first and foremost depended on soil disturbance and herbivory.
- For the first time, I have shown that soil quality may also play a relevant, albeit secondary role in OSR establishment success. Establishment was less successful on soils with low WHC and low SOM than on high-quality soils.
- Yet, microsites for establishment tended to disappear more quickly on very fertile soils. I therefore predict highest overall and long-term establishment success on ruderal soils with intermediate quality.
- OSR showed higher establishment success on ruderal soils than two weedy relatives, *B. nigra* and *R. raphanistrum*.
- The semi-dwarf hybrid PR45D03 produced either as many or less seeds than the tall cultivar Artus under stressors relevant on ruderal sites. Relative fitness was however context-dependent.

6.3.2 First insights
- This study showed that the persistence of dormant seeds may depend on soil pH.
- I also found indications that the meso- and small macrofauna has a positive effect on seed persistence.

6.3.3 Resulting research needs
I observed an overall low establishment success of OSR and can thus not explain the high frequency of feral populations observed in other studies. Further research should investigate whether persistent feral OSR populations show adaptations to relevant stressors. Most importantly, future studies need to determine the magnitude and sources of seed losses in transport, relevant seed dispersal vectors and frequency of suitable habitats at locations with high seed input. In particular, the magnitude of OSR dispersal via topsoil imports from agricultural areas should be investigated.

Based on my findings, future research should aim at further specifying optimal conditions for establishment success, including long-term survival. Indications that OSR long-term establishment success is highest on soils with intermediate quality need to be corroborated.
General Discussion

Studies should also determine the necessary disturbance regimes conducive to long-term survival on soils of varying quality. In retrospect, it would have been interesting to prolong the duration of the experiment in chapter 3 and simulate repeated disturbance events. Very importantly, ruderal seed banks need to be studied as one important aspect of long-term survival. Studies should identify both their frequency and conditions which favour seed bank formation and persistence. My study provided first insights on this topic, pointing at both abiotic and biotic factors which can be important. The reproducibility of these results and the responsible mechanisms behind them need yet to be determined (chapter 5.4.3).

My study also showed that OSR has a higher potential for weediness than certain weedy relatives. This raises concern regarding its self-sustained persistence in the environment. Further research is needed to complete my assessment, as there are indications that OSR might be “less weedy” in traits which could be of great importance. Specifically, resistance to herbivory and dormancy levels should be compared. Such studies should also aim to identify whether initial high fecundity or high dormancy rates contribute most to long-term survival.

This study provided knowledge of possible restrictions to OSR weediness. The most important next step, in my opinion, is to appraise whether more aggressive genotypes could develop through inter- and intraspecific hybridisation, novel GM traits and gene stacking. I strongly encourage further research to determine if OSR could acquire resistance against herbivory (e.g. through higher glucosinolate content), higher dormancy levels, an increase in competitive ability or higher tolerance of mowing. Gains in these traits might indeed generate a troublesome weed with limited weed control options. In view of recent developments to combine resistance traits (e.g. against multiple herbicides or against herbicide and insects), special attention must be paid to the effect which these single and combined traits will have on OSR fitness.

Before concluding from my results that dwarfed cultivars at least pose no additional risk by increasing the fitness of ruderal populations, further research should determine whether the reduced fitness observed in PR45D03 also applies to the specific dwarfed cultivars intended for transgenic mitigation. After all, I did not assess the effect of dwarfing per se but differences between two cultivars which were not near-isogenic. In addition, the effects of dwarfing need to be studied under different environmental conditions. Dwarfing might yet be advantageous on high-quality ruderal soils and under conditions which limit plant compensation to mowing (see 6.1.5). Varying mowing heights should be included to
determine if dwarfs have an inherently lower compensatory ability and if biomass loss and fecundity are related in an optimum curve.

**6.3.4. Implications for GMO risk assessment**

Based on the limited establishment success I have observed, I agree with previous assessments (Devos et al. 2012, Squire et al. 2010) that feral OSR is at present not weedy and widespread enough to contribute significantly to the violation of impurity thresholds (in contrast to volunteers growing in or close to cultivation areas). Weediness of existing genotypes outside cultivation will likely be restricted to disturbed ruderal sites with specific conditions. However, I also demonstrated that reproductive output of ruderal OSR under favourable conditions is high enough to enable self-sustained population growth, and persistence in ruderal environments can be expected to equal that of weedy relatives. Ruderal populations may still play an important role when it comes to providing opportunities and uncontrolled refuges for hybridisation and gene stacking (6.2.3). Even if such events may be rare, they can become relevant when extrapolated to the larger scale of nationwide long-term GMHR OSR cultivation. I therefore recommend monitoring of ruderal OSR populations, including those in urban areas. My results provide information which may help to identify conditions favouring feral OSR persistence and thereby potential hotspots for a well-targeted monitoring and management. Successful establishment can be expected in the absence of slug and rabbit herbivory and at appropriate levels of well-timed, repeated disturbance which creates gaps for seedling recruitment but does not prevent individuals from reaching maturity. Soils with intermediate water-holding capacity and soil organic matter content are more likely to sustain long-lived populations, and seed banks will be most persistent in soils with pH ~5. The accuracy of models predicting OSR population dynamics could be improved by including these factors (6.2.6).

As part of the monitoring programme, feral OSR should be screened for the occurrence of transgenes, hybridisation and stacking of GM traits in order to detect the evolution of problematic genotypes. My findings suggest that OSR weediness could be increased by gaining certain traits (6.2.4). This should be kept in mind when assessing the risks of novel transgenes or cultivars with multiple transgenes. Concerning possible approaches of mitigating transgene escape, it appears from my results that dwarfed cultivars indeed pose a lower risk than tall cultivars. Dwarfing will however not prevent transgene escape, and the fact that the semi-dwarf hybrid reached similar yield as the tall cultivar under some conditions questions the efficacy of this method.
Ferality of OSR and long-term persistence in the seed bank imply that HR traits will become an irretrievable part of the environment in case of GMHR OSR cultivation. Glyphosate-based herbicides are frequently used on railway tracks, industrial sites, pavements and road verges. As these are typical habitats for feral OSR and wild relatives, alternative weed control methods would have to be found in the long term. HR traits already complicate the control of volunteer OSR in subsequent crops in Canada (Mauro & McLachlan 2008), and OSR volunteers have repeatedly developed resistance against multiple herbicides (Beckie et al. 2003, Hall et al. 2000). Problems may also arise from other weed species which have been shown to acquire resistance to glyphosate both through transgene introgression and natural evolution under the strong selection pressure created by the widespread use of glyphosate (1.3.2). Total herbicide use has consequently increased since the introduction of GMHR crops in the U.S., where glyphosate-resistant crops are cultivated intensively (Benbrook 2012). Negative effects on the environment through non-target effects are a likely consequence. In response to the occurrence of glyphosate-resistant weeds, the industry is currently developing crops with resistance against multiple herbicides (Feng et al. 2010, Green & Castle 2010). 2,4 D-resistant corn and soybean as well as dicamba-resistant soybean have already been approved for cultivation in Canada (ISAAA 2013b). Both herbicides are considerably more toxic than glyphosate (Peterson & Hulting 2004), so that the likely widespread use could pose severe risks to human health and the environment (Benbrook 2012). They are further likely to cause damage in neighbouring crops through drift (Mortensen et al. 2012). Crops with multiple HR traits would have to be well-managed in the interest of sustainability: Sequential use of herbicides until loss of efficiency would greatly favour the evolution of multiple HR weeds (Green & Owen 2010, Mortensen et al. 2012). Long-term sustainable weed management requires a high diversity of weed management practices (Green & Owen 2010, Powles 2008). However, the introduction of HR crops has led to a neglect of research on and the implementation of integrated weed management (IWM) (Mortensen et al. 2012), so that strong reliance on multiple resistance crops for weed control could result in a “transgenic treadmill”, a new form of herbicide intensification (Bnimelis et al. 2009). These and other consequences and risks need to be considered and weighed against the benefits of HR OSR (see chapters 1.3.1 and 1.3.2) before introducing these crops to the EU. This study supports that this step will likely lead to the irreversible presence and self-perpetuation of feral populations, albeit probably at low levels. I recommend focusing on the more sustainable approaches of integrated weed and pest management, rather than the introduction of resistance traits.
Résumé

- Weediness of OSR is presently limited to very specific conditions and does thus not indicate major weed problems outside cultivation.

- Yet, there is a clear potential for self-sustained population growth, increased weediness and the evolution of problematic genotypes which needs to be considered in risk assessment and merits further research.

- A dwarfed cultivar shows lower establishment success, indicating that transgenic mitigation through dwarfing could reduce transgene escape. However, it would not prevent it effectively.

- Feral populations should be monitored as possible refuges for the evolution of problematic genotypes.

- Further research should focus on ruderal seed banks, vectors of seed input, likely sources from which OSR can acquire problematic traits (e.g. also future GM genotypes with multiple resistances) and further comparisons with weedy relatives.
Appendix I: Failed Experiments

This chapter gives an overview of additional experiments which were carried out but failed to yield information on the investigated questions. These experiments are presented in due shortness with the underlying hypotheses and methods and a short explanation why the experiments failed.

I.1 Is dwarfed oilseed rape (Brassica napus L.) less competitive than conventional OSR?

I.1.1 Objective

Two major objectives were pursued with this experiment. Firstly, it was meant to extend the comparison between the semi-dwarf hybrid cultivar PR45D03 and the tall cultivar Artus described in chapter 4. Al-Ahmad et al. (2006) found that dwarfing in OSR is disadvantageous under competitive conditions. I wished to verify this with a different variety (see chapter 4 for comparisons under non-competitive conditions). Secondly, I wished to get further insights into the effects of soil quality on OSR establishment success in the presence of competing vegetation. My previous findings (chapter 2) suggested that positive direct effects of a rise in soil quality (increase in soil organic matter content (SOM) and water-holding capacity (WHC)) on fecundity were counteracted by indirect negative effects if soil quality was very high: On very fertile agricultural soils, the percentage of fruiting OSR plants was reduced as competing vegetation invaded microsites for establishment more quickly. As a result, I hypothesised that establishment success is highest at intermediate soil quality if competing vegetation is present. Therefore, the addition of fertiliser should be beneficial on low-quality soils, but negative on high-quality soils.

I compared the performance of the semi-dwarf hybrid cultivar PR45D03 with that of the tall cultivar Artus in the presence of competing vegetation and tested whether fertilisation had different effects on soils with low and high quality. I raised the following hypotheses:

1) In the presence of competing vegetation, establishment success is lower for the semi-dwarf cultivar than for the tall cultivar Artus.

2) In the absence of competing vegetation, fertilisation has a positive effect on OSR establishment success both on low-quality and high-quality soils.

3) Grown in competing vegetation, OSR establishment success is
   a) increased by fertilisation of low-quality soil
   b) reduced by fertilisation of high-quality soil
I.1.2 Methods

The experiment was set up on the Siedenburg dump site (Appendix II.8) in containers previously used for the experiment in chapter 3 (3.2.2). Nine experimental blocks of four containers each were remodelled so that each block held two containers with sand and humous soil, respectively (see 3.2.1 for substrate properties). One container per substrate received NPK compound fertiliser at a dosage of 90 g·m\(^{-2}\) (as described in 2.2.3 for the fertilisation treatment). Three treatments were established per container: 1) cultivar Artus sown into competing vegetation 2) cultivar PR45D03 sown into competing vegetation and 3) cultivar PR45D03 sown into bare soil without competing vegetation. 40 seeds were sown per replicate. Competing vegetation was thinned to a cover of 50-70% directly before sowing OSR on 10-11 October 2008. Fences excluded small mammals and slugs from the complete experimental area (see 3.2.2 for details). Competing vegetation was regularly removed from treatments without vegetation throughout the study.

I.1.3 Results and discussion

Most plants in my experiment did not survive the winter (Fig. I.1), which can be explained by a period of severe frost in January (see Appendix II.5.3 Fig. II.16), which also caused relatively high mortality in plants from chapter 4. It is likely that the combined stress of frost and vegetation amplified winter mortality in the present study.

![Fig. I.1](image-url): Surviving plants in spring 2009 in % of seeds sown of the tall cultivar Artus and the semi-dwarf hybrid PR45D03. Only containers with vegetation are shown. Means ± 95 % CI (confidence interval) were back-transformed from arcsine-square-root-transformed data, n = 9.
In an attempt to save the experiment, I planted OSR plants reared in a climate chamber into the containers in July 2009 (20 plants per replicate). Only one of the replaced and none of the remaining plants flowered due to high levels of slug herbivory, so the experiment was terminated.

**I.2 The effect of Collembola on OSR seed persistence**

**I.2.1 Objective**

In chapter 5, I found a positive effect of small macro- and mesofauna on the persistence of buried OSR seeds. Previous studies have attributed similar effects to the presence of Collembola, which feed on fungal hyphae and can thereby reduce fungal-induced seed mortality in grassland species (Mitschunas et al. 2006, 2008). I suspected that the same mechanism was behind the effects described in chapter 5, but lacked evidence (5.4.3). The aim of the present experiment was therefore to investigate the effect of Collembola and fungi on the persistence of OSR seeds in the soil. There is some evidence that seed-attacking fungi are most severe at intermediate moisture content (Wagner & Mitschunas 2007). I would therefore expect that Collembola also have the highest impact on seed persistence at intermediate moisture levels.

I incubated OSR seeds on soil in a climate chamber and included treatments with fungicide or Collembola. The effects were tested under three different moisture levels. I tested the following hypotheses:

The proportion of OSR seeds persisting viable in the soil is
1) reduced by seed-infecting fungi
2) increased in the presence of Collembola
3) The effects of seed-infecting fungi and Collembola are most pronounced at intermediate soil moisture.

**I.2.2 Methods**

Prior to the experiment, I induced dormancy in seeds of cultivar Smart (5.2.3) by incubating them in dry soil for six weeks. After this, seeds were lightly brushed over sandpaper to damage the testa and thus favour fungal infection. The test was conducted in PE containers with 216 mg defaunated soil (dry mass) and closed lids. 50 seeds were placed into each test vessel in close proximity in a circular soil depression. Depending on treatment, the soil had
been watered to one of three moisture levels: dry (18% maximum water-holding capacity, \(WHC_{\text{max}}\)), intermediate (24% \(WHC_{\text{max}}\)) and wet (42% \(WHC_{\text{max}}\)). At each moisture level, three treatments with eight replicates each were prepared: 1) pure soil, 2) soil + Collembola and 3) soil + fungicide. 25 adult individuals of the Collembola \textit{Parisotoma notabilis} were added to the containers of treatment 2. For treatment 3, seeds were coated with the fungicide captan (compare 5.2.3), which was also added to the soil. One fungal-infected OSR seed was placed into each container to accelerate fungal spread.

Seeds were incubated in the respective containers for nine months starting on 22 December 2009 and were kept in darkness or green light at 15 °C so as not to break seed dormancy. All seeds which germinated within the first week were replaced. Containers were watered weekly and were checked for the presence of seedlings, which were counted and removed. As I observed no growth of fungi during the incubation phase, containers were exposed to drying and re-wetting after four and five months to favour fungal growth. Containers were kept with an open lid to dry for 3*12 hours, after which samples were watered. After five months, I re-introduced 25 \textit{Parisotoma notabilis} to treatments with Collembola, which appeared to have died. I also re-introduced one fungal-infected OSR seed to each container. At the end of the incubation phase, seeds were tested for viability with a germination test, followed by a tetrazolium test (see 3.2.5 for both).

I.2.3 Results and discussion

My visual inspections throughout the seed incubation phase indicated that there was no spread of fungi in the test vessels and no seeds were observed to suffer from fungal infection. This is confirmed by my results, which showed seed mortality of at maximum 11% and no effect of fungicide addition (Fig. I.2). I also observed no impact of Collembola on seed persistence. With regard to my hypotheses, I could not show that fungi have a relevant impact on OSR seed mortality. This is contrary to my findings in chapter 4, where fungi caused 4.4% mortality in buried OSR seeds, and contrary to observations made in preliminary and germination tests where fungi did cause some mortality. I obviously did not achieve to create conditions which are conducive to fungal seed infection. For one thing, fungal spread in preliminary and germination tests was most severe when these were conducted in Petri dishes. Possibly, oxygen and carbon dioxide concentrations differed in the Petri dishes due to the smaller volume of air. This may have had an impact on fungal growth. I did not conduct the test in Petri dishes as germinating seeds would have needed to be removed more frequently (otherwise, growing seedlings would have pushed the lids off the dishes), which could not be
Failed experiments

done due to time constraints. Secondly, the soil microfauna was probably reduced by my defaunation treatment. Thirdly, I used commercially produced seeds. Although they were not coated with fungicide, infestation with seed-borne fungi was probably lower than for seeds which have not undergone quality control. However, commercially produced seeds were also used for my burial study where fungi did cause mortality. Regarding the impact of fungi on OSR seed persistence, I can conclude that they were not relevant under the presented conditions, but have some relevance in other situations.

The lack of a Collembola effect could have two possible reasons: 1) Collembola reduce fungal-induced seed decay and therefore have no impact when fungi do not cause seed mortality, 2) Collembola influence seed persistence by yet unknown mechanisms not applying in my test system or 3) Collembola were not present in sufficient number throughout the experiment. The Collembola certainly did not survive for the whole duration of the test, probably due to the shortage of food. I extracted no live Collembola at the end of the test, though I found some exuvia. Therefore, it would be premature to draw any conclusions from my Collembola treatment.

Fig. I.2: Proportion of viable seeds retrieved from the test vessels depending on soil moisture content and on the presence of fungicide or Collembola. Means ± SE, n = 7-8.
Appendix II: Methods and site data

II.1 Experimental sites chapter 2

Fig. II.1: Location of Bremen (shaded in dark grey) in North-West Germany.

Fig. II.2: Location of the ruderal experimental sites in Bremen. Topographical map printed with kind permission of the Landesamt für Geoinformation und Landentwicklung Niedersachsen.
Methods and site data

Fig. II.3: Location of the agricultural sites in Lower Saxony.

Fig. II.4: Low-quality sites with low vegetation cover used for the spring sowing of 2007.
**Fig. II.5:** Low-quality sites with high vegetation cover used for the spring sowing of 2007.

**Fig. II.6:** High-quality sites with low vegetation cover used for the spring sowing of 2007 (upper picture in right-hand corner taken several weeks after sowing).
Fig. II.7: High-quality sites with high vegetation cover used for the spring sowing of 2007

Fig. II.8: Agricultural fields used for the spring sowing of 2007 (lower picture in left-hand corner taken several weeks after sowing).
II.2 Soil analyses

II.2.1 Chapter 2

Samples were taken from a depth of 0 to 15 cm with a corer of 2 cm Ø. Four soil cores from each block of the respective site were combined for analysis which was based on air-dried samples sieved to 2 mm. Two replicate measurements were made for pH and organic matter content. Water-holding capacity and content of selected nutrients were generally determined for one replicate and only verified with a second measurement if errors in the first measurement appeared likely. Total carbon and nitrogen content was assessed with an elemental analyser (EuroEA 3000, HEKAtech GmbH, Wegberg, Germany). Plant-available phosphorus (P) and potassium (K) were extracted with ammonium lactate and measured photometrically by flow injection analysis based on the molybdenum-blue reaction with stannous chloride as a reducing agent (P) or with Atomic Absorption Spectrophotometry (K). See Appendix II.2.5 and II.2.6 for details of the respective methods.

II.2.2 Chapter 3

Soil variables were assessed for a sample mixed from three 10 L buckets taken from the respective soil before containers were filled. Measurements were replicated three times. Water-holding capacity, soil organic matter content and pH were measured according to Appendix II.2.5, grain size distribution was estimated after (Dunger & Fiedler 1997).
II.2.3 Chapter 4

See II.2.2.

II.2.4 Chapter 5

Three soil cores (Ø = 5 cm) were taken in each of the experimental sites from a depth of 0 to 7.5 cm on 23-27 June 2008 when the buried seeds were exhumed. All cores taken on one site were analysed as a mixed sample. I determined soil organic matter content, water-holding capacity and pH with two replicate samples per site according to Appendix II.2.5.

II.2.5 Standard methods

Soil analyses were generally made for air-dried samples sieved to 2 mm. The following standard methods were used for the following soil variables:

- water-holding capacity: ISO 11269-2
- pH: DIN 19684 T1
- soil organic matter content: DIN 19684

II.2.6 Nutrient content of soil samples

Preparation of soil samples for analysis

Soil samples were air-dried and sieved to 2 mm (large pieces were crushed with mortar and pestle before sieving). Remaining roots were removed and the water content of the sample was determined gravimetrically (DIN 19683) so that nutrient content could be set in relation to soil dry mass. Samples for C/N analysis (approx. 5 g per sample) were further ground in a beater mill for 30 seconds (IKA® A11 basic).

Analysis of plant-available phosphate (P) and potassium (K)

The concentration of plant-available nutrients in the soil can be determined by extracting nutrients from soil samples with organic acids (ammonium lactate acetic acid) which are also exuded by plants (Schlichting et al. 1995).

I measured the concentration of extracted potassium by Atomic Absorption Spectroscopy (AAS). The extract is sprayed into a gas flame which dissociates the chemical compounds to atoms. Light of an element-specific wavelength is sent through the activated atoms with a hollow-cathode lamp and partly absorbed by the atoms. The attenuated light is
measured photometrically and the concentration of atoms of the respective element can be determined from the degree of light absorption (Schlichting et al. 1995).

Phosphate content of sample extracts was assessed via Flow Injection Analysis (FIA), in which the sample extract is injected into a continuously flowing carrier stream which mixes with a reagent through convection diffusion (Karlberg & Pacey 1989). The stream then passes a flow-through detector.

**Soil extracts**

5.0 g air-dried soil was weighed in 100 ml PE-bottles, and 100 ml extraction solution (9.01 g lactic acid, 18.75 g acetic acid and 7.75 g ammonium acetate) were added. The bottle was shaken to mix soil and solution and then placed onto a rotary shaker for 4 h. The content was then poured into fresh PE-bottles through a fluted filter (the first drops were discarded until the filtered solution was clear). The filtered extract was analysed with the AAS.

**Determination of phosphate content**

Calibration standards from 0.5 to 11 mg/L were prepared from potassium dihydrogen phosphate solved in ammonium lactate acetic acid solution. All solutions used in the analysis were degassed by membrane filtration. Soil extracts were injected into a carrier solution (5 g ammonium heptamolybdate and 17.5 ml concentrated sulfuric acid to which bi-distilled water was added up to 500 ml) in the FIA where phosphate from the extract reacted with ammonium molybdate to molybdophosphoric acid. Contact with a reagent (0.1 g Tin(II) chloride, 1 g hydrazinium sulfate and 17.5 ml concentrated sulfuric acid to which bi-distilled water was added up to 500 ml) led to the reduction of molybdophosphoric acid to phosphormolybdenum blue (Karlberg & Pacey 1989). The phosphate concentration in mg P/L extract was assessed by photometric detection of the blue complex at 509 or 690 nm. The following formula was used to calculate P concentrations in the soil samples:

\[
\text{soil P content [mg/100 g soil DM]} = \frac{a}{b} \times 10 \times \text{water factor}
\]

where

- \(a\) = mg P/L extract
- \(b\) = weight of air-dried soil sample
- water factor = \(\frac{100}{(100 - \text{soil water content [\% DM]})}\)

Four sample extracts with high phosphate concentrations were diluted 1:1 prior to analysis to achieve better accuracy. Determined soil phosphate concentrations were multiplied by factor 2 for these samples.
**Determination of potassium content**

Several standards which encompassed the concentration of the samples were used to calibrate the AAS. Prepared sample extract solutions were directly measured at a wavelength of 766.5 nm, which provided the K concentration in mg/L extract. K concentrations in the soil samples were calculated with the following formula:

\[
\text{soil K content [mg/100 g soil DM]} = [(a/b)*10]*\text{water factor}
\]

\[a = \text{mg K/L extract}\]
\[b = \text{weight of air-dried soil sample}\]
\[\text{water factor} = \frac{100}{(100 - \text{soil water content [% DM]})}\]

**Analysis of C and N content**

5-15 mg of ground soil samples were weighed into tin capsules and analysed for C and N content with an elemental analyser (EuroEA 3000, HEKAtech GmbH, Wegberg, Germany). The analysis is based on the principle of spontaneous dynamic combustion combined with the separation of compounds by gas-chromatography. Combustion of the soil sample at 1010 °C in an oxygen atmosphere releases the gaseous products NO\(_x\), CO\(_2\) and H\(_2\)O. The gas stream flows over a stationary phase of copper which binds the remaining O\(_2\) and reduces NO\(_x\) to N\(_2\) (Dunger & Fiedler 1997). The carrier gas helium further sweeps the gaseous compounds over magnesium perchlorate to bind H\(_2\)O and through the GC-column which separates N\(_2\) and CO\(_2\). A thermal conductivity detector then detects the gases exiting the GC-column. Measurements for C and N content are given in % and were multiplied with the water factor to obtain C and N content in % soil DM.

**II.3 Railway tracks and mini-sites**

Several sites were included which were not adequate for experimental manipulations due to size or substrate, but which were also considered potentially suitable for OSR growth. These sites were included to provide additional data on basic establishment success. Some sites were abandoned or infrequently used railway tracks (Fig. II.10). Other sites were classified as mini-sites with either stony substrate (paving stones, gravel) or soil as substrate. The first sowing in spring 2007 included five railway tracks and 32 mini-sites. Seeds of the cultivar Artus were sown into three replicate undisturbed plots of 23*23 cm. The minimum distance between plots was 0.5 cm. Many sites without successful establishment were excluded or replaced for the fall sowings of 2007 and 2008, but the number of replicates per site was raised to five
Appendix II

(two railway tracks and eight mini-sites sown in fall 2007, and five mini-sites sown in fall 2008). Unlike in the larger experimental sites, seeds were not mixed with sand prior to sowing but broadcast onto the bare soil. As establishment success was similar to establishment on the larger experimental sites, these data are only presented in the Appendix (see Appendix III.1.1).

Fig. II.10: Examples of mini-sites used for the spring sowing of 2007 (pictures in the middle show flowering OSR plants originating from the sowing).

II.4 Additional site conditions chapter 2

Table II.1: Additional variables describing site conditions on ruderal sites for the three sowing events of spring 2007, fall 2007 and fall 2008. Cover of competing vegetation at the first and second census of seedling emergence (VC1 and VC2), seedling herbivory assessed as the percentage of completely defoliated seedlings and external disturbance as the number of plots (out of five) with external disturbance on more than 30% of the plot area. Mean values ± SE are displayed, n = 60-84 (spring 2007, fall 2007) or n = 30-40 (2007, 2008). Significant differences between the sowings of spring and fall 07 or between the sowings of 2007 and 2008 according to U-tests or T-tests are denoted by means in columns followed by different letters (brackets indicate trends).

<table>
<thead>
<tr>
<th>sowing event</th>
<th>VC 1</th>
<th>VC 2</th>
<th>seedling herbivory</th>
<th>external disturbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>spring 2007</td>
<td>4.1a ± 0.4</td>
<td>5.2a ± 0.5</td>
<td>5.9a ± 1.7</td>
<td>1.4a ± 0.5</td>
</tr>
<tr>
<td>fall 2007</td>
<td>5.5b ± 0.4</td>
<td>6.3b ± 0.5</td>
<td>5.8a ± 1.7</td>
<td>1.3a ± 0.5</td>
</tr>
<tr>
<td>2007</td>
<td>6.8a ± 0.5</td>
<td>—</td>
<td>0.9a ± 0.5</td>
<td>1.4a ± 0.7</td>
</tr>
<tr>
<td>2008</td>
<td>3.9a ± 0.8</td>
<td>—</td>
<td>2.5a ± 2.5</td>
<td>0.9a ± 0.8</td>
</tr>
</tbody>
</table>

1vegetation cover on undisturbed plots

Means sharing the same letter are not statistically different according to Friedman test (spring vs. fall or 2007 vs. 2008).
Methods and site data

Table II.2: Additional variables describing site conditions on ruderal sites with high or low soil quality and high or low vegetation cover as well as agricultural sites (AF = agricultural field, G = grassland). Cover of competing vegetation at the first and second census of seedling emergence (VC1 and VC2), seedling herbivory assessed as the percentage of completely defoliated seedlings and external disturbance as the number of plots (out of five) with external disturbance on more of 30% of the plot area. Mean values ± SE are displayed, n = 7 (ruderal site types), n = 4 (G) or n = 6 (AF). Significant differences between site types according to U-tests or T-tests are denoted by asterisks (* p ≤ 0.05), asterisks in brackets signify trends ((*) p ≤ 0.10), n.s. = not significant. Vegetation was assessed as percentage cover in intervals of 10% (1 = 0-10%).

<table>
<thead>
<tr>
<th>site qual. veg.</th>
<th>VC 1</th>
<th>VC 2</th>
<th>seedling herbivory</th>
<th>external disturbance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ruderal sites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL low low</td>
<td>1.2 ± 0.1</td>
<td>2.1 ± 0.7</td>
<td>10.9 ± 6.2</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>LH low high</td>
<td>6.9 ± 0.5</td>
<td>9.2 ± 0.3</td>
<td>5.0 ± 2.2</td>
<td>1.1 ± 0.7</td>
</tr>
<tr>
<td>HL high low</td>
<td>2.3 ± 0.8</td>
<td>3.4 ± 1.0</td>
<td>26.3 ± 6.1</td>
<td>0.8 ± 0.7</td>
</tr>
<tr>
<td>HH high high</td>
<td>6.3 ± 0.7</td>
<td>8.1 ± 0.4</td>
<td>7.7 ± 3.3</td>
<td>1.3 ± 0.7</td>
</tr>
<tr>
<td><strong>agricultural sites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AF high low</td>
<td>1.7 ± 0.4</td>
<td>5.7 ± 1.1</td>
<td>26.4 ± 8.9</td>
<td>1.7 ± 1.1</td>
</tr>
<tr>
<td>G high high</td>
<td>8.9 ± 0.4</td>
<td>9.7 ± 0.1</td>
<td>21.2 ± 7.6</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td><strong>site comparisons</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>high vs. low qual.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>(*)</td>
<td>*</td>
</tr>
<tr>
<td>high vs. low veg.</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>agric. vs. rud.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>high VC</td>
<td>(*)</td>
<td>*</td>
<td>(*)</td>
<td>(*)</td>
</tr>
<tr>
<td>low VC</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

1 significant on high-quality sites
II.5 Climate data of the study period

Bremen is characterised by a North Atlantic climate with moderate changes in temperature, comparably wet summers and drier winter periods. Annual precipitation amounted to 725 mm in 2007-2009 (Fig. II.11), with a mean temperature of 10.2 °C (Fig. II.12), compared to a long-term average of 694 mm and 8.8 °C (DWD 2010). See the paragraphs below for descriptions of the weather during each experiment.

![Fig II.11: Cumulative precipitation (bars) per month over the experimental period of this thesis compared with the long-term average (line) from the period from 1961 to 1990 at Bremen Airport (DWD 2010).](image-url)
Precipitation in the study period was very variable, reaching from 2.2 mm in April 2007 to 127 mm in July of the same year. Months with extremely dry conditions were April 2007, May 2008 and December 2008. Other months showed a very high precipitation of more than 100 mm, namely May and July 2007, January 2008 as well as July and August 2008. Temperatures were nearly always at least as high as the long-term average mean (Fig. II.2). Several periods stand out in which temperatures were unusually high: spring and early summer of 2007, the winter months of 2007/2008, late spring and summer 2008 as well as spring 2009.
Figures II.13 to II.15 show the daily precipitation following the three sowing events. For the spring sowing of 2007 and the fall sowing of 2009, an extremely dry period ensued after the sowing phase, whereas rainfalls occurred directly after (and during) the sowing of 2007. The two weeks following the first substantial rainfalls after sowing showed a very high precipitation in spring 2007 (average precipitation of 5.5 mm per day from 6-19 May) and moderate precipitation levels in fall 2007 and 2008 (mean of 3.8 mm per day from 23 September until 6 October 2007 and a mean of 3.0 mm per day from 2-15 September).

**Fig. II.13:** Cumulative precipitation (bars) per day at Bremen Airport following the first sowing in spring 2007 (DWD 2010).
Fig. II.14: Cumulative precipitation (bars) per day at Bremen Airport following the second sowing in fall 2007 (DWD 2010).

Fig. II.15: Cumulative precipitation (bars) per day at Bremen Airport following the third sowing in fall 2008 (DWD 2010).
II.5.2 Chapter 3

During the period of this experiment (October 2007 – August 2008), temperatures were largely higher than the long-term average monthly means, with a particularly warm winter (monthly mean temperatures of 3.4 to 5.2 °C) and slightly warmer summer months with means of 16.7 to 18.4 °C (Fig. II.12). Temperatures in May 2008 were also by 2.6 °C higher than the long-term mean.

Precipitation varied considerably between months (Fig. II.11). Fall and winter months mostly ranged between 48 and 75 mm, with the exception of an unusually wet January 2008 with 110 mm. A particularly dry phase followed from April to June 2008, with a monthly maximum of 42 mm (June) and a nearly dry May. From July to August 2008, precipitation was much higher than the long-term mean with 122-124 mm.

II.5.3 Chapter 4

The experimental period (September 2008 – August 2009) was characterised by temperatures conforming largely to the long-term average monthly means (Fig. II.12), with mean monthly winter temperatures between 0.2 and 2.3 °C. A period of severe frost occurred in January (Fig. II.16). Spring and summer temperatures were slightly higher than the long-term mean (except June 2009), with an especially warm April 2009 (mean temperatures of 12.8 °C). My study fell into an overall very dry period: during the months of September and December 2008 and April and May 2009, precipitation lay far below the long-term average means (Fig. II.11), while July 2009 was the only month with an unusually high precipitation (117 mm).
**II.5.4 Chapter 5**

This experiment comprised two periods in which seeds were buried. Burial 1 lasted from late September 2007 until late June 2008. Temperatures in fall were similar to the long-term mean, but the winter months greatly exceeded it, by 4.4 °C in January and 3.2 °C in February 2008 (Fig. II.12 and II.17). The summer period was also slightly warmer than average. Precipitation was comparably high in fall and winter months and nearly always reached or exceeded the minimum long-term mean (Fig. II.11 and II.18). September and January were particularly wet, with a precipitation by 26.3 and 54.2 mm higher than the long-term mean. In contrast, May and June 2008 were unusually dry months, with only 10 mm of rainfall in May.

In the study period of burial 2 (mid-November 2008 until the middle of August 2009), temperatures were close to the long-term mean in winter (Fig. II.12, Fig. II.17) but largely higher than average in spring and summer, with a particularly warm April (12.8 °C) and a relatively hot August (18.7 °C). The period was further uncommonly dry: Precipitation mostly lay below the long-term mean and was particularly low in December.
Fig. II.17: Deviation of monthly mean temperature during burial 1 and burial 2 from the long-term monthly mean of the period from 1961 to 1990 at Bremen Airport (DWD 2010). Mean monthly temperatures over the two burial periods are given as text.

Fig. II.18: Deviation of monthly precipitation during burial 1 and burial 2 from the long-term monthly mean of the period from 1961 to 1990 at Bremen Airport (DWD 2010). Mean monthly precipitation over the two burial periods are given as text.
### II.6 List of censuses chapter 2

Table II.3: Censuses on which variables of establishment success were assessed.

<table>
<thead>
<tr>
<th>variables</th>
<th>spring 2007</th>
<th>fall 2007</th>
<th>fall 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>seedling establishment 1</td>
<td>19.-25.05.2007</td>
<td>19.09.-28.09.2007</td>
<td>20.-22.10.2008</td>
</tr>
<tr>
<td>seedling establishment 2</td>
<td>16.-22.06.2007</td>
<td>31.10.-13.11.2007</td>
<td>—</td>
</tr>
<tr>
<td>reproducing individuals 1</td>
<td>04.-09.05.2008</td>
<td>04.-09.05.2008</td>
<td>16.-17.05.2009</td>
</tr>
<tr>
<td>reproducing individuals 2</td>
<td>02.-07.06.2008</td>
<td>02.-07.06.2008</td>
<td>—</td>
</tr>
<tr>
<td>reproducing individuals 3</td>
<td>29.06.-01.07.2008</td>
<td>29.06.-01.07.2008</td>
<td>09.-10.07.2009</td>
</tr>
</tbody>
</table>

### II.7 Split-plot designs

Split-plot designs have originated from agricultural experiments and are a special form of randomised complete block design, where factors are applied to experimental units at different scales. If these scales are spatial, one or more factors are applied to whole plots (between-plot factors), which are replicated, and others vary only within the plots (within-plot factors) (Quinn & Keough 2007). Split-plot analyses use different error terms for the effect of between-plot and within-plot factors, as measurements made within plots are pseudoreplicates as far as the between-plot factors are concerned, but true replicates for the within-plot factors (Crawley 2005) (see II.7.1).

#### II.7.1 Chapter 2

For the sowing of spring 2007, sites represent the plots, to which the between-plot factors soil quality and vegetation cover were applied (28 sites \(\rightarrow\) 7 independent replicates for each combination of soil quality and vegetation cover, Fig. 2.1 in chapter 2). Cultivar and disturbance are within-plot factors, which were further replicated within each site in five blocks (replicated split-plot ANOVA, 28 sites*5 blocks \(\rightarrow\) 140 independent replicates for each combination of cultivar and disturbance). All data points can be used in split-plot ANOVA, as different error terms are applied for the between-plot and within-plot factors. Whenever I was restricted to one-factorial analyses, I used means per site for the effects of vegetation cover or soil quality (e.g. 14 independent replicates per soil quality status, pooled over vegetation cover), and all data points for the effects of disturbance and cultivar (e.g. 280 replicates per cultivar pooled over disturbance treatments).
II.7.2 Chapter 3

Containers (Fig. 3.1 in chapter 3) represent the plots. Substrate is the between-plots factor as each container was filled with one substrate only, and plant species is the within-plots factor, as all plant species were sown into each container.

II.7.3 Chapter 4

As in chapter 2, substrate is the between-plots factor and cultivar is the within-plots factor (Fig. 4.1 in chapter 4).

II.8 Site description chapters 3 and 4

The Siedenburg dump site in Bremen, Northern Germany, 53°7’21”N and 8°47’33”E (Fig. II.19), was used for depositing non-hazardous building rubble and excavation material from construction work until 1977. After this period, the rubble was covered with a 60 to 100 cm thick layer of various disturbed soils (Koehler & Müller 2003). The upper layer was bulldozed in 1980 (Koehler, H., pers. communication). Since 1979, the dump site has served as a site for ecological research, focusing on the documentation of a mostly undisturbed succession (Koehler & Müller 2003). Having largely been overgrown by bramble (*Rubus armeniacus*), it was bulldozed at the end of September 2007 to remove the vegetation layer, so that this experiment could be set up on the bare soil cover layer, which is a sandy loam. The experimental site is located on the plateau at an elevation of approximately 20 m above the surrounding ground level without access to ground water (Koehler & Müller 2003).
II.9 Censuses chapter 3

Several censuses were necessary to determine establishment success and reproductive potential (Table II.4). Censuses of reproducing individuals were conducted at different times for different species as the time to flowering varied greatly. Census duration varied also, as some species were so poorly developed that counting was very quick.

<table>
<thead>
<tr>
<th>variables</th>
<th>date of census</th>
</tr>
</thead>
<tbody>
<tr>
<td>seedling establishment</td>
<td></td>
</tr>
<tr>
<td>all species</td>
<td>14.-16.11.2007</td>
</tr>
<tr>
<td>all species</td>
<td>12.-14.12.2007</td>
</tr>
<tr>
<td>all species</td>
<td>22.-23.05.2008</td>
</tr>
<tr>
<td>flowering</td>
<td></td>
</tr>
<tr>
<td>B. napus, B. rapa, R. raph.</td>
<td>22.-25.05.2008</td>
</tr>
<tr>
<td>B. nigra</td>
<td>25.07.2008</td>
</tr>
<tr>
<td>fruiting</td>
<td></td>
</tr>
<tr>
<td>B. rapa</td>
<td>16.-18.07.2008</td>
</tr>
<tr>
<td>B. napus</td>
<td>15.-16.07.2008</td>
</tr>
<tr>
<td>R. raph.</td>
<td>28.07.2008</td>
</tr>
<tr>
<td>B. nigra</td>
<td>25.08.2008</td>
</tr>
</tbody>
</table>
II.10 Tetrazolium test for seed viability

One commonly used method of checking if a seed is viable and thus has the potential to germinate is by staining the seed with a 2,3,5-triphenyltetrazolium chloride solution. Respiration processes of living tissues involve dehydrogenase enzymes which release hydrogen atoms. Hydrogen reacts with tetrazolium molecules, which produces the red pigment formazan. The resulting colour differences in the embryo tissues then allow for a differentiation between normal, weak and dead tissues (Moore 1973). Details of the procedure (e.g. staining time and embryo evaluation criteria) depend on the type of seed under investigation. The tetrazolium tests in this thesis were performed according to a protocol specifically designed for seeds of *Brassica spp.* (Duffy et al. 2007).

II.10.1 Test protocol

- seeds presoaked for 16 hours in tap water at 10 °C
- removal of seed coats: crosswise incision of the testa at one of the outer cotyledons, then pressing the seed gently out of the seed coat
- staining for 5 hours at 30 °C in a 1% tetrazolium chloride solution
- evaluation via the maximum area of unstained, flaccid or necrotic tissue (Fig. II.20, Table II.5)

![Fig. II.20: Evaluation guide for viable (V) and non-viable (NV) *Brassica spp.* Seeds after tetrazolium staining (illustrations include, on the left side, the separated outer cotyledon), from Duffy et al. (2007). Figures correspond with descriptions in Table II.5 (V1 = 1. Viable).](image-url)
Table II.5: Evaluation criteria of seed viability after tetrazolium staining (Duffy et al. 2007).

<table>
<thead>
<tr>
<th>Viable seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. VIABLE: Completely stained seed of a normal red colour.</td>
</tr>
<tr>
<td>2. VIABLE: Upper 1/3 of the cotyledons unstained (if pervading).</td>
</tr>
<tr>
<td>3. VIABLE: Upper 1/2 of each cotyledon unstained (if superficial).</td>
</tr>
<tr>
<td>4. VIABLE: Minor unstained spots on lower ½ of each cotyledon in areas other than at junction of radicle-hypocotyl axis and cotyledons.</td>
</tr>
<tr>
<td>5. VIABLE: Not more than 1/3 of the extreme tip of radicle-hypocotyl axis unstained whether or not this extends into or through the conducting tissue.</td>
</tr>
<tr>
<td>6. VIABLE: Unstained area in the radicle-hypocotyl axis, not extending into the conducting tissue.</td>
</tr>
<tr>
<td>7. VIABLE: Extreme of the radicle stained dark red, not extended into the conducting tissue.</td>
</tr>
<tr>
<td>8. VIABLE: Other examples not described here as viable.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-viable seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>9. NON-VIABLE: Unstained area of more than 1/3 of each cotyledon (if pervading).</td>
</tr>
<tr>
<td>10. NON-VIABLE: Unstained area of more than 1/2 of each cotyledon (if superficial).</td>
</tr>
<tr>
<td>11. NON-VIABLE: Unstained area of cotyledons extending into the region where radicle-hypocotyl axis and cotyledons are attached (whether or not this includes the shoot meristem).</td>
</tr>
<tr>
<td>12. NON-VIABLE: Unstained area of radicle-hypocotyl axis extending into or through the conducting tissue.</td>
</tr>
<tr>
<td>13. NON-VIABLE: Unstained area involving more than 1/3 of extreme tip of radicle and extending into or through the conducting tissue.</td>
</tr>
<tr>
<td>14. NON-VIABLE: Completely green, brown or whitish yellow embryo.</td>
</tr>
<tr>
<td>15. NON-VIABLE: Seed completely unstained.</td>
</tr>
<tr>
<td>16. NON-VIABLE: Other examples not described here as non-viable.</td>
</tr>
</tbody>
</table>

II.11 Defaunation of soils

Soils which were defaunated were exposed to repeated freezing and thawing to kill the residing mesofauna, unless otherwise mentioned.

24 h at –20 °C

24 h at room temperature

48 h at –20 °C

II.12 Attempted resowing (substrate comparison chapter 4)

Winter mortality among plant rosettes was unexpectedly high, so I attempted to replace dead plants. I had anticipated some loss and set up an extra block to serve as a reserve from which plants could be taken to replace dead plants in the experimental blocks. Unfortunately, winter survival of these reserve plants was equally low so that a new sowing was preferred. Seeds were sown at each spot where a plant was missing on 24 March 2009. Containers were watered in the following drought period with 2 L per container every two to three days to ensure germination and seedling survival until 15 April, when seedlings were deemed strong
enough to survive under natural conditions. The drought period continued for another two weeks. The new seedlings were left out of the analysis as they developed poorly and did not flower.

II.13 Censuses chapter 4

Table II.6: Time schedule for assessment of variables in the substrate comparison and the simulated mowing experiment.

<table>
<thead>
<tr>
<th>variables</th>
<th>date of census</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>substrate comparison</td>
</tr>
<tr>
<td>seedling establishment</td>
<td>24.10.2008</td>
</tr>
<tr>
<td>pod production</td>
<td>14.-18.07. and 07.-11.09.2009*</td>
</tr>
<tr>
<td>height of seedlings</td>
<td>—</td>
</tr>
<tr>
<td>height of rosettes</td>
<td>—</td>
</tr>
<tr>
<td>height of bolting plants</td>
<td>—</td>
</tr>
<tr>
<td>height of flowering plants</td>
<td>25.05.2009</td>
</tr>
<tr>
<td>height of fruiting plants</td>
<td>—</td>
</tr>
</tbody>
</table>

* analysed as the maximum pod production over the two censuses

II.14 Location of experimental sites for chapter 5

Fig. II.21: Location of the experimental sites in used for seed burial in chapter 5 in Bremen and Niedersachsen.
II.15 Heat extraction of the mesofauna

Soil samples were kept at 15 °C for two days prior to extraction. The mesofauna was extracted from the soil with a heat extraction method modified after Macfadyen (1953). Soil cores (slightly broken up) were placed upside down onto sieves in plastic funnels. A gradient of heat and humidity drives the soil biota downwards through the funnel, below which they are caught in 70% ethanol. Samples were moistened at the beginning of the extraction and kept moist in the beginning by covering them with a lid. Temperatures were raised from 25 °C to 60 °C within 8 days, in steps of 5 °C every 24 h. Lids were removed at 40 °C. After the extraction, soil dry weight of each sample was assessed as a reference value for faunal densities.

II.16 Test for the effect of soaking on seed dormancy

Coating seeds with captan was done by soaking in aqueous captan solution for five minutes. Both the fungicide itself and the soaking procedure might have affected seed dormancy. I therefore included a test for the effect of soaking on seed persistence in the second burial. Seeds soaked in water for five minutes and then dried at room temperature were tested against untreated control seeds. I buried six minicontainer bars on site RL2, each equipped with one replicate (300 seeds in four containers, sealed with 20 µm gauze) per treatment. Seed persistence was approximately 88% and did not differ between soaked and control seeds (Mann-Whitney U-test, p = 0.818, n = 6).

II.17 Germination tests

Table II.22: Differences in test conditions for the two burials. WHC = water-holding capacity; TZ = tetrazolium test.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>test vessel</td>
<td>petri dish</td>
<td>plastic planting pot</td>
</tr>
<tr>
<td>soil content</td>
<td>6 mm high</td>
<td>179 g</td>
</tr>
<tr>
<td>WHC [%]</td>
<td>80%</td>
<td>70%</td>
</tr>
<tr>
<td>seed location</td>
<td>buried in soil</td>
<td>on soil surface</td>
</tr>
<tr>
<td>TZ test</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>visible fungal growth during test</td>
<td>yes</td>
<td>no</td>
</tr>
</tbody>
</table>
Appendix III: Results

III.1 Chapter 2

III.1.1 Establishment success on special sites

Establishment success on mini-sites and railway tracks was generally similar to experimental ruderal sites if plants grew on soil (see Table III.1). Sites with stony soil or railway tracks displayed very reduced to no chances for establishment.

Table III.1: Establishment and reproductive success of cultivar Artus on railway tracks and ruderal mini-sites with either stony substrate or soil for the three sowing events. Mean values per site (averaged over the 3-5 replicates) were used to calculate means of site types. Mean ± SE.

<table>
<thead>
<tr>
<th></th>
<th>spring 2007</th>
<th>fall 2007</th>
<th>fall 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>mean ± SE</td>
<td>n</td>
</tr>
<tr>
<td>seedling emergence [%]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>stony</td>
<td>9</td>
<td>2.28 ± 1.18</td>
<td>8</td>
</tr>
<tr>
<td>soil</td>
<td>23</td>
<td>10.43 ± 2.89</td>
<td>11</td>
</tr>
<tr>
<td>railway track</td>
<td>5</td>
<td>0.33 ± 0.26</td>
<td>2</td>
</tr>
<tr>
<td>flowering individuals [%]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>stony</td>
<td>9</td>
<td>0.00 ± 0.00</td>
<td>8</td>
</tr>
<tr>
<td>soil</td>
<td>23</td>
<td>0.13 ± 0.10</td>
<td>12</td>
</tr>
<tr>
<td>railway track</td>
<td>5</td>
<td>0.00 ± 0.00</td>
<td>2</td>
</tr>
<tr>
<td>fruiting individuals [%]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>stony</td>
<td>9</td>
<td>0.00 ± 0.00</td>
<td>8</td>
</tr>
<tr>
<td>soil</td>
<td>23</td>
<td>0.10 ± 0.06</td>
<td>12</td>
</tr>
<tr>
<td>railway track</td>
<td>5</td>
<td>0.00 ± 0.00</td>
<td>2</td>
</tr>
</tbody>
</table>
**III.1.2 Establishment success on ruderal sites**

**Fig. III.1**: Flowering individuals [% of seeds sown] for the three sowing events, spring 2007 (plot a)), fall 2007 (plot b)) and fall 2008 (plot c)). Note that flowering individuals are presented on a log-scaled y-axis. Data of the spring sowing include the cultivars Artus and Smart sown on low-quality vs. high-quality sites with low or high vegetation cover and disturbed (D) or undisturbed (U) plots. Data of the fall sowings comprise cultivar Artus only, and sites were no longer divided into low and high vegetation cover sites. For the sowing of fall 2008, sites were also not divided into low- and high-quality soils. Mean ± SE, n = 35 (spring 07), n = 45 (fall 07) and n = 30 (fall 08).

**Fig. III.2**: Relationship between the density of seeds sown per 0.05 m² and the number of flowering individuals. Results refer to cultivar Artus. Each data point represents the mean of three plots on the respective site (RT = railway track, AF = agricultural field, RH = ruderal high-quality site, RL = ruderal low-quality site), n = 28.
Fig. III.3: Seedling emergence of seeds of cultivar Artus sown into disturbed (D) or undisturbed (U) plots on the same seven low-quality and five high-quality sites (five blocks each) in spring 2007 and fall 2007. Mean ± SE, n = 25 and 35.

Fig. III.4: Seedling emergence of seeds of cultivar Artus sown into disturbed (D) or undisturbed (U) plots on the same six sites (five blocks each) in fall 2007 and fall 2008. Mean ± SE, n = 30.

Fig. III.5: Flowering individuals [%] of cultivar Artus in disturbed (D) or undisturbed (U) plots on the same seven low-quality and five high-quality sites (five blocks each) in spring 2007 and fall 2007. Mean ± SE, n = 25 and 35.

Fig. III.6: Flowering individuals [%] of cultivar Artus in disturbed (D) or undisturbed (U) plots on the same six sites (five blocks each) in fall 2007 and fall 2008. Mean ± SE, n = 30.
II.1.3 Summary of results

Table III.2: Summary of results for the differences between agricultural and ruderal environments. My hypothesis was that establishment success would be higher on agricultural sites. Results refer to both cultivars, Artus and Smart. √ = hypothesis confirmed; n.s. = no significant results; ! = the opposite result was true. Results are generally displayed for the complete dataset including externally disturbed plots. Round brackets ( ) denote trends, square brackets [ ] give the result of analyses based on the dataset in which externally disturbed plots were excluded (shown only if the result differed from the result given by analysis of the complete dataset).

<table>
<thead>
<tr>
<th>variable</th>
<th>all sites</th>
<th>high vegetation cover</th>
<th>low vegetation cover</th>
</tr>
</thead>
<tbody>
<tr>
<td>seedling emergence</td>
<td>n.s.</td>
<td>n.s. [!]</td>
<td>n.s.</td>
</tr>
<tr>
<td>flowering individuals [%]</td>
<td>!</td>
<td>(1)</td>
<td>(1)</td>
</tr>
<tr>
<td>fruiting individuals [%]</td>
<td>(1) [n.s.]</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>
Table III.3: Summary of results of the effects of the factors soil quality, vegetation cover, disturbance and cultivar on establishment success of oilseed rape for the three sowing events. Results refer to both cultivars, Artus & Smart (spring 2007) or cultivar Artus only (fall 2007 & fall 2008). See Table III.2 for explanations of the characters displayed. Veg. cov. = vegetation cover.

<table>
<thead>
<tr>
<th>variable</th>
<th>sowing event</th>
<th>high-quality &gt; low-quality?</th>
<th>low veg. cov. &gt; high veg. cov.?</th>
<th>disturbed &gt; undisturbed?</th>
<th>Smart &gt; Artus?</th>
</tr>
</thead>
<tbody>
<tr>
<td>seedling emergence</td>
<td>spring 2007</td>
<td>✓</td>
<td>n.s.</td>
<td>✓</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>fall 2007</td>
<td>n.s.</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>fall 2008</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>flowering</td>
<td>spring 2007</td>
<td>n.s.</td>
<td>n.s.</td>
<td>✓ [n.s.]</td>
<td>n.s.</td>
</tr>
<tr>
<td>individuals [%]</td>
<td>fall 2007</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>fall 2008</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>fructifying</td>
<td>spring 2007</td>
<td>n.s.</td>
<td>(1) [n.s.]</td>
<td>(✓) [n.s.]</td>
<td>n.s.</td>
</tr>
<tr>
<td>individuals [%]</td>
<td>fall 2007</td>
<td>—</td>
<td>—</td>
<td>✓</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>fall 2008</td>
<td>—</td>
<td>—</td>
<td>✓</td>
<td>—</td>
</tr>
<tr>
<td>pods produced</td>
<td>spring 2007</td>
<td>✓</td>
<td>n.s.</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>per plant</td>
<td>fall 2007</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>fall 2008</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
Table III.4: Summary of results for the effects of fertilisation and manipulated seed density on cultivar Artus. See Table III.2 for explanations of the characters displayed.

<table>
<thead>
<tr>
<th>variable</th>
<th>fertilised &gt; unfertilised?</th>
<th>higher establishment at higher seed density?</th>
</tr>
</thead>
<tbody>
<tr>
<td>seedling emergence</td>
<td>n.s.</td>
<td>☑</td>
</tr>
<tr>
<td>flowering individuals [%]</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>fruiting individuals [%]</td>
<td>☑</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Table III.5: Summary of results for the differences between the sowing events (spring versus fall 2007 and fall 2007 versus fall 2008) for cultivar Artus. See Table III.2 for explanations of the characters displayed.

<table>
<thead>
<tr>
<th>variable</th>
<th>spring 07 &lt; fall 07 ?</th>
<th>fall 07 &gt; fall 08?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>all sites</td>
<td>disturbed</td>
</tr>
<tr>
<td>seedling emergence</td>
<td>!</td>
<td>!</td>
</tr>
<tr>
<td>flowering individuals [%]</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>fruiting individuals [%]</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>
III.2 Chapter 3

### III.2.1 Plant species differences

Table III.6: Plant species effects on variables of plant establishment and reproductive potential tested with one-way analyses for each substrate separately. Degrees of freedom (d.f.), sample sizes per factor level (n), significance levels (p) and F values (F) of one-way blocked ANOVAs or Welch’s ANOVAs are given for the plant species effect (d.f. = 2-3). Significant effects (p ≤ 0.05) are shown in bold type.

<table>
<thead>
<tr>
<th></th>
<th>shallow</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f.</td>
<td>n</td>
<td>p</td>
<td>F</td>
<td>d.f.</td>
<td>n</td>
<td>p</td>
<td>F</td>
</tr>
<tr>
<td>seedling emergence [%]</td>
<td>3</td>
<td>20</td>
<td>&lt;0.001</td>
<td>322.72</td>
<td>2c</td>
<td>3</td>
<td>20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>flowering individuals [%]</td>
<td>3</td>
<td>20</td>
<td>&lt;0.001</td>
<td>453.51</td>
<td>2c</td>
<td>3</td>
<td>20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>viability of seeds</td>
<td>2</td>
<td>5-6</td>
<td>&lt;0.001</td>
<td>27.85</td>
<td>2c</td>
<td>3</td>
<td>18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>F1 seedlings per seed sown</td>
<td>3</td>
<td>18</td>
<td>&lt;0.001</td>
<td>48.03</td>
<td>3a</td>
<td>18</td>
<td>&lt;0.001</td>
<td>70.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 log-transformed for analysis  
2 arcsine-square-root-transformed for analysis  
3 rank-transformed for analysis

![Fig. III.9: Seedling emergence of B. napus & B. rapa (cultivated species) vs. B. nigra & R. raphanistrum (wild species) on the four substrates. sh = shallow humous soil; h = humous soil; m = mixed soil; s = sand. Different letters indicate significant differences between plant species on the respective substrate but do not signify substrate differences (Table III.6, Tukey or Tamhane post-hoc tests). Back-transformed means ± 95 % CI (confidence interval). Mean ± SE, n = 20](image-url)
Fig. III.10: Percentage of flowering individuals per seed sown for *B. napus* & *B. rapa* (cultivated species) vs. *B. nigra* & *R. raphanistrum* (wild species) on the four substrates. sh = shallow humous soil; h = humous soil; m = mixed soil; s = sand. Different letters indicate significant differences between plant species on the respective substrate but do not signify substrate differences (Table III.6, Tukey or Tamhane post-hoc tests). Back-transformed means ± 95 % CI (confidence interval). Mean ± SE, n = 20

Fig. III.11: Viability of seeds produced by *B. napus* & *B. rapa* (cultivated species) vs. *B. nigra* on the four substrates. Viability could not be assessed for *R. raphanistrum*. sh = shallow humous soil; h = humous soil; m = mixed soil; s = sand. Different letters indicate significant differences between plant species on the respective substrate but do not signify substrate differences (Table III.6, Tukey or Tamhane post-hoc tests). Back-transformed means ± 95 % CI (confidence interval). Mean ± SE, n = 5-6
Fig. III.12: Estimated number of F1 seedlings per seed sown for B. napus & B. rapa (cultivated species) vs. B. nigra (wild species) on the four substrates. No estimate of R. raphanistrum was made as seed viability could not be assessed. sh = shallow humous soil; h = humous soil; m = mixed soil; s = sand. Different letters indicate significant differences between plant species on the respective substrate but do not signify substrate differences (Table III.6, Tamhane post-hoc tests). Original values are shown rather than back-transformed ones as different transformations were used for the analysis on different substrates. Mean ± SE, n = 18

### III.2.2 Substrate effects

Table III.7: Substrate effects on variables of establishment success and reproductive potential tested with one-way analyses for each plant species. Degrees of freedom (d.f.), sample sizes per factor level (n), significance levels (p) and F values (F) of one-way blocked ANOVAs, Welch’s ANOVAs or Kruskal-Wallis H-test are given for the substrate effect (d.f. = 3). Significant effects (p ≤ 0.05) are shown in bold type.

<table>
<thead>
<tr>
<th>substrate effect for each plant species</th>
<th>d.f.</th>
<th>n</th>
<th>B. napus</th>
<th>B. rapa</th>
<th>B. nigra</th>
<th>R. raph.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>p</td>
<td>F</td>
<td>p</td>
<td>F</td>
</tr>
<tr>
<td>seedling emergence [%]</td>
<td>3</td>
<td>20</td>
<td>0.021</td>
<td>3.45 $^{2d}$</td>
<td>$&lt;0.001$</td>
<td>16.15 $^{2c}$</td>
</tr>
<tr>
<td>flowering individuals [%]</td>
<td>3</td>
<td>20</td>
<td>0.053</td>
<td>2.67 $^{2d}$</td>
<td>$&lt;0.001$</td>
<td>6.19 $^{2d}$</td>
</tr>
<tr>
<td>viability of seeds</td>
<td>3</td>
<td>5-6</td>
<td>0.580</td>
<td>0.67 $^{2d}$</td>
<td>$&lt;0.001$</td>
<td>18.97 $^{2d}$</td>
</tr>
<tr>
<td>F1 seedlings per seed sown</td>
<td>3</td>
<td>18</td>
<td>0.002</td>
<td>5.35 $^{1d}$</td>
<td>$&lt;0.001$</td>
<td>16.40 $^{1d}$</td>
</tr>
</tbody>
</table>

1 log-transformed for analysis
2 arcsine-square-root-transformed for analysis
3 rank-transformed for analysis
a Welch's ANOVA
b Kruskal-Wallis H-test
c blocked ANOVA
d unblocked ANOVA
Table III.8: Variables of plant establishment and reproductive potential as percentages of seeds sown for the four different species on the four different substrates. Back-transformed means in bold and 95 % CI (confidence interval) in brackets. F1 seedlings are shown as original means ± SE since no transformation was used for analysis. Different letters indicate significant differences between substrates for the respective plant (Table III.7, Tukey post-hoc test, p < 0.05) but do not signify plant effects. Flowering individuals of *R. raphanistrum* were analysed with a Kruskal-Wallis H-test followed by U-tests (n.s. after Bonferroni correction). n = 5-20 (see Table III.7).

<table>
<thead>
<tr>
<th>B. napus</th>
<th>B. rapa</th>
<th>B. nigra</th>
<th>R. raph.</th>
</tr>
</thead>
<tbody>
<tr>
<td>seedling emergence [%]</td>
<td>shallow</td>
<td>81.2&lt;sup&gt;a&lt;/sup&gt; (78.8-83.5)</td>
<td>70.1&lt;sup&gt;a&lt;/sup&gt; (67.1-73.0)</td>
</tr>
<tr>
<td></td>
<td>humous</td>
<td>79.0&lt;sup&gt;a&lt;/sup&gt; (76.0-81.9)</td>
<td>69.4&lt;sup&gt;a&lt;/sup&gt; (66.4-72.4)</td>
</tr>
<tr>
<td></td>
<td>mixed</td>
<td>83.0&lt;sup&gt;ab&lt;/sup&gt; (80.0-85.8)</td>
<td>66.6&lt;sup&gt;a&lt;/sup&gt; (61.3-71.6)</td>
</tr>
<tr>
<td></td>
<td>sand</td>
<td>84.8&lt;sup&gt;b&lt;/sup&gt; (81.9-87.4)</td>
<td>56.0&lt;sup&gt;b&lt;/sup&gt; (51.8-60.2)</td>
</tr>
<tr>
<td>flowering individuals [%]</td>
<td>shallow</td>
<td>62.6&lt;sup&gt;a&lt;/sup&gt; (58.6-66.4)</td>
<td>55.5&lt;sup&gt;a&lt;/sup&gt; (51.6-59.5)</td>
</tr>
<tr>
<td></td>
<td>humous</td>
<td>61.5&lt;sup&gt;a&lt;/sup&gt; (57.8-65.0)</td>
<td>57.2&lt;sup&gt;a&lt;/sup&gt; (53.6-60.8)</td>
</tr>
<tr>
<td></td>
<td>mixed</td>
<td>67.9&lt;sup&gt;a&lt;/sup&gt; (63.0-72.7)</td>
<td>54.4&lt;sup&gt;a&lt;/sup&gt; (50.6-58.3)</td>
</tr>
<tr>
<td></td>
<td>sand</td>
<td>66.0&lt;sup&gt;a&lt;/sup&gt; (63.4-68.6)</td>
<td>46.3&lt;sup&gt;b&lt;/sup&gt; (41.6-51.1)</td>
</tr>
<tr>
<td>viability of produced seeds [%]</td>
<td>shallow</td>
<td>96.1&lt;sup&gt;a&lt;/sup&gt; (89.6-99.5)</td>
<td>94.0&lt;sup&gt;ab&lt;/sup&gt; (88.2-97.9)</td>
</tr>
<tr>
<td></td>
<td>humous</td>
<td>96.3&lt;sup&gt;a&lt;/sup&gt; (93.9-98.2)</td>
<td>92.7&lt;sup&gt;a&lt;/sup&gt; (87.1-96.8)</td>
</tr>
<tr>
<td></td>
<td>mixed</td>
<td>98.5&lt;sup&gt;a&lt;/sup&gt; (94.6-100.0)</td>
<td>98.1&lt;sup&gt;b&lt;/sup&gt; (95.4-99.6)</td>
</tr>
<tr>
<td></td>
<td>sand</td>
<td>97.3&lt;sup&gt;a&lt;/sup&gt; (93.1-99.6)</td>
<td>79.7&lt;sup&gt;c&lt;/sup&gt; (75.3-83.9)</td>
</tr>
<tr>
<td>F1 seedlings per seed sown</td>
<td>shallow</td>
<td>31.2&lt;sup&gt;a&lt;/sup&gt; ± 8.3</td>
<td>81.2&lt;sup&gt;a&lt;/sup&gt; ± 16.0</td>
</tr>
<tr>
<td></td>
<td>humous</td>
<td>73.8&lt;sup&gt;a&lt;/sup&gt; ± 19.0</td>
<td>279.7&lt;sup&gt;a&lt;/sup&gt; ± 26.3</td>
</tr>
<tr>
<td></td>
<td>mixed</td>
<td>71.6&lt;sup&gt;a&lt;/sup&gt; ± 10.9</td>
<td>266.3&lt;sup&gt;b&lt;/sup&gt; ± 41.6</td>
</tr>
<tr>
<td></td>
<td>sand</td>
<td>30.5&lt;sup&gt;a&lt;/sup&gt; ± 5.4</td>
<td>148.6&lt;sup&gt;a&lt;/sup&gt; ± 35.6</td>
</tr>
</tbody>
</table>

Fig. III.13: Estimated number of seeds produced per plant by *B. napus* & *B. rapa* (cultivated species) vs. *B. nigra* (wild species) on the four substrates. Original values are shown, which differ greatly from the back-transformed means displayed in the results section (Table 3.9 in chapter 3.3.3) as a result of a few strong outliers in *B. nigra* and *R. raphanistrum*. Mean ± SE, n = 6-18
II.2.3 Summary of results

Table III.9: Summary of results of the comparison between cultivated and weedy species concerning the hypothesis that cultivated species perform worse than weedy species on low-quality ruderal soils. ✓ = hypothesis confirmed; n.s. = no significant results; ! = the opposite result was true. All parameters of establishment success and reproductive potential are first displayed for the means over all substrates and then for the individual substrates (sh = shallow humous soil; h = humous soil; m = mixed soil; s = sand).

<table>
<thead>
<tr>
<th></th>
<th>B. napus cultivated &lt; wild?</th>
<th>B. rapa cultivated &lt; R. raph?</th>
<th>B. napus cultivated vs. cultivated</th>
<th>B. napus B. rapa cultivated &lt; wild?</th>
</tr>
</thead>
<tbody>
<tr>
<td>seedling emergence</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>!</td>
</tr>
<tr>
<td>sh</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>!</td>
</tr>
<tr>
<td>h</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>!</td>
</tr>
<tr>
<td>m</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>!</td>
</tr>
<tr>
<td>s</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>!</td>
</tr>
<tr>
<td>flowering individuals [%]</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>!</td>
</tr>
<tr>
<td>sh</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>!</td>
</tr>
<tr>
<td>h</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>!</td>
</tr>
<tr>
<td>m</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>!</td>
</tr>
<tr>
<td>s</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>!</td>
</tr>
<tr>
<td>fruiting individuals [%]</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>✓</td>
</tr>
<tr>
<td>sh</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>✓</td>
</tr>
<tr>
<td>h</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>✓</td>
</tr>
<tr>
<td>m</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>✓</td>
</tr>
<tr>
<td>s</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>✓</td>
</tr>
<tr>
<td>seeds produced per plant</td>
<td>n.s.</td>
<td>n.s.</td>
<td>!</td>
<td>✓</td>
</tr>
<tr>
<td>sh</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>h</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>✓</td>
</tr>
<tr>
<td>m</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>✓</td>
</tr>
<tr>
<td>s</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>✓</td>
</tr>
<tr>
<td>seeds produced per seed sown</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>✓</td>
</tr>
<tr>
<td>sh</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>✓</td>
</tr>
<tr>
<td>h</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>✓</td>
</tr>
<tr>
<td>m</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>✓</td>
</tr>
<tr>
<td>s</td>
<td>n.s.</td>
<td>!</td>
<td>!</td>
<td>✓</td>
</tr>
<tr>
<td>seed viability [%]</td>
<td>n.s.</td>
<td>n.s.</td>
<td>!</td>
<td>!</td>
</tr>
<tr>
<td>sh</td>
<td>n.s.</td>
<td>!</td>
<td>n.s.</td>
<td>!</td>
</tr>
<tr>
<td>h</td>
<td>n.s.</td>
<td>!</td>
<td>n.s.</td>
<td>!</td>
</tr>
<tr>
<td>m</td>
<td>n.s.</td>
<td>!</td>
<td>n.s.</td>
<td>!</td>
</tr>
<tr>
<td>s</td>
<td>n.s.</td>
<td>✓</td>
<td>!</td>
<td>!</td>
</tr>
<tr>
<td>F1 seedlings per seed sown</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>!</td>
</tr>
<tr>
<td>sh</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>!</td>
</tr>
<tr>
<td>h</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>!</td>
</tr>
<tr>
<td>m</td>
<td>!</td>
<td>!</td>
<td>!</td>
<td>!</td>
</tr>
<tr>
<td>s</td>
<td>n.s.</td>
<td>!</td>
<td>!</td>
<td>!</td>
</tr>
</tbody>
</table>
Table III.10: Summary of results of the substrate effect concerning the hypothesis that performance on low-quality soils (sand, mixed) is reduced. ✓ = hypothesis confirmed; n.s. = no significant results; ! = the opposite result was true. SOM = soil organic matter content, WHC$_{\text{max}}$ = maximum water-holding capacity.

<table>
<thead>
<tr>
<th>variable</th>
<th>effect</th>
<th>negative effects of low SOM / WHC$_{\text{max}}$?</th>
<th>B. napus</th>
<th>B. rapa</th>
<th>B. nigra</th>
<th>R. raph</th>
</tr>
</thead>
<tbody>
<tr>
<td>seedling emergence</td>
<td>sand &lt; mixed?</td>
<td>n.s.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>sand &lt; humous?</td>
<td><img src="https://example.com" alt="✓" /></td>
<td><img src="https://example.com" alt="✓" /></td>
<td>✓</td>
<td><img src="https://example.com" alt="✓" /></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mixed &lt; humous?</td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td></td>
</tr>
<tr>
<td>flowering individuals [%]</td>
<td>sand &lt; mixed?</td>
<td>n.s.</td>
<td><img src="https://example.com" alt="✓" /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td></td>
</tr>
<tr>
<td></td>
<td>sand &lt; humous?</td>
<td><img src="https://example.com" alt="✓" /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mixed &lt; humous?</td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fruiting individuals [%]</td>
<td>sand &lt; mixed?</td>
<td>n.s.</td>
<td><img src="https://example.com" alt="✓" /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td></td>
</tr>
<tr>
<td></td>
<td>sand &lt; humous?</td>
<td><img src="https://example.com" alt="✓" /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mixed &lt; humous?</td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>seeds produced per plant</td>
<td>sand &lt; mixed?</td>
<td>n.s.</td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td></td>
</tr>
<tr>
<td></td>
<td>sand &lt; humous?</td>
<td><img src="https://example.com" alt="✓" /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mixed &lt; humous?</td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>seeds produced per seed sown</td>
<td>sand &lt; mixed?</td>
<td><img src="https://example.com" alt="✓" /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td></td>
</tr>
<tr>
<td></td>
<td>sand &lt; humous?</td>
<td><img src="https://example.com" alt="✓" /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mixed &lt; humous?</td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>seed viability [%]</td>
<td>sand &lt; mixed?</td>
<td>n.s.</td>
<td><img src="https://example.com" alt="✓" /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>sand &lt; humous?</td>
<td><img src="https://example.com" alt="✓" /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mixed &lt; humous?</td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1 seedlings per seed sown</td>
<td>sand &lt; mixed?</td>
<td><img src="https://example.com" alt="✓" /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>sand &lt; humous?</td>
<td><img src="https://example.com" alt="✓" /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mixed &lt; humous?</td>
<td><img src="https://example.com" alt="n.s." /></td>
<td><img src="https://example.com" alt="n.s." /></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table III.11: Summary of results of the substrate effect concerning the hypothesis that performance on low-quality soils (shallow soil) is reduced. ✓ = hypothesis confirmed; n.s. = no significant results; ! = the opposite result was true.

<table>
<thead>
<tr>
<th>variable</th>
<th>effect</th>
<th>B. napus</th>
<th>B. rapa</th>
<th>B. nigra</th>
<th>R. raph</th>
</tr>
</thead>
<tbody>
<tr>
<td>seedling emergence</td>
<td>shallow &lt; sand ?</td>
<td>n.s.</td>
<td>!</td>
<td>!</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>shallow &lt; mixed?</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>shallow &lt; humous ?</td>
<td>n.s.</td>
<td>n.s.</td>
<td>!</td>
<td>n.s.</td>
</tr>
<tr>
<td>flowering individuals [%]</td>
<td>shallow &lt; sand ?</td>
<td>n.s.</td>
<td>!</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>shallow &lt; mixed?</td>
<td>n.s.</td>
<td>n.s.</td>
<td>✓</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>shallow &lt; humous ?</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>fruiting individuals [%]</td>
<td>shallow &lt; sand ?</td>
<td>n.s.</td>
<td>!</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>shallow &lt; mixed?</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>shallow &lt; humous ?</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>seeds produced per plant</td>
<td>shallow &lt; sand ?</td>
<td>n.s.</td>
<td>✓</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>shallow &lt; mixed?</td>
<td>n.s.</td>
<td>✓</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>shallow &lt; humous ?</td>
<td>n.s.</td>
<td>✓</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>seeds produced per seed sown</td>
<td>shallow &lt; sand ?</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>shallow &lt; mixed?</td>
<td>✓</td>
<td>✓</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>shallow &lt; humous ?</td>
<td>✓</td>
<td>✓</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>seed viability [%]</td>
<td>shallow &lt; sand ?</td>
<td>n.s.</td>
<td>!</td>
<td>✓</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>shallow &lt; mixed?</td>
<td>n.s.</td>
<td>n.s.</td>
<td>✓</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>shallow &lt; humous ?</td>
<td>n.s.</td>
<td>n.s.</td>
<td>✓</td>
<td>n.s.</td>
</tr>
<tr>
<td>F1 seedlings per seed sown</td>
<td>shallow &lt; sand ?</td>
<td>n.s.</td>
<td>n.s.</td>
<td>✓</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>shallow &lt; mixed?</td>
<td>✓</td>
<td>✓</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>shallow &lt; humous ?</td>
<td>n.s.</td>
<td>✓</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>
Results

III.3 Chapter 4

III.3.1 Substrate comparison

**Table III.12**: Viability of seeds [%] produced by the tall cultivar Artus and the semi-dwarf hybrid PR45D03 in two experiments. The different treatments in the substrate comparison included humous, mixed and shallow humous soil (n = 5-6), and the mowing experiment compared unmown control plots with fall- and spring-mown plots (n = 6). Means (in bold) back-transformed from arcsine-square-root-transformed data with 95% CI (confidence interval) in brackets.

<table>
<thead>
<tr>
<th>experiment</th>
<th>treatment</th>
<th>Artus (%)</th>
<th>PR45D03 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>substrate comparison</td>
<td>humous</td>
<td>98.8 (95.5-100.0)</td>
<td>97.5 (96.0-98.6)</td>
</tr>
<tr>
<td></td>
<td>mixed</td>
<td>95.6 (88.7-99.4)</td>
<td>95.9 (92.8-98.2)</td>
</tr>
<tr>
<td></td>
<td>shallow</td>
<td>94.7 (91.5-97.2)</td>
<td>96.6 (95.5-97.5)</td>
</tr>
<tr>
<td>mowing experiment</td>
<td>control</td>
<td>96.4 (94.0-98.2)</td>
<td>98.1 (95.1-99.7)</td>
</tr>
<tr>
<td></td>
<td>fall</td>
<td>97.5 (93.8-99.6)</td>
<td>94.2 (91.0-96.8)</td>
</tr>
<tr>
<td></td>
<td>spring</td>
<td>98.4 (97.8-99.0)</td>
<td>96.6 (91.1-99.5)</td>
</tr>
</tbody>
</table>

**Table III.13**: Effect of cultivar and treatment on the viability of seeds [%] produced by cultivar Artus and the semi-dwarf hybrid PR45D03 in the two experiments. Significance levels (p) and variance ratios (F) are displayed. The treatment term represents substrate effects for the substrate comparison and mowing effects for the mowing experiment. Data were arcsine-square-root-transformed for analysis. A two-factorial ANOVA was performed for the mowing experiment, but factors of the substrate comparison had to be analysed in separate one-way analyses (ANOVA and Welch’s ANOVA). Significant effects (p ≤ 0.05) are shown in bold type. In spite of the significant substrate effect, Tukey post-hoc analyses revealed no significant difference between substrates, and analyses of substrate effects separately for each cultivar showed no significant effect.

<table>
<thead>
<tr>
<th>effect</th>
<th>substrate comparison</th>
<th>mowing experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f.</td>
<td>F</td>
</tr>
<tr>
<td>treatment</td>
<td>2</td>
<td>3.700</td>
</tr>
<tr>
<td>cultivar</td>
<td>1</td>
<td>0.001</td>
</tr>
<tr>
<td>cultivar*treatment</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

a two-factorial ANOVA, error d.f. = 30, total d.f. = 35
b one-factorial ANOVA, error d.f. = 31, total d.f. = 33
c Welch’s ANOVA, d.f. 2 = 22.070
Fig. III.14: Winter mortality for the cultivars Artus and PR45D03 on the different substrates. Means ± SE, back-transformed from arcsine-square-root-transformed data, n = 12. Cultivars differed significantly in winter mortality, see Table III.14.

Table III.14: Effect of cultivar and treatment on winter mortality [%] of cultivar Artus and the semi-dwarf hybrid PR45D03 in the two experiments. Number of replicates per treatment (n), degrees of freedom (d.f.) and significance levels (p) are displayed. The treatment term represents substrate effects for the substrate comparison and mowing effects for the mowing experiment. Data were arcsine-square-root-transfomed for analysis. Significant effects (p ≤ 0.05) are shown in bold type.

<table>
<thead>
<tr>
<th>effect</th>
<th>substrate comparison</th>
<th>mowing experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>treatment</td>
<td>n</td>
<td>d.f.</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>cultivar</td>
<td>36</td>
<td>1</td>
</tr>
</tbody>
</table>

a Kruskal-Wallis H-test
b Mann-Whitney U-test

Fig. III.15: Height of flowering plants of the tall cultivar Artus and the semi-dwarf hybrid PR45D03 on humous, mixed and shallow humous substrates for the unmown control treatment. Means ± SE, n = 6-12. Effect of cultivar and substrate were not significant in a two-way split-plot ANOVA with substrate (d.f. = 2) as between-plots and cultivar (d.f. = 1) as within-plots factor (interaction also n.s.), total d.f. = 57.
### III.3.2 Mowing simulation

**Table III.15**: Mowing effects on variables of reproductive potential for Artus and the semi-dwarf hybrid PR45D03, analysed separately for each cultivar. Significance levels (p) and variance ratios (F) of one-way ANOVAs are displayed for the mowing effect (d.f. = 2). Fruiting individuals were analysed as the number of fruiting individuals out of four plants in a generalized linear mixed model with quasi-binomial distribution and logit link function (dispersion parameter estimated). F stands for Wald F statistic in this case. Denominator d.f. were 14 for Artus and 21 for PR45D03. n = 24 (fruited individuals); n = 20-24 (seeds per plant) and n = 23-24 (seeds per plot). Significant effects (p ≤ 0.05) are shown in bold type.

<table>
<thead>
<tr>
<th></th>
<th>Artus</th>
<th>PR45D03</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f.</td>
<td>p</td>
</tr>
<tr>
<td>fruiting individuals</td>
<td>2</td>
<td>0.006</td>
</tr>
<tr>
<td>seeds per plant</td>
<td>2</td>
<td>0.260</td>
</tr>
<tr>
<td>seeds per plot</td>
<td>2</td>
<td>0.015</td>
</tr>
</tbody>
</table>

1 square-root-transformed for analysis
2 unblocked ANOVA
a Generalized linear mixed model
b unblocked ANOVA
**Table III.16**: Significance of mowing effects on variables of reproductive potential separately for each cultivar * = significant mowing effect according to oneway analysis (Table III.15) and post-hoc tests (p ≤ 0.05); n.s. = not significant; ctr = unmown control plots; spring/fall = mown plots

<table>
<thead>
<tr>
<th></th>
<th>fruiting individuals</th>
<th>seeds per plant</th>
<th>seeds per plot¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Artus</td>
<td>PR45D03</td>
<td>Artus</td>
</tr>
<tr>
<td></td>
<td>ctr      spring fall</td>
<td>ctr      spring fall</td>
<td>ctr      spring fall</td>
</tr>
<tr>
<td>ctr</td>
<td>n.s.     n.s.</td>
<td>n.s.     n.s.</td>
<td>n.s.     n.s.</td>
</tr>
<tr>
<td>spring</td>
<td>n.s.     n.s.</td>
<td>n.s.     n.s.</td>
<td>n.s.     n.s.</td>
</tr>
<tr>
<td>fall</td>
<td>n.s.     *</td>
<td>n.s.     n.s.</td>
<td>n.s.     n.s.</td>
</tr>
</tbody>
</table>

¹ square-root-transformed for analysis

**Fig. III.16**: Winter mortality for the cultivars Artus and PR45D03 for the different mowing treatments. Means ± SE, back-transformed from arcsine-square-root-transformed data, n = 8. Cultivars did not differ significantly in winter mortality, see Table III.14. Winter mortality was not affected by mowing if pooled over both cultivars, but in cultivar Artus, fall-mown plots showed significantly higher winter mortality than control and spring-mown plots (Kruskal-Wallis H-test, p = 0.005, n = 8, and Mann-Whitney U-tests, n = 8, p = 0.011 (fall-mown vs. control) and p = 0.005 (fall- vs. spring-mown).
Table III.17: Effects of cultivar on variables of reproductive potential analysed in separate one-factorial analyses for each mowing treatment. Significance levels (p) and F values (F) of one-way blocked ANOVAs, Welch’s ANOVAs are given for the cultivar effect (d.f. = 1). Fruiting individuals were analysed as the number of fruiting individuals out of four plants in a generalized linear mixed model with quasi-binomial distribution and logit link function (dispersion parameter estimated). F stands for Wald F statistic in this case. Denominator d.f. were 7 for spring-mown, 6.9 for fall-mown and 7 for control plots. n = 8 (fruiting individuals); n = 5-8 (seeds per plant) and n = 7-8 (seeds per plot). Significant effects (p ≤ 0.05) are shown in bold type.

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>fall-mown</th>
<th>spring-mown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f. p F</td>
<td>d.f. p F</td>
<td>d.f. p F</td>
</tr>
<tr>
<td>fruiting individuals</td>
<td>1 0.736 0.12 a</td>
<td>0.176 2.28 a</td>
<td>0.056 5.25 a</td>
</tr>
<tr>
<td>seeds per plant</td>
<td>1 0.790 0.07 b</td>
<td>0.813 0.06 b</td>
<td>0.014 9.094 c</td>
</tr>
<tr>
<td>seeds per plot</td>
<td>1 0.699 0.16 1b</td>
<td>0.751 0.11 1b</td>
<td>≤ 0.001 19.57 1b</td>
</tr>
</tbody>
</table>

† square-root-transformed for analysis  a Generalized linear mixed model  b unblocked ANOVA  c Welch’s ANOVA

III.3.3 Summary of results

Table III.18: Summary of results of the cultivar effect concerning the hypothesis that reproductive potential is higher for PR45D03 than for Artus a) on low-quality soils b) on mown plots. ∆ = hypothesis confirmed; n.s. = no significant results; † = the opposite result was true.

<table>
<thead>
<tr>
<th></th>
<th>semi-dwarf &gt; tall cultivar?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>on shallow/mixed soil</td>
</tr>
<tr>
<td>fruiting individuals [%]</td>
<td>!</td>
</tr>
<tr>
<td>seeds produced per plant</td>
<td>!</td>
</tr>
<tr>
<td>seeds produced per plot</td>
<td>!</td>
</tr>
<tr>
<td>seed viability [%]</td>
<td>n.s.</td>
</tr>
</tbody>
</table>
III.4 Chapter 5

Fig. III.17: Relationship between soil pH (log-transformed, note logarithmic scale of the x-axis) and the arcsine-square-root-transformed proportion of viable seeds retrieved from a 9-month burial period in 2007/2008 (outlier-site AF3 included). Each data point represents one site (n = 9) and is the mean of all 15-18 samples taken per site. Results of a quadratic regression are shown. Intercept, slope and the quadratic term of the regression line are significantly different from zero (p = 0.042 and p = 0.025 and p = 0.022, respectively).

\[ Y = -514 + 1585 \log(X) - 1098 \log^2(X) \]

Table III.19: Summary of significant or near-significant treatment effects on the nine sites in burial 1 (2007/2008) and burial 2 (2008/2009). The significant difference on site RH3 is left out as it was between treatment 20 µm + F and ~2 mm, so that effects of mesh size and fungicide cannot be differentiated. My hypotheses were that mesofauna could have positive or negative effects on seed persistence and that fungicide coating should increase persistence. ✓ = hypothesis confirmed; ! = the opposite result was true. [ ] = weak statistical tendency.
References

This list includes all references from chapters 1 and 6 as well as from the Appendix. References from chapters 2-5 are given at the end of each respective chapter.


References


References


References


References


Hulme, P. E. (1998a) Post-dispersal seed predation and seed bank persistence. Seed Science Research, 8, 513-519.


212


References


References


Acknowledgements

Above all, I wish to thank Juliane Filser, who gave me the opportunity to carry out this Ph.D. thesis. Juliane, you have supported me well throughout this long journey with both professional and personal advice and I could always turn to you in moments of major and minor crisis. Thank you for your inspiring enthusiasm, and my warmest thanks for all the opportunities you’ve given me to test my strength in teaching and other projects.

Martin Diekmann examined this thesis, gave professional advice on questions of vegetation ecology and encouraged my progress with moral support. Martin, many thanks!

Heartfelt thanks to Broder Breckling, who corrected countless pages and was a valuable source of information on genetically modified organisms and oilseed rape. Broder, thank you also very much for your moral support.

Susanne Pfeffer was my better half at work and helped me in countless questions. Susi, thank you for the good times we had together, for many discussions, help with practical work and for proof-reading all my experimental chapters. I miss our regular pep talks.

Stephan Hackmann spent countless hours helping me with the field work. Stephan, many thanks for creating soil disturbances for me during my sowing experiment, scaring rabbits away with a fence, planting OSR seedlings and moving several barrels with water all the way up to the dump site. I could count on you, no matter how hot or cold the day was.

I am further grateful to Alexander Seup, Katharina Stepputis, Nadine Korzeniowsky, Steffi Wolgast, Teresa Tonn, who assisted me in field and lab work, counting seeds, seedlings or pods.

Many thanks to Ute Uebers, Iris Burfeindt and Annemarie Kissling for helping with many little things in the lab, for ordering materials, watering plants or assisting me in field work.

I owe thanks to my wonderful working group for providing a very nice atmosphere with mutual support, friendship, shared social activities and scientific discussions. Special thanks to Birthe Schröder for correcting some of these pages and to Yvonne Sakka and Thomas Buse for moral support.

Several people gave me much-appreciated professional advice at some time or other: Gertrud Menzel, Annette Kolb, Josef Müller, Hartmut Köhler, Sabine Gruber and Ulrike Middelhoff.
I will be eternally grateful to some very strong women, Lena Nietschke, Heidi Wolters, Nadine Mitschunas, Susanne Pfeffer and Sonja Schaper, for moving 17 m$^3$ of soil with me during the creation of my experimental site on the Siedenburg dump site.

Many thanks to several farmers who kindly allowed me to sow OSR on side strips of their acres: Hermann Meyer-Toms, Jürgen Büttelmann, Jürgen Kramer, Volker Ehlers, Michael Bode-Kirchhoff and Ralf Borgmann from the research station Wehnen.

Ralf Möller from the Umweltbetrieb Bremen helped me a lot by providing sites on road verges which stayed exempt from mowing.

I wish to thank Norbert Binder from Bremenports and his team at the dump site for sluiced sand in Seehausen for the creation of three soil embankments for my sowing experiment.

Joseph Müller, Hartmut Köhler, the “Bremischer Deichverband am rechten Weserufer“ and the “Umweltbetrieb Bremen” (Werner Jorzick) organised the clearing of the Siedenburg dump site of its former vegetation and helped to defeat invading brambles – many thanks.

I am further grateful to Rolf Schäfer and his BRAS team of the University of Bremen for moving sand from the site at the UFT into a container for transport.

Marion Ahlbrecht analysed the nutrient content of soil samples for me – many thanks.

I thank Raimund Kesel for his help with the preparation of maps in GIS software.

RAPOOL-RING GmbH, Isernhagen, Syngenta Seeds, Bad Salzuflen and Pioneer Hi-Bred, Buxtehude kindly gave numerous OSR seeds to me free of charge.

Enno Dietrich kept faith in me during this long and sometimes meandering journey. Enno, I thank you for all your love and support, for being my shoulder to lean on and the one to challenge me when my inner workaholic takes over too much of our time. Last but not least for proof-reading and solving my graphics-crisis.

A big hug to my wonderful family, Norbert, Maidi and Helge Seeger, for their loving support. Maidi, I especially thank you for your tireless efforts in sharing the load: counting seeds, making data entries, correcting my English… Norbert, you have been an invaluable help by helping me to focus and not to lose my nerves.
Curriculum vitae

Jana Seeger was born on 29 February 1980 in Emsdetten, Germany. In 1999, she served a Voluntary Ecological Year in environmental education at the Wadden Sea nature conservation centre in Norddorf, Amrum. She began her studies of biology at the University of Bremen in 2000, focusing on ecology, marine biology and zoology. In 2004, Jana went to the U.S. for an internship at the Smithsonian Environmental Research Center in Edgewater, Maryland, where she worked in the Marine Invasions Lab.

For her diploma thesis, she joined the workgroup General and Theoretical Ecology at the Centre for Environmental Research and Technology (UFT) at the University of Bremen. Here, she investigated the role of honeydew as a carbon source for soil organisms and graduated in 2006. Jana started her PhD in November 2006 under supervision of Juliane Filser at the UFT. Her work on the establishment success of oilseed rape on ruderal sites in view of risk assessment of genetically modified plants is subject of the present thesis and was completed in December 2013. During her PhD, Jana was also active in teaching Bachelor and Master courses in ecology at the University of Bremen and completed her training as a nature, forest and experiential education specialist at the Institute for Training and Consulting (ibt), Hamburg, Germany. From 2010 to 2013, she worked as a research associate at the UFT on the project “HydRes”, where she participated in the development and testing of a water reservoir module for trees.

Publications


**Conference contributions**

Seeger, J. & Filser, J.: Down from the top: honeydew as a carbon source for soil organisms. Poster at the 36th Annual Conference of the Ecological Society of Germany, Switzerland and Austria (GfÖ), Bremen, Germany, 10.-14.09.2007

Seeger, J.: Who does better on ruderal soils: oilseed rape or wild relatives? Oral presentation at the Second International Conference on Implications of GM Crop Cultivation at Large Spatial Scales (GMLS) 2010, Bremen, Germany, 25.-26.03.2010


**Versicherung**

Hiermit versichere ich, dass ich die vorliegende Arbeit ohne unerlaubte fremde Hilfe angefertigt, ausschließlich die angegebenen Quellen und Hilfsmittel benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Bremen, den 02.12.2013