The handle http://hdl.handle.net/1887/32954 holds various files of this Leiden University dissertation.

**Author:** Moshgani, Mahmoud  
**Title:** Effects of slug herbivory on seedling recruitment in Brassica napus and B. rapa  
**Issue Date:** 2015-05-07
Success of hybrids of *Brassica napus* and its wild relative *Brassica rapa* in experimental conditions.

Mahmoud Moshgani and Tom J. de Jong
Institute of Biology Leiden, Leiden University
P. O. Box 9505, 2300 RA Leiden, the Netherlands
m.moshgani@biology.leidenuniv.nl
t.j.de.jong@biology.leidenuniv.nl

Key words:
Genetically modified crops, gene flow, transgene, hybridization, canola, back cross, F2, unpaired C-chromosome, DNA value, flow cytometry, slug herbivory, introgression
When crops can cross with their wild relatives there is a potential for gene flow from crop to wild species. Whether crop genes are maintained in natural populations depends on the fitness of hybrids and subsequent backcrosses. We investigated the growth of F1, F2 and BC1 hybrids derived from a cross between Brassica napus (cultivar Hornet) and the wild species B. rapa (accession Maarssen) under controlled conditions. We compared the dry mass of seedlings derived from hybrids and their parents under both favourable growing conditions and under conditions with drought stress. Our aim was to estimate the effect of unpaired C-chromosomes on growth and early survival of BC1 and F2 generations. The hybrids were all tested for ploidy level by flow cytometry. We found no difference between the amount of DNA measured and predicted for BC1 that was the mean of that in B. rapa and in the F1. This implies that there was no selective abortion against BC1 individuals carrying many C-chromosomes. The F2 individuals had more DNA than their F1 parents indicating that individuals with low C-chromosome numbers were selectively aborted which is in contrast with our hypothesis.

The growth of B. napus in favourable conditions was higher but its survival was lower than that of B. rapa. Contrary to our prediction, in drought stress conditions, neither the growth of BC1 and F2 nor their survivals were lower than those of B. rapa. The survival of B. napus under drought stress was the lowest. Similar to the results of the experiment under favourable growing conditions, under drought stress we did not find selective abortion against hybrids with many C-chromosomes in the BC1 but selection against offspring with few C-chromosomes in the F2. Finally we studied the herbivory of the slug Arion lusitanicus on seedlings. Results showed that slugs had a negative yet different impact on the recruitment of all Brassica seedlings. They fed most on B. napus and least on B. rapa. F2 and BC1 hybrids were consumed 3 and 2.3 times more than B. rapa respectively. These findings indicate that the presence of C-chromosomes in BC1 and F2 hybrids is not a determining factor for their failure to establish in the wild and that the pressure of slug herbivory may play an important role.

Abstract

There are many studies indicating that F1 individuals may have equal or even higher fitness than their parents depending on the novel genotype and their interaction with the environment (Arnold and Hodges 1995, Arnold 1997, Whitney et al. 2010). Some studies have proposed hybridization as a mechanism behind ecological adaptation and speciation (Rieseberg et al. 2003, Whitney et al. 2010). After a cross between a crop and a wild relative, crop genes may be maintained in the wild population (introgression) and this may lead to changes in wild populations. Especially for transgenes in genetically modified (GM) crops this point has led to concern (Ellstrand et al. 1999, Andresson and de Vicente 2010) since these crops could become invasive themselves or contribute to the evolution of more invasive plants by hybridization (Ellstrand, 2003). Many domesticated species have wild relatives near agricultural fields. For instance, one third of the 31 domesticated species in the UK hybridize spontaneously with one or more wild relatives in the local flora (reviewed in Raybould and Gray 1993). This is the case for Brassica napus L. (oilseed rape) that has been found to hybridize and introgress with its wild relative Brassica rapa (Jorgensen and Andersen 1994, Mikkelsen et al. 1996). Oilseed rape has become increasingly important as a crop in recent years, and its area of cultivation has extended across Europe and other continents (e.g. Northern America). This is due to the development by plant breeders of oilseed rape seeds with reduced contents of glucosinolates that are known to have unwanted effects on human and animal health (Falvey et al. 2001). At present, oilseed rape is used as oil for human nutrition, as a source of protein and energy for animals and for some industrial purposes.

Introduction

Over the past years, there have been several reports of the occurrence of hybridization of B. napus. Warwick et al. (2008) reported the presence of a herbicide resistance trait in F1 (B. napus × B. rapa) and backcrossed generations under natural conditions in Canada. Hansen et al. (2003) also detected F1 and backcross hybrids in an abandoned agricultural field in Denmark. In contrast, Luijten et al. (2014), found only F1 hybrids and no backcrosses in the Netherlands, which could show, in their opinion, that the later generation hybrids were less fit in the field.

There may be two reasons for the scarcity of later generation hybrids in the wild. In the first place, the presence of unpaired C-chromosomes may cause hybrids to perform less well than their parents. Secondly, there could be a higher pressure of herbivory on hybrids than on their wild parent. There is a close genomic similarity between B. rapa (AA: 2n=20) and B. oleracea (CC: 2n=18) and hybridization between those species (after duplication of the AC-genome) resulted in the allotetraploid B. napus (AACC: 2n=38) (U 1935). This species is thought to have originated in the early Middle Ages in Europe. Hybridization between B. rapa and B. napus gives a triploid F1-hybrid (AAC: 2n = 29). Therefore, F1 hybrids include 10 pairs of chromosomes from the A set and all 9 C-chromosomes derived from B. napus. After crossing the hybrids with B. rapa the BC1 hybrids have 10 pairs of A-chromosomes accompanied by 0 to 9 unpaired C-chromosomes. These extra C-chromosomes could disturb the chromosomal balance and result in low fitness. Therefore, there could be some barriers for gene flow between species with different chromosome numbers if the aneuploid offspring are less fit. De Jong and Hesse (2012) suggested that although the F1 grew well and produced seeds in natural conditions in the field, the further generations, BC1 and F2, would be much less fit and perform poorly. The chance for a specific unpaired C-chromosome to be passed on to a gamete is theoretically 50%. That
is, when an AAC hybrid containing all nine C-chromosomes crosses with *B. rapa* (AA) it is expected that the number of C-chromosomes in the BC1 will follow a binomial distribution with a mean of 4.5, i.e. there is 50% transmission of the C-chromosomes. Some authors, however, have observed only a 40% transmission rate of C-chromosomes to the next generation (Lu et al. 2002) while others found no selection and maintained that 50% is passed on (Fantes and Mackay 1978, Leflon et al. 2006). Based on these observations we can conclude that there is no evidence for strong selection in the gametogenesis or embryonic stage. This does not rule out however, that C-chromosome numbers may be negatively related to plant growth and survival. Only a few papers have reported a relationship between the number of C-chromosomes and the survival of further generations in *Brassica*. Some studies made crosses but mostly in a context of plant breeding (Choudhary and Joshi 2012) and rarely in an ecological context (Hauser et al. 1998). Choudhary and Joshi (2012) found a relationship between the number of C-chromosomes and the survival of further generations in numbers may be negatively related to plant growth and survival. Only a few papers have reported that the number of C-chromosomes in the BC1 will follow a binomial distribution with a mean of 4.5. Hauser et al. (1998) observed that the F2 had the lowest fitness and suggested that these less fit second-generation hybrids would decrease gene flow from transgenic *B. napus* to *B. rapa*. Other studies have focused on the measurement of introgression in the field using AFLP markers (Hansen et al. 2001, Luijten et al. 2014) or theoretical models (de Jong and Hesse 2012). In the opinion of De Jong and Hesse (2012) the absence of BC1 and F2 hybrids in the field is due to selection against plants with unpaired C-chromosomes.

In this study we analyzed the performance of F1, F2 and BC1 after seed formation. In particular, we investigated the effect of the presence of unpaired C-chromosomes on growth and survival both under favourable conditions and under drought stress. We also considered that apart from the potentially negative effects of the unpaired C-chromosome on growth and survival, there could be additional factors that could lead to selection against F1 and BC individuals in the wild. For example, the differences observed by Hesse and de Jong (unpublished data) in the seedling survival of 29 different accessions of *B. napus* and *B. rapa* sown in the field could be attributed to herbivory on seedlings since slugs were present in the field plots. However, no quantification of their effects on seedling survival was made at that time.

Slugs are considered to be highly selective and could potentially have large effects on the early life stages of some species in plant communities (Buschmann et al. 2005, Strauss et al. 2009). Some authors have observed that slug damage is very relevant in *B. napus* (Glen et al. 1990, Frank and Friedli 1999). We thus studied slug herbivory, choosing the slug *Arion lusitanicus*, which has become very common in Europe since the 1990s (Quinteiro et al. 2005, Soroka et al. 2009).

In this study we addressed the following questions:

- Does *B. napus* have a lower survival and growth than *B. rapa* under favourable or drought stress conditions?
- Do hybrids and backcrossed individuals have a lower growth and survival than *B. rapa* under favourable or drought stress conditions?
- If so, is this related to the presence of unpaired C-chromosomes?
- Are hybrids with unpaired C-chromosomes selectively aborted in a cross between *B. napus* × *B. rapa*?
- Does the slug *Arion lusitanicus* prefer *B. napus* and hybrids and backcrossed individuals to *B. rapa*?

---

**Material and methods**

**Plant material.** Hybrids. We conducted all experiments in controlled conditions (20°C, 10 hours dark, 14 hours light and 70% RH). We made a cross between the canola cultivar, *B. napus* (cultivar Hornet) and the wild species *B. rapa* (accession Maarssen). The total GS (the sum of aliphatic and indole GS) content for seeds of the cultivar and the wild species, *B. rapa*, was 16.2 (μmole/g DW) and 78.7 (μmole/g DW), respectively. An F1 was produced by transferring pollen from *B. napus* to the *B. rapa*. We self-pollinated flowers on *B. rapa* but none of these flowers set fruit so we concluded that *B. rapa* was self-incompatible under our experimental conditions. Using *B. napus* as the paternal parent and *B. rapa* as the maternal parent, we attempted to mimic the natural situation in which a rare self-incompatible *B. rapa* individual that is near a crop of *B. napus* would very likely be pollinated by the abundant pollen of the crop. We checked the ploidy level of the F1 plants by flow cytometry, confirming that the F1 was always between the mean of its parents. We then made a back cross (BC) to the wild *B. rapa* by transferring pollen from one F1 hybrid to flowers of two *B. rapa* plants. The seeds of a single mother plant were used here. When the F1 was flowerling it became apparent that there was one hybrid plant (F1) that spontaneously self-pollinated and produced many viable seeds. The obtained seeds from this single F1 plant were collected and are represented here as F2.

**Experiment 1:**

**Evaluation of the growth of *B. napus*, *B. rapa* and hybrid seedlings under good conditions**

We used 50 seedlings of *B. rapa* Maarssen, 50 seedlings of *B. napus* Hornet, 100 from the F2- hybrid, 100 from BC1 and 20 from the F1 (the total number of F1 seeds available was limited to about 70). Seedlings were grown in small pots (7 × 7 × 6 cm) filled with normal potting soil (RHP soil: www.horticoop.nl). We watered the plants every other day. After 5 weeks, we harvested a fresh leaf from all BC1 and F2 plants and one F1 hybrid and cut it in half. The leaf halves were weighed separately and one of each was immediately transferred to a plastic zip-lock bag and sent to iribov (http://www. iribov.nl) as blind samples for flow cytometry (including *B. rapa* Maarssen and *B. napus* Hornet leaves as references). This method is a convenient and reliable tool to calculate ploidy level and chromosome numbers (Givan 2001). The fresh and dry mass of the second half of each leaf was determined to calculate its dry matter content (DMC). With this value we could also estimate the dry mass of the other half of the leaf that had been sent for flow cytometry. We also measured the dry mass of the remaining plant material of all hybrids and the fresh weight and dry mass of all plants not involved in the collection of leaves for flow cytometry. Three plants were excluded from the analysis because of experimental failure.

**Experiment 2:**

**Evaluation of the growth of *B. napus*, *B. rapa* and hybrid seedlings under drought stress conditions**

We grew 50 seedlings of *B. rapa* Maarssen, 50 seedlings of *B. napus* Hornet, 100 from the F2- hybrid, 100 from the BC1 and 20 from the F1 in identical conditions to those described for Experiment 1. The seedlings were watered 3 times only during the first week after which we suspended the watering during 11 days. At this point a substantial fraction of the plants had shrunk and died. After this we resumed watering for 5 days to observe which plants recovered and survived. We then harvested all surviving plants and collected leaf samples from all hybrids for flow cytometry. A further 7 plants were excluded from the experiment after this, due to technical errors during flow cytometry.
**Experiment 3: Comparison of slug herbivory between hybrids and parents**

The slugs used in this study were the third generation of *Arion lusitanicus* reared in the lab in 2013. They had been fed only with lettuce. Seeds were germinated on humid filter papers in petri dishes and seedlings were transplanted into 12 trays with 8 × 8 cells (dimensions of each cell: 4 × 4 × 6 cm) filled with potting soil. We placed each tray with 64 randomly-distributed seedlings (16 F2, 16 BC1, 16 *B. napus* and 16 *B. rapa*) into a larger container. When the seedlings were 7 days old, we introduced one slug onto each tray, covered it with plastic foil to prevent it leaving the tray and allowed it to feed for 4 days. Each tray was controlled every morning to measure the damage by slugs and photographed. We used these pictures to estimate the fraction of slug damage on the plants. The fraction cumulative damage on a plant in one tray was calculated as the mean of 16 replicate individuals of each category. Since the size of seedlings was different (the *B. napus* seedlings were initially bigger), we weighed seedlings from 2 control trays without slugs. We then calculated the mean dry mass of seedling groups and multiplied it by the fraction damage per plant to obtain an absolute measure of herbivory (g dry mass). Four trays in which the slug did not feed were excluded from the study.

**Statistical analysis**

For data analysis, we used the R software package version 2.15.3 (R Core Team 2014). The correlation between the dry mass of F2 plants and their DNA content was examined with the Spearman rank correlation. We also tested the rank correlation between dry mass of BC1 plants and their DNA amount. To test whether the average dry mass of hybrids and parents were different, we used an ANOVA test with the dry mass as the response variable and plant type (*B. napus*, *B. rapa*, BC1, F2, F1) as the factor. The residuals of these models were all normally distributed. To compare averages of 1.75 (one sample t-test: t = 8.66, df = 82, P<0.001). This implies that gametes or embryos with a high C-chromosome number had a higher chance of being transferred to the next generation, which was the opposite to what we expected. Among all plant categories the lowest DNA content was 1.97 found for F2 hybrids was much higher than the predicted value of 1.75 (one sample t-test: t = 1.26, df = 90, P = 0.21). This fitted the prediction that no selective abortion occurs against seeds with many C-chromosomes in the BC1. However, unexpectedly, the DNA value of 1.97 found for F2 hybrids was much higher than the predicted value of 1.75 (one sample t-test: t = 8.66, df = 82, P<0.001). This implies that gametes or embryos by a high C-chromosome number had a higher chance of being transferred to the next generation, which was the opposite to what we expected. Among all plant categories the lowest DNA content was found in *B. rapa* (1.05) and the highest in *B. napus* (2.43) (Table 1).

**Results**

**Do *B. napus*, *B. rapa* and their hybrids differ in survival and growth under good conditions?**

The mean DNA value of the BC1 plants (1.37) did not differ from the expected values based on the mean of the parents (1.40) (One sample t-test: t = −1.26, df = 90, P = 0.21). This fitted the prediction that no selective abortion occurs against seeds with many C-chromosomes in the BC1. However, unexpectedly, the DNA value of 1.97 found for F2 hybrids was much higher than the predicted value of 1.75 (one sample t-test: t = 8.66, df = 82, P<0.001). This implies that gametes or embryos by a high C-chromosome number had a higher chance of being transferred to the next generation, which was the opposite to what we expected. Among all plant categories the lowest DNA content was found in *B. rapa* (1.05) and the highest in *B. napus* (2.43) (Table 1).

**Table 1. Comparison of survival and success, dry mass and DNA amount for 5 plant categories F1, F2, BC1 *B. rapa* and *B. napus* when grown under favourable growing conditions.**

<table>
<thead>
<tr>
<th>Category</th>
<th>Survival (%)</th>
<th>Dry mass average(SD)</th>
<th>DNA average(SD)</th>
<th>Success</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. rapa</em> Maarsen</td>
<td>96</td>
<td>0.30 (0.17)</td>
<td>0.004-0.59</td>
<td>0.29</td>
</tr>
<tr>
<td><em>B. napus</em> Hornet</td>
<td>90</td>
<td>0.41(0.23)</td>
<td>0.03-0.86</td>
<td>0.37</td>
</tr>
<tr>
<td>F2</td>
<td>84</td>
<td>0.23(0.15)</td>
<td>0.01-0.65</td>
<td>0.19</td>
</tr>
<tr>
<td>BC1</td>
<td>92</td>
<td>0.23(0.17)</td>
<td>0.01-0.76</td>
<td>0.21</td>
</tr>
<tr>
<td>F1</td>
<td>95</td>
<td>0.39(0.13)</td>
<td>0.19-0.59</td>
<td>0.37</td>
</tr>
</tbody>
</table>

The different letters indicate there is significant difference among dry mass in the Tukey test

\*Success was calculated as dry mass × survival fraction

Finally, we found no significant correlation between the relative DNA content of F2 and BC1 plants and their dry mass respectively (Spearman rank correlation: r = 0.16, P = 0.15, n = 83) (Spearman rank correlation: r = 0.11, P = 0.28, n = 91).
Do B. napus, B. rapa and their hybrids differ in survival and growth under conditions of drought stress?
The number of individuals that survived due to drought stress varied significantly between categories (Chi-squared test: χ² = 58.76, df = 4, P<0.001). A very low survival was observed for B. napus (18%) followed by 50% for both B. rapa and F1, and a notably higher survival for both BC1 and F2. These results showed that there was no correlation between lower number of extra C-chromosomes and higher survival rates even under drought stress conditions (Table 2).

Table 2. Comparison of survival and success, dry mass and DNA amount for 5 plant categories F1, F2, BC1, B. rapa and B. napus grown under drought stress conditions.

<table>
<thead>
<tr>
<th>Category</th>
<th>Survival (%)</th>
<th>Dry mass average(SD)n</th>
<th>Dry mass (min.-max.)</th>
<th>D N average(SD)n</th>
<th>A DNA (min.-max.)</th>
<th>Success*</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. rapa Maarsen</td>
<td>50</td>
<td>0.25(0.12) 25</td>
<td>0.00-10.65</td>
<td>NA*</td>
<td>NA</td>
<td>0.13</td>
</tr>
<tr>
<td>B. napus Hornet</td>
<td>18</td>
<td>0.28(0.12) 9</td>
<td>0.11-0.49</td>
<td>NA</td>
<td>NA</td>
<td>0.05</td>
</tr>
<tr>
<td>F2</td>
<td>75</td>
<td>0.27(0.17) 75</td>
<td>0.01-0.90</td>
<td>1.98(0.21) 73</td>
<td>1.35-2.31</td>
<td>0.20</td>
</tr>
<tr>
<td>BC1</td>
<td>76</td>
<td>0.23(0.12) 76</td>
<td>0.02-0.61</td>
<td>1.33(0.21) 72</td>
<td>1.04-1.68</td>
<td>0.17</td>
</tr>
<tr>
<td>F1</td>
<td>50</td>
<td>0.26(0.09) 10</td>
<td>0.14-0.40</td>
<td>1.72(0.03) 2*</td>
<td>1.70-1.74</td>
<td>0.13</td>
</tr>
</tbody>
</table>

*Success was calculated by dry mass × survival fraction
*NA: no or only few samples analyzed in this trial (see Table 1 for expected values)

There was no significant difference in the mean dry mass of plants of the five categories (ANOVA, F = 0.81, df = 4, P = 0.52) (Table 2). While the crop B. napus outperformed the wild B. rapa under normal experimental conditions, B. rapa survived much better under drought stress. Both BC1 and F2 had a much higher success rate (defined as the product of fraction dry mass and survival) than both parents.

Are hybrids between B. napus × B. rapa with unpaired C-chromosomes selectively aborted under drought stress conditions?
For BC1, we found a significant difference between the average DNA value (1.34) and our prediction (1.4); (One sample t-test: t = -2.43, df = 71, P = 0.02). However, the 95% confidence interval of the mean DNA for BC1 was between 1.29 and 1.39 indicating that the deviation was actually small. As in experiment 1, we found a higher DNA amount in the F2 hybrids (1.98) than our expected value (1.75) that was the mean of the F1 (One sample t-test: t = 9.25, df = 72, P<0.001).

Neither F2 nor BC1 plants showed a correlation between their dry mass and DNA content in stress conditions (Spearman rank correlation: r = 0.20, P = 0.08, n = 73 for F2 and r = 0.13, P = 0.26, n = 72 for BC1).

Does the slug Arion lusitanicus have a different preference for B. napus, B. rapa and their hybrids?
Slugs fed most from B. napus (mean ± SE = 0.34 ± 0.10) and least from B. rapa (mean ± SE = 0.03 ± 0.01) and the consumption of the hybrids was between the mean of the parents (mean ± SE=0.09 ± 0.03 and 0.07 ± 0.02 for F2 and BC1 respectively). While the difference was not significant (Friedman rank sum test: chi-squared = 6.58, df = 3, P = 0.09), it is noticeable that slugs consumed 3 and 2.3 times more from F2 and BC1, respectively, than from B. rapa.

Discussion
Selective abortion of C-chromosome numbers
Our results showed that the DNA content of F1 and BC1 hybrids was between the mean of their parents. In the case of BC1 plants, there was no significant difference between the measured DNA values and those predicted for the case in which there was no selection against C-chromosomes. Thus, it can be concluded that there is no selective abortion against BC1 hybrids with more C-chromosomes. With respect to the F2, we found a higher amount of DNA than expected, implying that in this case, embryos with low C-chromosome numbers were selectively aborted. We had expected the DNA value of the F2 to be similar to that of F1 AAC(1.75). In our data, however, it was 12.57% higher than that of F1. The distribution of DNA in F2 individuals is rather puzzling because if no C-chromosomes were passed on to the F2, the expected DNA amount should be that of AA (1.04). On the other hand, if both gametes contained the maximum of 9 C-chromosomes, the F2 could contain 18 C-chromosomes that are paired as in the tetraploid B. napus AAC(2.43). Surprisingly, we did not find any F2 that lost all C-chromosomes to establish AA. The lowest DNA value in the F2 was 1.43 (good conditions) and 1.35 (drought stress), showing that the whole lower range of the distribution towards 1.04 is missing. It should be recalled that B. rapa is self-incompatible and the rare selfing of an F1 individual (AAC) created the F2. This could lead to many deleterious alleles coming together in the F2. It is possible that the presence of C-chromosomes compensates for the lack of some functional genes in the A genome due to severe inbreeding depression. It is important to note that we found a similar distribution of the DNA amount in the F2 plants in both experiments.

Difference in growth and survival compared to parents
The early survival did not depend on the amount of DNA. Under drought stress conditions the wild B. rapa survived much better than B. napus. We had expected wild Brassica accessions to be more tolerant to drought stress than hybrids, but our findings revealed that they actually survived less than BC1 and F2 hybrids. While the success (defined as the product of fraction survival and dry mass) of B. napus in good conditions was 94.7% and 76.2% higher than that of F2 and BC1 respectively, this was dramatically inverted in drought conditions in which BC1 and F2 were actually 240 and 300% more successful. In the case of B. rapa, under favourable conditions its success rate was 52.6% and 38.1% higher than that of F2 and BC1, but again in drought stress conditions, these were actually 30.7% and 53.8% higher, respectively than that of B. rapa respectively. This implies that, contrary to the common assumption that BC1 and F2 do poorly due to the presence of C-chromosomes, they perform better than their parents. Thus, there is no indication that the presence of unpaired C-chromosome reduces survival and growth and thus it cannot explain the absence of BC1 and F2 hybrids in natural populations.

Difference in slug preference compared to parents
The level of slug damage was notably higher in B. napus than B. rapa. Intermediate levels were observed for the hybrids F2 and BC1 but they were much higher than for the wild B. rapa (200% and 133% more respectively). Our results suggest that the pressure of slug predation on B. napus could prevent the establishment of feral B. napus in the wild and also explain the absence of F2 and BC1 in Netherlands or UK.

De Jong and Hesse (2012) modeled the accumulation of C-chromosomes in wild populations using
B. rapa and B. napus. Their model showed that unless the fitness of aneuploid hybrids is below 30% that of B. rapa, C-chromosomes would accumulate in the population. As they did not find C-chromosomes in wild populations they suggested that the establishment of hybrids must somehow be problematic. We found, without slug herbivory, that the success of the BC1 was almost equal (Table 1) or even higher than that of B. rapa (Table 2) which could imply that C-chromosomes do not play a major role in reducing hybrid success. In the case of our second hypothesis, the potential influence of slug herbivory, the preference for hybrids was clearly higher than for the wild B. rapa, suggesting that this could be an important factor that hinders the establishment of hybrids in the field. Naturally, it is necessary to carry out field tests to confirm these hypotheses.


