

The development of a model to help predict the genetic changes affecting wild radish populations in regions where GM oilseed rape is grown

Henri Darmency

French National Institute for Agricultural Research (INRA) Biology and Management of Weeds, Dijon, France

darmency@dijon.inra.fr

Yann Tricault

INRA Biology and Management of Weeds, Dijon, France

Gaëlle Gueritain

INRA Biology and Management of Weeds, Dijon,

Abstract

Gene flow from oilseed rape to wild radish seems to occur at very low frequency. Linking of herbicide-resistance genes with genes favouring interspecific crosses could lead to gradual changes in the wild radish populations. This could progressively result in plants difficult to distinguish from natural wild radish that can spread in the whole region and cause trouble to farmers that never grown GM oilseed rape. We present a simple model to account for this effect together with the fitness cost of hybridization and the fitness benefit of herbicide resistance.

Introduction

Much of the research addressing the coexistence concern between genetically modified (GM) and conventional crops aims at quantifying GM seed admixture in non-GM harvest and the appropriate management strategies to contain it. Less attention has been paid to quantifying other descriptors of coexistence and its agronomic consequences for farmers. In particular, a more and more detailed field of investigation provides data on the escape of transgenes through interspecific hybridization between crops and their wild relatives (Chandler and Dunwell, 2008). Hybrid seeds and their pollen can move from the GM field and spread in the region, thus initiating the enrichment of regional weed populations in beneficial genes such as those conferring herbicide-resistance, insect-resistance, drought-resistance, etc. Thus, unwanted modified weeds could cause trouble to farmers that never grow a GM crop and that are not aware of the possible presence of these plants in their field.

Wild radish, *Raphanus raphanistrum*, is a weed infesting fields of oilseed rape, *Brassica napus*. It is difficult to control because it belongs to the same botanical family as the oilseed rape, the Brassicaceae, and therefore it shares the same innate herbicide resistances as the crop and very few selective herbicides have been discovered so far. The use of GM varieties in which a herbicide-resistance gene has been transferred from a foreign organism creates a selectivity of action preserving the crop but killing wild radish. However, risk assessment studies of GM crops have recently brought evidence of a low spontaneous hybridization frequency of oilseed rape with wild radish, which could result over time in new herbicide-resistant weedy relatives. The question is to determine how long it will take to turn a wild radish population from completely susceptible to resistant enough to cause trouble to farmers in the field.

Under agronomic conditions, the frequency of interspecific Brassicaceae hybrids is very low (Darmency et al., 1998; Chèvre et al., 2000; Rieger et al., 2001), but the hybridisation rate varies according to the rape cultivar (Baranger *et al.*, 1995), and even abundant hybrids can be produced on male sterile oilseed rape (Eber *et al.*, 1994). In addition, a polymorphism was demonstrated to occur among wild radish plants for interspecific cross-compatibility (Guéritaine and Darmency, 2001; Guéritaine et al., 2003) so that differences between populations could result in local gene flow intensity varying among regions (Ammitzbøll and Jørgensen, 2006). However, since this polymorphism was observed within populations, it is likely that those wild radish plants exhibiting the fewest barriers to hybridization would become the parents of interspecific hybrids. Thus, hybrids would display both low-barrier genes and the transgene. In the case of a beneficial transgene such as herbicide-resistance, hybrids would survive better than wild plants, and thus enrich the population with those low-barrier genes (hitchhiking effect). Consequently, gene flow would be enhanced up to a barrier-free level that would speed up gene flow and change drastically the biology of the wild species.

As an opposing force, the formation of hybrids and hybrid progeny was shown to be associated with functional imbalance and low fitness, which would reduce the risk of gene escape and establishment of the GM wild species in the field. The F₁ hybrids showed a lower seedling emergence, a significant delay in emergence, and a lower

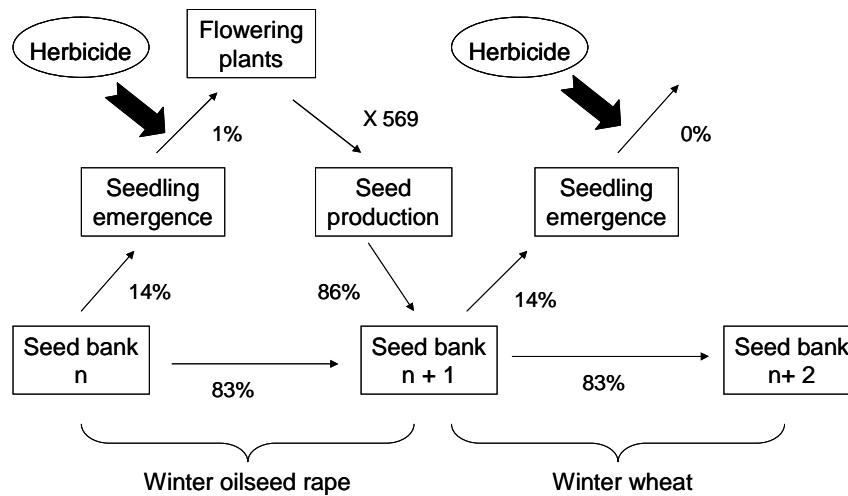
survival than both parents. Rosette diameter and dry matter of hybrid plants were reduced relative to those of both parents, especially when they grew in the field and under competition conditions (Guéritaine *et al.*, 2003). The seed production of F₁ was very weak (Chèvre *et al.*, 1997, 1998; Darmency *et al.*, 1998; Benabdelmouna *et al.*, 2003). The progeny are produced through backcrossing, which, generation after generation, progressively results in plants with restored fertility that are difficult to distinguish from natural wild radish (Chèvre *et al.* 1999; Guéritaine *et al.*, 2002). However, even at advanced backcross generations, in the unique case described today, the presence of the transgene still lowered the rate of flowering, significantly delayed the onset of flowering and decreased pollen fertility, seed output and seedling emergence (Guéritaine *et al.*, 2002). These differences were probably due to caryological problems accompanying the presence of a remnant oilseed rape chromosome with non-Mendelian hereditary transmission, only reaching 10 % of the gametes (Al Mouemar and Darmency, 2004).

The transition phase between the hybrid status and the release of progeny looking like genuine wild radish is therefore of prime importance for a transgene to be fixed in a population. The weakest step of that process is the establishment and reproduction of interspecific hybrids. It is suggested that a lag time could occur before the visible onset of a GM wild radish population because of the low probability of hybrids producing viable seeds in the field and low fitness of the first backcross generations. However, herbicide resistance is a highly selected trait when using herbicide, which may boost gene flow through preserving hybrid individuals that would otherwise die. Therefore, it would be important to predict the long-term changes of the genetic structure of the target population in order to estimate the coexistence situation in the future and prevent the occurrence of highly fit hybrids. The present paper considers the various opposing forces and uses a simple modelling approach to estimate the lag-time between the first interspecific hybrids and the appearance of a troublesome resistant population via a gradual change of the mating system.

A simple model

Let us imagine a cropping system with a winter oilseed rape - winter wheat rotation, and superficial cultivation just before crop sowing. Including additional wheat and barley in the rotation has no effect on the qualitative evolution of the wild radish population, because we assume that all wild radish seedlings emerging in cereals are killed by the herbicides used, but this simply delays the kinetics of the development of herbicide-resistance in the population in per-year terms. In order to account for the dynamics of wild radish populations in an arable field, we use a simple transition model from seed bank in year n to seed bank in year $n + 2$ (Figure 1). It includes seedling emergence, longevity of the remaining seeds in the soil seed bank, mortality due to herbicide spray, seed production by the adult plants, and seed predation. All the germinated seeds give rise to emerged seedlings, and there is no plant competition.

Figure 1. Simplified two-year model of the life cycle of wild radish in an oilseed rape – wheat rotation.



Interspecific hybrids are generated in every oilseed rape crop. Since Guéritaine and Darmency (2001) described genetic variation for interspecific cross-compatibility within the wild radish population, we hypothesized that the rate of interspecific hybridization could evolve with time. Four phenotypes were described on the basis of stigma surface/pollen compatibility and pollen tube guidance to the micropyle. Phenotype A showed no oilseed rape pollen germinating on stigma. Phenotype B showed pollen germination but pollen tubes had no tropism toward the micropyle. Phenotype C had pollen tubes mostly with tropism to the micropyle. A few plants, described as phenotype D, had the same fertilization efficiency as when pollinated by a con-specific wild radish. We tentatively use here a parsimonious two-independent-genes interpretation of these processes. We assume that in both cases the lack of a barrier is endowed by recessive alleles at a gene conferring pollen/stigma compatibility (P_c) and at a gene conferring guidance to the micropyle (G_c). Table 1 shows the putative genotypes corresponding to the four phenotypes and their observed frequency in the population. These inheritance patterns are not incompatible with the data of the studied population in which both recessive alleles could have a frequency $p = 0.77$ (as calculated by a maximum likelihood analysis). Only the plants being homozygous recessive at the two loci can produce hybrids.

Table 1. Phenotypes for interspecific cross fertilization in a wild radish population

Phenotype	Frequency	Pollen/stigma	Micropyle guidance
A	0.40	$P+P+$ and $P+P_c$	unknown
B	0.23	P_cP_c	$G+G+$ and $G+G_c$
C+D	0.37	P_cP_c	G_cG_c

The hybrids and their progeny have the same life cycle as the wild radish except that the number of seeds produced per plant depends on the backcross generation. They participate in the reproduction of the whole population, thus enriching the gene pool with the recessive alleles for interspecific hybridization and the herbicide-resistance gene when the crop is oilseed rape. Because wild radish is a self-incompatible

species, and assuming no homogamy, we can expect Hardy-Weinberg equilibrium and calculate the allele proportions at each generation.

Setting the parameters

For the life cycle model (Figure 1), the default values are averaged values calculated from the literature: Roberts and Bodrell (1983) for an average 14 % seed germination and 18 % longevity after 5 years of burial in the soil (i.e. 83 % of the remaining seed in the soil survive to the next season); Guéritaine et al. (2002) for seed production in field conditions (see Table 2), and Barralis et al. (1977) for the wild radish density before herbicide spray (5 plants m⁻²). Since wild radish is known to be difficult to destroy in oilseed rape, particular attention was paid to herbicide application, which results in 99 % mortality regardless of whether a conventional herbicide program is used or an unselective herbicide on the herbicide-resistant genetically modified variety. Herbicide efficiency in wheat is 100 %, making this a simple seed-loss phase. An initial soil seed bank of 36 seeds m⁻² allows a density of 5 plants m⁻² and results in a roughly stable infestation of 500 plants ha⁻¹ in the first oilseed rape crop of the simulation. In order to keep this infestation stable over years, we set an arbitrary seed predation rate of 86 %.

In field experiments, using the wild radish population studied by Guéritaine and Darmency (2001), the rate of spontaneous crosses was 0.05 % (Darmency et al., 1998). According to our hypothesis, only phenotypes C and D could contribute to hybrid production. Since they constituted 37 % of the population, their actual interspecific crossing rate should be 0.15 %. At the beginning of the simulation, the two recessive alleles have a low frequency, 0.24, which corresponds to a field experiment where a very low frequency of hybrids was observed, 5 10⁻⁶ (Chèvre et al., 2000).

The herbicide-resistance is 100 % for the F₁. In the case study for which we have the data, the herbicide-resistance gene was never transferred to a wild radish chromosome, but rather inherited as a single remnant oilseed rape chromosome showing a non-Mendelian inheritance pattern (Al Mouemar and Darmency, 2004). Therefore, it decreases according to the decreased number of chromosomes from backcross generation BC₁ to BC₃: 82, 57 and 24 %, respectively (Chèvre et al. 1997). For further generations, its inheritance is calculated according to a 10 % transmission in the gametes (Al Mouemar and Darmency, 2004).

The seed production of the hybrid and its progeny through backcrossing with wild radish and/or open pollination is derived from data obtained from F₁ to BC₆ in several studies (see Table 2). In all the cases, the number of seeds per 100 flowers was recorded, but the number of seeds per plant was sometimes missing or could not be compared from generation to generation because the growth conditions were very variable. In particular, data from F₁ to BC₄ were obtained under non-competitive conditions, while those of BC₆ and wild radish came from field conditions. Therefore, it is likely that early hybrid generations produced many more flowers than could have occurred under competitive conditions. For instance, the BC₂ had 2900 flowers while a wild radish in the field had 1000 flowers, and the resistant BC₆ had only 650 flowers. However, because early hybrid generations produced few seeds, they

probably used more resources to make more flowers, a feature that would disappear as the generation advanced. Thus we decided to keep the values observed in the literature cited above for F₁ to BC₂ (Table 2). For further generations, because the transmission of the remnant oilseed rape chromosome is stabilized at 10 %, we assume a constant seed production as observed for the BC₆. The normal, non-GM BC₆ had similar seed production to the wild radish, while the resistant, GM BC₆ had seed production reduced by around one-third (Guéritaine et al., 2002).

Table 2. Values recorded for the number of seeds per 100 flowers and per plant in several experiments with resistant hybrid generations, and estimated values used in the model.

Generation	F ₁	BC ₁	BC ₂	BC ₃	BC ₄	BC ₅₋₆	Wild type
Seeds per 100 flowers	0.12a, 0.18e	1.4a	7.9a	27.5c	42c	55d	55d
Observed seeds per plant	0.8a, 1.8b	11a, 15.2b	229a	NR	NR	356d	569d
Estimated seeds per plant	1.3	14	229	356	356	356	569

a: Chèvre et al., 1998; b: Darmency et al., 1998; c: Chèvre et al., 1999; d: Guéritaine et al., 2002, e: Benabdelmouna et al., 2003 ; NR : not recorded.

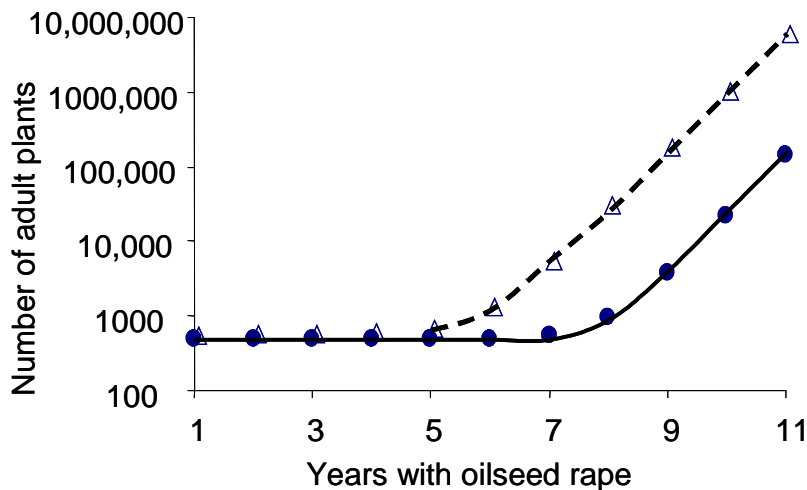
From hybrid to GM wild radish population

Under the conditions described above, nothing apparently occurs during a long lag-time (Figure 2). This is due to both the low reproduction value of the hybrids and the buffering effect of the soil seed bank. Even longer lag-times could be expected if the low competition value of the hybrid for seedling establishment were taken into account (Guéritaine et al., 2003). In contrast, hybrids growing on the border of the field, where there is less competition, reproduced more than those within the field, which could lead to more rapid spread of the resistance transgene. It was only when BC₃ plants appeared and reproduced that the resistant plants could represent more than 0.1 % of the total adult population. Only by the seventh rotation did the resistant plants represent 9 % of the adult plant population, and still only 0.1 % of the seed in the soil. Thereafter, the population evolves apparently very quickly as it double its size at the eighth rotation, with 45 % of plants resistant. At this stage, the farmer would probably still not recognize any reduced efficacy of the herbicide strategy. However, at the tenth rotation, there are 22 resistant plants m⁻², which is a serious problem. In addition, because the transgene has never been introgressed on a wild radish chromosome, but has still an erratic inheritance linked to the transmission of a remnant oilseed rape chromosome, there is also an increase in the susceptible fraction of the population. The soil seed bank has increased by 18 times and comprises 13 % resistant seeds.

In the case where the genetic structure of the population studied at Dijon was modeled, i.e. using $p = 0.77$ for the frequencies of the putative recessive alleles governing interspecific compatibility, a similar evolutionary curve appeared but two rotations earlier (Figure 2). There is no effect at the beginning of the simulation as the F₁, BC₁ and BC₂ hybrids have low pollen fertility, and therefore they cannot enrich the population with the recessive alleles. It is only when the BC₃ plants

contribute to the pollen cloud that the frequency of hybridization could increase. From this time on, the population becomes an important source of transgenic pollen, possibly to be transported to neighboring fields and waste places. And at the tenth generation, the frequency of the recessive alleles is nearly 1, which means that the population has definitively evolved to be reproductively compatible with oilseed rape and able to incorporate every new beneficial transgene.

Figure 2. Simplified scenario of the fate of appearance of herbicide-resistant hybrids and their progeny in a stable wild radish population of 500 plants ha⁻¹ after herbicide treatment (99 % efficiency on susceptible plants). Values of reproductive parameters are indicated in the text. Frequencies of putative recessive alleles for interspecific compatibility are $p = 0.24$ (solid line) and 0.77 (dotted line).

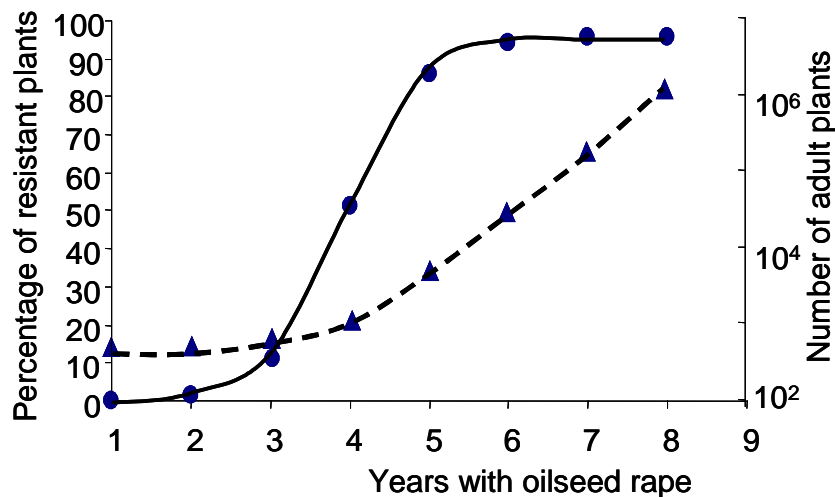


Consequences for coexistence

In a scenario of sustainable coexistence, the landscape-wide occurrence of hybrids is a given, and the challenge is to deal with the migration of seed and pollen of stabilized introgressants from field to field in the region. In our model, let us imagine a GM BC3 weed seed migrating to an oilseed rape field where the farmer uses for the first time the corresponding GM variety, and where the wild radish population has no genetic capability to hybridize with oilseed rape (i.e. not containing phenotypes C and D). This migrant GM wild radish represents 1/500 of the population after herbicide treatment. Although a resistant plant produces only 10 % gametes containing the remnant oilseed rape chromosome and releases one-third less seeds than the susceptible plants, the herbicide-resistant sub-population increases rapidly (Figure 3). It forms 50 % of the adult plant population at the fourth oilseed rape crop of the rotation, thus doubling the initial wild radish population size. At that stage, the one thousand plants per ha after herbicide application is probably not enough to trigger any reaction from the farmer. In addition, the overall herbicide efficacy is still 98 %, which is not an alarming signal. However, it is the beginning of an exponential increase of the wild radish density, and at the next oilseed rape crop the resistant phenotype constitutes 86 % of the population with a density of 0.5 adult plants m⁻². Thereafter, the percentage of resistant plants reaches a plateau at ca. 95 %, and

plant density exceeds 100 plants m⁻² at the eighth rotation. In these conditions, the agronomic trouble generated by the interspecific gene flow resulting in the development of herbicide-resistant plants occurs much earlier than in the case of the initial appearance of interspecific hybrids.

Figure 3. Simplified scenario of the fate of the frequency of resistant wild radishes after herbicide treatment, for eight generations, with only one resistant BC₃ plant on year 1 over 500 susceptible wild radishes alive after treatment, 99 % herbicide efficiency on susceptible plants, and values of reproduction parameters as indicated in the text. Solid line: percentage of resistant plants; dotted line: total number of wild radishes per ha.



It is interesting to note that the population cannot reach 100 % resistant plants. First, 1 % of susceptible plants escape the herbicide spray because of the umbrella effect of a bigger plant, or late germination, or dose variation due to the sprayer. Second, we use data from the unique published example of interspecific hybrids between a GM oilseed rape and wild radish. In that case, the transgene is not located on a radish chromosome, but it remains on an oilseed rape chromosome or a piece of chromosome up to at least BC₁₁ (Al Mouemar and Darmency, 2004), which causes permanent trouble for reproduction. Thus, the continuous release of susceptible gametes by the resistant plants explains the plateau. It also explains why the population size increases for both the resistant and the susceptible plants. Even with a huge quantity of resistant plants at the eighth rotation, the herbicide efficacy is still 81 %.

Conclusion

Obviously, the present model is over-simplified and uses fragmentary data to set parameters. For instance, seed longevity, germination and emergence data of hybrids are mostly lacking (Chadoeuf et al, 1998); chromosome inheritance and distribution along a series of backcrosses certainly are more complex than exposed here (Chèvre et al., 2007); the reproduction data are obtained in non-competitive conditions; the short rotation and the exclusive use of only one herbicide in oilseed rape makes the model a worst case scenario, etc. However, the model shows that

the weak nature of the interspecific hybrid between wild radish and oilseed rape generates a low fitness value that allows several years of tranquility before transgenic weeds do occur and spread in the region. It can be adapted to different kind of transgenes conferring beneficial traits other than herbicide resistance. The interest of modeling gene flow is to identify the different weed management strategies which can delay a regional infestation. In contrast, monitoring programs looking for hybrids are probably little adapted to detect the onset of the invasion, or they would do so too late. A mechanistic demography model belonging to the GENESYS model family (Colbach et al., 2001), RAPRASYS, is under construction. It will include climatic data, longer crop rotation and more varied crops, which may affect the differential fitness of hybrids and wild radish.

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