

New frontiers in weed science: from transgenics to genomics

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Summary Weed science and weed management are being changed and challenged on several fronts by biotechnology. Transgene flow from crops to volunteer crops and weeds can introduce novel fitness enhancing genes—from insect resistance to herbicide tolerance and beyond—into the weed landscape. The case-in-point of multiple herbicide tolerance in canola (*Brassica napus* L.) comes to mind as one new problem. As much as transgene flow issues might resonate in the minds of regulators and the general populace, a more prominent problem is, and will be, the evolution of herbicide tolerance in weeds. Of greatest concern is the evolution of glyphosate resistance in ryegrass and horseweed. More species are certain to follow. Compared with new herbicide resistance conferred by crop-to-weed transgene flow, spontaneous evolution of glyphosate resistance is far and away a greater problem for at least two reasons. First, the acreage affected is much greater—the US state of Tennessee alone has approximately a half-million hectares of glyphosate resistant horseweed. This one case could lead to new regulatory oversight in herbicide resistance management. Second, the global resource of glyphosate as an effective and safe herbicide, and enabler of reduced tillage practices is at risk. Thus, the paradoxical combination of new weed problems as the result of biotechnology and new biotechnology and genomics techniques lead us to the opportunity and need to dissect the genome of some model weeds (weed scientists know that *Arabidopsis* is not a *real* weed). Developing weed genomic models could not only help us understand the molecular mechanisms of herbicide tolerance, but should also lead to the elucidation of the genetic basis of increased competitiveness and other weediness traits. Experience with *Brassica* crops and weeds such as horseweed should prove illustrative as to the future role of biotechnology in weed science.

Keywords *Brassica napus*, *Brassica rapa*, *Conyza canadensis*, gene flow, introgression, herbicide tolerance, glyphosate, functional genomics.

INTRODUCTION

In the ‘good old days’ the paradigm of spray and pray was simple. In the really good old days, ploughs, mules or hoes were the dominant weed technology. How times have changed. There is no doubt that the most technologically challenging days for the weed scientist lie ahead. Both emerging problems and solutions will have technology at their respective cores. Seemingly, weed scientists must worry about hybridisation and

introgression from transgenic crops to weeds and the evolution of herbicide resistant biotypes. The combination of a deployment of herbicide tolerant crops and subsequent weed evolution for resistance to the herbicide of choice is especially troubling for farmers, biotechnology companies and regulators alike. Fortunately, the news is not entirely bad. Biotechnologies have many more benefits than risks, and new genomics technologies hold the promise to better understand weed biology. Thus, this paper will analyse the state of the science, and prognosticate as to the direction we should go. As is customary, problems shall be explored first, then probable solutions will be proffered.

GENE FLOW FROM CROPS TO WEEDS: ARE TRANSGENES MORE WICKED THAN REGULAR GENES?

Newspapers and non-government organisations (NGOs) alike have focused intensely on gene flow as a route of transgene escape from crops into weeds. The fear is not totally without merit. Stewart *et al.* (2003) reviewed the likelihood of transgenes introgressing from crops to their wild relatives and also assessed potential problems associated with certain phenotypes conferred by appropriate transgenes. The transfer of herbicide tolerance traits from crops to related weeds has been of special interest for regulators and weed scientists. Certainly, hybridisation and introgression from transgenic crops to weeds is possible, especially in the following crops and weeds. The following crop/weed combinations fall under the moderate risk category for introgression: alfalfa (*Medicago sativa* L. ssp. *sativa*)/wild alfalfa (*Medicago sativa* L.); bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* L. ssp. *turgidum*)/wild *T. turgidum* and *Aegilops* species; canola (*Brassica napus* L.)/wild turnip or field mustard (*Brassica rapa* L., to slightly confuse the matter, canola quality *B. rapa* also exists); and sunflower (*Helianthus annuus* L.)/wild sunflowers (*Helianthus* species). The single plant in the high-risk category for transgene introgression is sorghum (*Sorghum bicolor* (L.) Moench.)/johnsongrass (*Sorghum halepense* (L.) Pers.). This last combination is of special interest because of the noxiousness of johnsongrass. In the high-risk category it is quite unlikely that any transgenic crop sorghum will ever be engineered, and certainly not for herbicide tolerance. The other combination of special interest worth noting here is canola/wild turnip. Hybridisation in an agronomic situation has occurred between these two

plants in the field in Canada, where herbicide tolerant canola varieties have been extensively planted (Warwick *et al.* 2003). Thus, the first crop \times wild hybrids for glyphosate tolerance have arisen in the field with the potential for backcross (BC) hybrids (back to wild *B. rapa*) as reality.

For several years my lab and collaborators have sought to better understand the relationship between transmission genetics (e.g., linkage effects, probability of transfer, etc.), transgene expression, and competition of transgenic weeds in this canola/*B. rapa* system. We have not been investigating herbicide resistance, but rather, insect resistance (conferred by a *Bacillus thuringiensis* (Bt) *cry1Ac* gene, which kills certain caterpillars) along with a green fluorescent protein (GFP) marker to facilitate fieldwork. Simply, the GFP marker gene can be tracked and quantified in the field using several techniques (Halfhill *et al.* 2001, Stewart 2001 and Millwood *et al.* 2003).

Multiple transgenic events have been produced in canola with these transgenes, and hybridisation and introgression (back to several accessions of weedy *B. rapa*) experiments have been performed in the greenhouse and field. The transgenes are expressed in both genetic backgrounds (Halfhill *et al.* 2002, 2003), and introgression rates are variable, indicating that there is a transgenic event and *B. rapa* accession effects in transgene transmission (Halfhill *et al.* 2001, 2002). One recently published study used controlled handcrosses moving pollen from transgenic plants to *B. rapa* to produce hybrids and up to 4 BC generations (Zhu *et al.* 2004). Segregation distortion was observed with certain transgenic events beginning in the BC₂ generation and persisting through BC₄. Thus, the inference is that transgenes located on certain genomic blocks (*B. napus* or canola is AACC) might be transmitted at less than expected frequencies to the *B. rapa* (AA) wild relative.

In the field, hybridisation rates between crop and weed (at multiple locations at different years) was 10% (with a large range among transgenic events) under a high crop:weed ratio (600:1) and 2% under a lower (180:1) ratio (Halfhill *et al.* 2004a). Backcrossing occurred much less frequently in the field (when *B. rapa* plants served as the maternal parent) – frequencies were 0.088% and 0.060% (Halfhill *et al.* 2004a). Even though BC₁ rates were low and introgression might be decreased using transgenic events in which the transgene is located on a chromosomal locus that is introgressed at a lesser frequency, it appears as if there is a fair chance that transgenes might persist in the wild relative (Stewart *et al.* 2003).

That said, recent studies muddy the water of this system. Competition experiments were performed in

which transgenic BC₂F₂ *B. rapa* plants were competed against wheat, and the biomass and yield of wheat were used as a competitive (weediness) index (Halfhill *et al.* 2004b). Transgenic weeds did not compete better than canola, non-transgenic *B. rapa*, or the non-transgenic BC₂F₂ *B. rapa* plants, and in one case, transgenic weeds even performed worse than these controls as competitors. In looking more closely at the transgenic BC₂F₂ plants, it is apparent that although they have identical chromosome numbers as *B. rapa* (20), they contain between 15 and 29% *B. napus*-specific amplified fragment length polymorphic markers (Halfhill *et al.* 2003). Thus it can be concluded that the transgenic weeds are less weedy and more ‘croppy’ (genomically-speaking) than wild type *B. rapa* weeds. These recent results cast some doubt as to persistence of fitness-enhancing transgenes in weeds as the result of gene flow. I contend that, considering even herbicide resistance traits, we have over-focused our research energy on gene flow and not enough on spontaneous evolution of herbicide resistance.

A BIGGER PROBLEM: SPONTANEOUS EVOLUTION OF HERBICIDE RESISTANCE

The spontaneous evolution and selection of herbicide tolerance *de novo* in weeds has caused new weed problems (Gressel 2002). An emerging problem, probably the result of an over-reliance on one herbicide, is glyphosate resistance in weeds. For example, in 2000 the first glyphosate resistance in a dicot weed (*Coryza canadensis* L.), horseweed, was described in Delaware (VanGessel 2001). A few resistant plants also appeared in Tennessee. This past field season, over 1,000,000 hectares of cropland were infested with glyphosate resistant horseweed in the US and new resistant populations seemed to be spreading (Main *et al.* 2004). It appears as if the resistance gene is not a 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) mutation (such as the transgenic CP4 resistance gene engineered into soybean and other crops), but is transmitted as if it were located on a single locus (Mueller *et al.* 2003 and Feng *et al.* 2004). The molecular mechanism of resistance is still unknown. Scientists at Monsanto and Tennessee are performing experiments to attempt to elucidate the resistance gene and the population genetics behind the spread. So, in contrast to land infested with introgressed herbicide resistance in *B. rapa* (tens of hectares), the amount of resistant horseweed is daunting. Thus, spontaneous evolution of herbicide resistance would appear, at first glance, to be orders of magnitude more important than introgressed herbicide tolerance from crops to related weeds. This problem illustrates the need for advanced development of tools and knowledge in weed genomics.

WEED GENOMICS: NEW TOOLS TO DEFEAT OLD ENEMIES

The maxim rings true: to defeat your enemy you must first know him. Genomics research holds the promise to know weeds more intimately than ever; from the parts list to the wiring diagram. Weed genomics research is necessary to dissect weediness traits and provide the tools needed to understand the evolution of herbicide resistance as well as to identify important genes conferring resistance (Basu *et al.* 2004).

Comparative genomics between plant model species and weeds, map-based approaches, genomic sequencing and functional genomics research can play vital roles in better understanding weed biology. Some candidate genomics approaches and what-could-be model weeds will be introduced.

Comprehensive genomics research performed on the ultimate model weed, *Arabidopsis thaliana* (L.) Heynh., has been a booming success. Even though *Arabidopsis* is a lousy weed, it is, however, an excellent model plant as the result of its small size, small genome, quick generation time, and ease of genetic transformation. *Oryza sativa* (rice) is the most prominent model crop for genomics, because it also possesses many of these qualities. Thus, we would emulate these successes in arguing for the weed science community to establish certain model genomic weeds. In developing this argument, we shall also discuss some of the methodologies that might help identify weediness genes.

All the following have moderate-sized genomes under 1×10^9 bases and are potential weed genomics candidates: *Amaranthus* species (also known as pigweeds), horseweed (*Conyza canadensis*), wild turnip (*Brassica rapa*) and weedy red rice (*Oryza sativa* L.). *Amaranthus* spp. are vigorous summer annuals that have widespread agronomic importance in crops. Common waterhemp (*Amaranthus rudis* J.D.Sauer) is a dominant weed in the midwestern US infesting several million hectares. Palmer pigweed (*Amaranthus palmeri* (S.) Wats.) is a dominant weed in the southern US over millions of hectares. *Amaranthus* species are, however, global pests. Smooth pigweed (*Amaranthus hybridus* L.) might serve as the best pigweed genomic model since, unlike the others, it is monoecious and self-compatible.

The second weed candidate, horseweed, has already been discussed, and would be a good model to understand the genomics of herbicide resistance. *Amaranthus* spp. and *C. canadensis* should ultimately be studied using the full suite of genomic tools, but are probably not the appropriate first choices to dissect weediness using genomics because of the cost of genome-wide sequencing. However, if funds were available, it seems that the first full-blown genomics project on a weed should be performed on an *Amaranthus* species because

of its rapid evolution, widespread dominance, and economic importance. It is the consummate weed.

Wild turnip and red rice are potentially the best models because of an inordinate amount of latent genomics information within arm's reach. *B. rapa*, as discussed above is of concern because of transgene introgression, but its close relatedness to *A. thaliana* in the Brassicaceae is the genomic attraction. As a result of extensive synteny, much of the *Arabidopsis* genomics would be directly relevant to *Brassica* (Paterson *et al.* 2001) (for more information on the *Brassica* genome initiative visit: <http://www.brassica.info>). In addition, there is a large number of agronomically important *B. rapa* crop types (e.g. canola, Polish rape, and turnip) that could be compared with weedy *B. rapa* types.

Finally, weedy red rice is usually the same species as cultivated Asian rice, which is a genomics model in its own right. While important *Oryza* species have several genomes, *O. sativa* (red and cultivated) shares the AA genome (a different AA genome than *Brassica*). With regard to weediness and domestication rice research is probably most advanced and discussed in various sections below. By first investigating wild turnip and red rice for weediness genes, sequence data, microarrays, and other genomic tools might be immediately applied to better understand the molecular basis of weediness.

Genomic techniques such as QTL (quantitative trait loci) mapping using available maps and genomic information from rice and other crops has already been fruitful in elucidating important QTL for weediness traits, such as dormancy, described more fully in Basu *et al.* (2004). For example, a weedy rice biotype that had increased seed dormancy was crossed with a crop breeding line to produce BC₁ individuals and QTL for dormancy were identified (Gu *et al.* 2004). Six QTL explaining significant dormancy and epistases led the researchers to infer that dormancy in rice has complex control networks, which might have important implications for genomics research in weeds. In another example, three QTL associated with vegetative dispersal by rhizomes, a weediness trait, were predicted in johnsongrass (*Sorghum halepense*). Progeny resulting from a cross between cultivated and wild species of *Sorghum* were screened using maps and important QTL were identified (Paterson *et al.* 1995). In another study, major QTL that were responsible for perennial habit were identified in johnsongrass and rice, indicating convergence in these traits (Hu *et al.* 2003).

Comparative genomics seem to be the entrée into weed genomics and other tools such as microarray analyses and transgenic knockouts should be helpful as the science develops. My lab has produced transgenic

B. rapa and *C. canadensis* (unpublished data) on the way to using transgenic approaches to facilitate weed genomics research.

CONCLUSIONS

Exciting and challenging times are on the horizon for weed science as well as invasives biology. Biotechnology and genomics will certainly solve more problems than they create in these areas and adaptability and cooperation will be valuable traits for weed scientists in the 21st century as new demands on agriculture production and environmental stewardship are imposed.

ACKNOWLEDGMENTS

I am indebted to my colleagues who have performed so much research that writing such a chapter is made relatively easy.

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