INSIGHTS

Does genetic variability in weeds respond to non-chemical selection pressure in arable fields?

H DARMENCY (iD)

UMR Agroécologie, AgroSup Dijon, INRA, Univ. Bourgogne Franche-Comté, Dijon, France

Summary

At a time when herbicide use is being challenged by the selection of herbicide-resistant weeds, reliance on other, innovative weed control strategies is becoming increasingly necessary. However, one may question the sustainability of these novel farming practices if weeds adapt rapidly to these non-chemical selection pressures. Although farmers and agronomists impose many selective processes through farming practices, there is a paucity of literature demonstrating these selection cases in arable fields. In contrast to the relatively simple case of herbicide resistance, random trait association and variability in selection pressures in field conditions could explain why there are so few clear examples of adaptive processes to non-chemical control in arable fields.

Keywords: experimental selection, adaptation, arable fields, weeds.

Introduction

Weeds have been adapting to the within-arable field habitat for centuries and continue to do so as farming systems change and agricultural land use intensifies (Vigueira *et al.*, 2013). Herbicide resistance provides an undisputed example of adaptive evolution in response to selection in arable fields (Délye *et al.*, 2013; Heap, 2018). Much less is understood about responses to selection pressures other than herbicides, although there is increasing evidence in the literature for weed adaptation to non-chemical control. For instance, there are many examples of within-population variation in, and artificial selection for, seed dormancy (see references in Darmency *et al.*, 2017), including the observation that populations of *Lolium rigidum* in Australia delay germination in response to intensive cropping (Owen *et al.*, 2015 and references therein). There is also concern about the selection for shorter plants or earlier seed shedding in response to harvest weed seed control (HWSC, Walsh *et al.*, 2018). This has not yet been confirmed in *Lolium rigidum,* but there are demonstrations of experimental selection for early- and late-flowering variants in *Raphanus raphanistrum* (Ashworth *et al.*, 2016). Cases of adaptation include mimicry with crop when weeds display appropriate genetic variation (Barrett, 1983) and via gene flow by introgression from crops to related weeds of 'field-preadapted' genetic diversity, such as observed in response to a farmer's adoption of a red coloured rice cultivar to discriminate from green weedy rice to allow hand weeding: weedy rice stem subsequently turned

red, thus jeopardising the innovation (Oka & Chang, 1959). In other cases, gene flow from crops could lead to plants displaying novel traits, such as *Raphanus* species in California (Campbell *et al.*, 2009). More generally, gene flow among weed populations may propagate resistance and adaptive alleles (Délye *et al.*, 2010), but in turn, it also dilutes the selection effect by providing unselected migrants, therefore delaying selection, but preserving the genetic diversity: the persistence of the soil seedbank has a similar buffering effect on weed adaptation.

To my knowledge, only one experiment has provided clear evidence of a direct causal link between these population-level shifts and cropping practices. Following up on an observation of genetic adaptation for seed dormancy (Naylor & Jana, 1976), Jana and Thai (1987) sowed artificial populations of mixed dormant and non-dormant *Avena fatua L.* (wild oat) in fields managed under summer-fallow or continuous-cropping regimes. The result was rapid selection for completely dormant types, because seeds of non-dormant plants displayed suicide germination in the fallow year. In most, if not all, other studies, adaptation has been inferred from co-occurrence of a selection pressure and a high frequency of a given trait. Why is there so little experimental evidence for exactly how effectively non-chemical selection pressures act on intra-specific weed variability? Is it because it is difficult to prove experimentally or because it is not happening? If the latter is the case, we need not worry. However, if it is happening, what are the obstacles, gaps or needs that constrain experimental approaches needed to quantify adaptive processes in weeds? When herbicides used to control weeds are challenged by development of, and selection pressure from herbicide-resistant weeds, reliance on other, innovative weed control strategies becomes necessary. The global need for sustainable agriculture increases the requirement to determine time spans over which weed species adaptations to changing farming practices and new agricultural systems occur. Here, I review the experimental obstacles to answering these important questions and discuss potential solutions.

Non-chemical versus herbicide selection pressure

Baker (1974) suggested that agriculture, as a complex association of farmer practices, was a major determinant of weed selection through 'local race formation' at the species level, rather than simple colonisation of land. However, before the 1970s, no clear-cut link was established between within-species variability and weed adaptation. Phenotypic variability was just a matter

for taxonomical disputes (McNeill, 1976). The so-called 'general purpose genotype' was believed to respond to most arable field variations (Baker, 1974). At that time, the first reports of adaptation in response to herbicide use were being published (McNeill, 1976), demonstrating the adaptive potential of weeds. There is no doubt that massive and repeated use of the same chemical is likely to lead to rapid selection of resistant plants, especially when only one gene is involved. In addition, non-target site resistance mechanisms, involving several genes, generally occur in a second step (Délye *et al.*, 2013). However, there are also many examples of rapid selection of resistant plants under reduced selection pressure at low doses and/or sub-lethal treatments (Neve & Powles, 2005). Thus, high versus low selection pressure may not be the only cause of the different occurrence of herbicide versus non-chemical selection.

Does herbicide resistance receive too much attention, leading us to not see the forest for the trees, in terms of weed adaptation? Or is it really the only significant factor? Are centuries-old general purpose genotypes already sufficiently adapted to persist in response to more recent changes in farming practices (including conservation agriculture, precision agriculture, diversified rotations, direct seeding, delayed sowing), or are there ongoing adaptations to management change? Does the addition of herbicide resistance selection swamp the previous cocktail of non-chemical selection pressures such that it is the only process that matters in determining weed genotypes? Few examples of interactions between these two classes of selection forces have been published that may help answer these questions. One example, however, is the case of *Poa annua,* a grass weed that shows a prostrate perennial ecotype adapted to undisturbed habitats and an erect short-lived, opportunist ecotype adapted to open and disturbed habitats, such as cropped fields. Initially appearing on a triazine-treated running track around a sports field colonised by the prostrate ecotype, resistant plants escaped to the surrounding urban zone on roadsides and pavements previously colonised by the erect type. Triazine treatment in that new habitat rapidly resulted in a perennial resistant population at the expense of the erect type, which showed the strength of the chemical selection pressure and masked the previous habitat selection pressure. However, molecular markers indicated a large proportion of hybrids between the two ecotypes. Moreover, some pure erect type plants were resistant. Triazine resistance is cytoplasmically inherited, indicating that an active pollen-mediated gene flow occurred between the two types. Therefore, herbicide selection initially masked the usual open habitat type selection, but these

low selective forces reappeared later (Darmency & Gasquez, 1983).

In contrast, is it possible for a wild species to become a weed thanks to a herbicide resistance gene and without a 'Bakerian' 'general purpose' genotype? Although only one occurrence of triazine-resistant *Epilobium tetragonum* is known (Heap, 2018), this species is noteworthy for not occurring in maize fields: the only trait that led to it colonising a cultivated maize field was the resistance gene that allowed development in a weed-free, triazine-treated field. A similar case was documented along treated roadsides: the first arrived resistant plant was *Brachypodium distachyon*, not known to be a dangerous weed, but other herbicide-resistant weeds later evolved in the same habitat and outcompeted the grass that consequently disappeared from roadsides (Gressel & Kleifeld, 1994). Thus, herbicide resistance may temporarily turn a plant into a weed, but other traits that play determinant roles for long-term adaptation make it necessary to scrutinise the long-term selection forces of combined chemical and non-chemical management. The expansion of *Amaranthus palmerii* and *A. rudis* throughout cotton and maize fields in the United States is probably a result of both conservation tillage and glyphosate resistance (and overuse of glyphosate), because these species were not previously known as important weeds in these regions (Ward *et al.*, 2013).

In the past, every time a new herbicide was released, the potential for the occurrence of evolving resistant weeds was ignored or disregarded. Only in recent times has this possibility had to be evaluated in the dossiers for market regulation. From a simple curiosity in the sixties, variation in herbicide response within a species became a major threat in contemporary agriculture. We are now in a similar situation with non-chemical control. How can we demonstrate that adaptation to cultural practices is happening, and under which conditions? How can we estimate the potential damages as well as preventive and curative strategies? Modelling is a possibility, but it must be fed with data obtained from long-term experiments. Such an approach has a practical importance for weed research, but it may push our discipline to the field of evolutionary biology and be recognised too on theoretical grounds (Neve *et al.*, 2009).

Genuine versus controlled field conditions

Are arable fields actually appropriate systems to test adaptive processes to multiple, diffuse selection pressure through experimental approaches? Usually, researchers in the evolutionary domain handle simplified or closed environments and use fast cycling organisms; one of these is *Arabidopsis thaliana*, but this weed is rarely put in a genuine arable situation (Kawecki *et al.*, 2012). In genuine fields, however, farmers choose a range of factors that may serve as evolutionary forces, including soil perturbation and fertilisation, dominant plant species (i.e. the crop itself), plant density and coverage, and periods of vegetation growth and harvest. Only environmental variables are uncontrolled, though artificial water supply and shading may be managed. Even pest and pollinator frequency may be modulated via pesticide use. Therefore, it may be argued that arable fields provide a man-made habitat where it is possible to use unbiased experimental approaches and where growth conditions may be modelled, making it possible to study how a few evolutionary forces act on weed characteristics. How, then, do we explain the lack of experimental field studies on weed adaptation to non-chemical selection pressures? On the one hand, the lack of experimental studies on plant adaptation in arable fields may be due to a lack of interest in genetics and ecology on the part of agricultural scientists and agronomists. As stated above, gene flow among populations experiencing diverse selection pressures, combined with a diversity of crop rotation and the buffering effect of the seedbank, makes weed selection difficult to quantify, thus reducing its attractiveness as both basic and applied research questions. On the other hand, the complexity of genetic and environmental variables, especially when climatic data are involved, may also discourage applied studies, by comparison with more stable or simplified environments.

For the later, the inconclusive results of a study aimed at mimicking natural selection driven by climatic conditions triggered the present discussion and could serve to illustrate that point. Adaptive selection to regions with new climatic conditions showed that adaptation to climate change may be successful (Clements & DiTommaso, 2011). In our case, frost resistance was purported to be the cause of the increasing frequency of an *A. fatua* winter type observed in a field population monitored over six years of continuous winter crop rotation (Darmency & Aujas, 1986). In an attempt to validate this hypothesis, an experiment using the same *A. fatua* population was set up over nine years under the same winter crop rotation under genuine farming conditions. However, no clear-cut population shift was observed (unpublished results). Firstly, the weakness of the selection pressure during the experiment (i.e. the poor number of frost days during winter) partly explained the negative result. However, low selection pressures are not really an obstacle to selection, even under short time: experiments under controlled conditions showed that herbicide resistance

was rapidly selected using sublethal herbicide doses (e.g. Neve & Powles, 2005), and evolution of populations with polygenic, non-target-site resistance mechanisms occurred in the fields (Délye *et al.*, 2013).

Secondly, the choice to mimic the actual observed field case resulted in the use of plant types belonging to the very field where the population shift was observed, with each plant type displaying several linked differences with regard to various response traits: frost resistance, vernalisation requirements, seed dormancy characteristics, competitive ability and possibly herbicide sensitivity. Avoiding confusing effects not directly related to the studied trait or selection pressure is often difficult under genuine field conditions because selection is multifactorial and weeds have the genetic make-up to respond to many stimuli. Preparing isogenic lines with or without frost resistance could have more clearly highlighted the selection process, avoiding any interaction with differential dormancy, germination properties and herbicide sensitivity. However, preparing such plant material is time-consuming and makes the experiment still longer, and it is far to what might happen in real arable field populations where plants vary across many traits, selection pressure is discontinuous, and the soil seedbank buffers the adaptive process.

Thirdly, the choice to mimic genuine farmers' field conditions, again, made it necessary to change one component of the farming system halfway into the experiment: initially without grass weed herbicide, it became necessary to apply grass weed herbicides to control *A. fatua* because the weed density became so great that it was irrelevant to normal arable field conditions. As a consequence, there was an additional possible herbicide selection, and the population size decreased dramatically, resulting in poor accuracy of the estimates. This highlights the challenge of attributing causality to individual non-chemical selection pressures over several generations of a weed.

Perspectives

In addition to herbicide resistance and, to a lesser extent, seed germination processes, there is a bulk of traits whose selection is embedded in a diversity of constraints. Given the genetic diversity of weed populations in the face of a wide diversity of farming practices, the adaptive response of weeds to biotic and abiotic stresses, including changed practices (e.g. higher sowing density, new tools for precision weeding, mulch, false seedbed, later/earlier sowing and harvest), is not as straightforward and rapid to observe as modelling simulations indicate because of the complexity of causal relationships throughout the life cycle. It is noteworthy that very few models aiming at simulating the development and management of herbicide-resistant weeds include individual genotype performances (see for instance differential fitness of various ACCase mutants in Colbach *et al.*, 2016). Paradoxically, although farmers control nearly everything in the field, man-made selection pressures led to difficulties in isolating the effect of individual weed management factors on weed adaptation because of the resulting genetic diversity at multiple loci of the plant populations. The buffering effect of the soil seedbank, variable seed dormancy, farmers' evaluation of the weediness of their field and resulting farmers' practices makes it necessary to carry out pluriannual experiments in which variability is intrinsic. To overcome this problem, one could develop pedigreed seed family approaches and quantitative genetics to estimate components of fitness in genuine, annual field conditions and then incorporate them into simulation models that take account of such complex patterns of quantitative trait determination, trade-offs among traits and interactions with the environment (Neve *et al.*, 2009). However, *in silico* selection needs to be complemented by robust data to anticipate adaptive potential and speed of population shift when changing farming systems.

Acknowledgements

References

Ashworth MB, Walsh MJ, Flower KC, Vila-Aiub MM & Powles SB (2016) Directional selection for flowering time leads to adaptive evolution in *Raphanus raphanistrum* (wild radish). *Evolutionary Application* **9**, 619–629.

Baker HG (1974) The evolution of weeds. *Annual Review of Ecology and Systematics* **5**, 1–24.

Barrett SH (1983) Crop mimicry in weeds. *Economic Botany* **37**, 255–282.

Campbell LG, Snow AA & Sweeney PM (2009) When divergent life histories hybridize: insights into adaptive life-history traits in an annual weed. *New Phytologist* **184**, 806–818.

Clements DR & DiTommaso A (2011) Climate change and weed adaptation: can evolution of invasive plants lead to greater range expansion than forecasted? *Weed Research* **51**, 227–240.

Colbach N, Chauvel B, Darmency H, Délye C & Le Corre V (2016) Choosing the best cropping systems to target pleiotropic effects when managing single-gene herbicide resistance in grass weeds. A blackgrass simulation study. *Pest Management Science* **72**, 1910–1925.

Darmency H & Aujas C (1986) Polymorphism for vernalization requirements in a population of *Avena fatua* L. *Canadian Journal of Botany* **64**, 730–733.

DARMENCY H & GASQUEZ J (1983) Interpreting the evolution of a triazine-resistant population of *Poa annua*. *New Phytologist* **95**, 299–304.

DARMENCY H, COLBACH N & LE CORRE V (2017) Review: relationship between weed dormancy and herbicide rotations: implications in resistance evolution. *Pest Management Science* **73**, 1994–1999.

DÉLYE C, CLEMENT JAJ, PERNIN F, CHAUVEL B & LE CORRE V (2010) High gene flow promotes the genetic homogeneity of arable weed populations at the landscape level. *Basic and Applied Ecology* **11**, 504–512.

DÉLYE C, JASIENUK M & LE CORRE V (2013) Deciphering the evolution of herbicide resistance in weeds. *Trends in Genetics* **29**, 649–658.

GRESSEL J & KLEIFELD Y (1994) Can wild species become problem weeds because of herbicide resistance? *Brachypodium distachyon*: a case study *Crop Protection* **13**, 563–566.

HEAP I (2018) The international survey of herbicide resistant weeds. Online Internet Friday, June 2018. Available www.weedscience.org

JANA S & THAI KM (1987) Patterns of changes of dormant genotypes in *Avena fatua* populations under different agricultural conditions. *Canadian Journal of Botany* **65**, 1741–1745.

KAWECKI TJ, LENSKI RE, EBERT D, HOLLIS B, OLIVIERI I & WHITLOCK MC (2012) Experimental evolution. *Trends in Ecology and Evolution* **27**, 547–560.

MCNEILL J (1976) The taxonomy and evolution of weeds. *Weed Research* **16**, 399–413.

NAYLOR JM & JANA S (1976) Genetic adaptation for seed dormancy in *Avena fatua*. *Canadian Journal of Botany* **54**, 306–312.

NEVE P & POWLES S (2005) Recurrent selection with reduced herbicide rates results in the rapid evolution of herbicide resistance in *Lolium rigidum*. *Theoretical and Applied Genetics* **110**, 1154–1166.

NEVE P, VILA-AIUB M & ROUX F (2009) Evolutionary-thinking in agricultural weed management. *New Phytologist* **184**, 783–793.

OKA HI & CHANG WT (1959) The impact of cultivation on populations of wild rice, *Oryza sativa* f. *spontanea*. *Phyton* **13**, 105–117.

OWEN MJ, GOGGIN DE & POWLES SB (2015) Intensive cropping systems select for greater seed dormancy and increased herbicide resistance levels in *Lolium rigidum* (annual ryegrass). *Pest Management Science* **71**, 966–971.

VIGUEIRA CC, OLSEN KM & CAICEDO AL (2013) The red queen in the corn: agricultural weeds as models of rapid adaptive evolution. *Heredity* **110**, 303–311.

WALSH MJ, BROSTER JC, AVES C & POWLES SB (2018) Influence of crop competition and harvest weed seed control on rigid ryegrass (*Lolium rigidum*) seed retention height in wheat crop canopies. *Weed Science* **66**, 627–633.

WARD SM, WEBSTER TM & STECKEL LE (2013) Palmer amaranth (*Amaranthus palmeri*): a review. *Weed Technology* **27**, 12–27.