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Gene technologies in weed management: a technical feasibility analysis

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With the advent of new genetic technologies such as gene silencing and gene drive, efforts to develop additional management tools for weed management is gaining significant momentum. These technologies promise novel ways to develop sustainable weed control options because gene silencing can switch-off genes mediating adaptation (e.g. growth, herbicide resistance), and gene drive can be used to spread modified traits and to engineer wild populations with reduced fitness. However, applying gene silencing and/or gene drive is expected to be inherently complex as their application is constrained by several methodological and technological difficulties. In this review we explore the challenges of these technologies, and discuss strategies and resources accessible to accelerate the development of gene-tech based tools for weed management. We also highlight how gene technologies can be integrated into existing management tactics such as classical biological control, and their possible interactions.

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Introduction

Despite the ongoing use of gene technologies in plants for crop improvement, their utility in combating weeds or invasive plants has received relatively little attention [1,2]. Broadly, gene technologies can be categorized into two broad concepts based on their delivery mechanism for practical applications in weed/pest management: exogenous gene silencing and self-perpetuating gene drive. Gene silencing can be achieved through topical application of double-stranded RNA (dsRNA) which can alter the target organism to suppress phenotypic traits. For instance, dsRNA specific to target genes can be developed and topically applied to silence genes mediating functional traits (e.g. growth and development pathways, herbicide resistance) through RNA-interference (RNAi). Alternatively, gene drives can conceivably be engineered to spread particular traits (that could be modified through gene silencing or genome editing) among populations via distorted segregation. For instance, gene drive could possibly spread non-lethal genetic modifications among populations by releasing trait-modified (e.g. herbicide susceptible) individuals into the wild [1].

Recent advances and access to genetic tools that can reliably manipulate genes within the plant genome offers the option of modifying-specific traits in weeds and to drive those traits among populations to suppress or replace the entire population. However, gene silencing and gene drive are inherently complex, and several factors can affect developing these technologies. Neve [2] has outlined different gene drive systems and discussed some of the factors (transformation systems, DNA repair mechanism and weed biology) that potentially affect gene drives in weed management. Our aim is to explore-specific methodological complexities associated with gene silencing and gene drive research, and to discuss plant ecological traits that might affect deployment of gene technologies in weed management.

We focus initially on challenges associated with gene silencing and discuss strategies for efficient silencing. We then summarize challenges associated with gene drive and provide strategies to circumnavigate those constraints. In the remainder of the review, we focus on generic challenges common for both gene silencing and gene drive, which include lack of genomic and transcriptomic resources, and discuss risks associated with gene technologies. Finally, potential interactions, both positive and negative, of the two technologies with biological control agents will be briefly discussed.

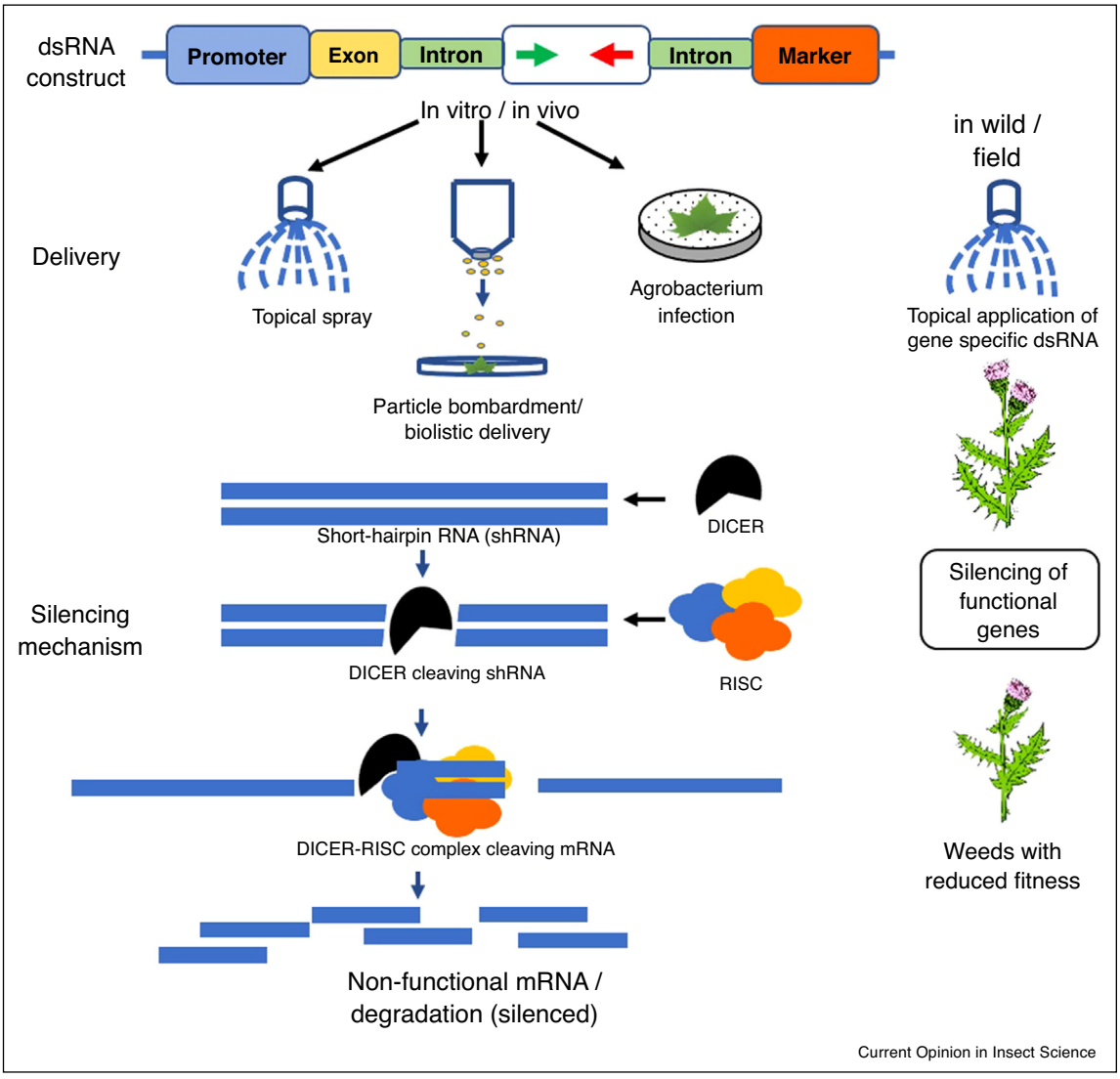
Gene silencing

Genes can be transcriptionally silenced by genome imprinting, paramutation, transposons and RNA-directed DNA methylation [3,4]. However, post-transcriptional silencing or RNAi through microRNA and small-interfering RNA (siRNA) has revolutionized gene silencing attempts because of its precision, efficiency and ability to be induced exogenously through topical application. siRNAs especially have been extensively used in crop improvement and crop protection research [4]. Steps involved in siRNA-mediated RNAi (Figure 1), major considerations and strategies for improvement in each step to adapt to weed management are discussed below.

Stability and efficiency of dsRNA constructs

The criteria for selecting an siRNA sequence for a dsRNA construct are summarized in Agrawal *et al.* [5]. Briefly, sequences from 50 and 100bp downstream of the start codon, with 30–70% of GC content, of 21 nt length and with 5′-phosphate and 3′-hydroxyl group are efficient. dsRNA constructs containing hairpin RNAs (hpRNA) can produce transient silencing of target genes [6], and these can be made as intron or intron-less, anti-sense and co-suppression constructs each with varying stability and efficiency [4,7]. Among these, constructs with introns in the spacer regions have been found to be stable and efficient in a wide range of plant species [8]. These vectors appear to help

Figure 1



Schematic representation of RNAi workflow. RNAi either neutralizes targeted mRNA molecules thereby inhibit translation or degrades mRNA post transcription. Exogenous RNAi is most commonly mediated by delivery of double-stranded RNA (dsRNA) and associated proteins into plants that are converted into small-interfering RNAs (siRNA) by DICER enzyme. After incorporation into multi-subunit ribonucleoprotein complex, siRNA leads to mRNA degradation.

align the arms of the hpRNA for duplex formation better than intron-less hpRNA (Figure 1) [9]. Several intron-splicing vector systems (e.g. pHANNIBAL, pKANNINAL, pHELLSGATE, pANDA, pIPK) are available to design efficient constructs [10]. These vectors were made such that they can be readily used to construct dsRNA and can be delivered into plants.

Delivery mechanisms

Endogenous delivery of dsRNA into plant tissues can be achieved by a diversity of methods including viral infection, *Agrobacterium* infection or direct delivery using gene gun approaches [7]. Kodama and Komamine [11••] synthesized detailed protocols for these delivery mechanisms. Several plant viruses (e.g. Potato virus X, barley stripe mosaic virus) have been used as vectors for RNA silencing [12], so a weed-virus combination capable of carrying siRNA expression cassettes could be used for delivery. Similarly, plant viruses used for delivery of cargo molecules in plants in the laboratory and completely synthetic virus-like particles that self-package their own RNA and potentially cargo RNAi molecules could also be modified for RNAi delivery [13,14]. More recently, cell-penetrating peptides and chemical modification of dsRNA have been found to enhance the longevity of dsRNA [15,16].

Exogenous/topical application and uptake of dsRNA into plant cells have been constrained for many years by lack of effective delivery mechanisms. Multiple factors affect the efficient uptake of dsRNA by recipient plant tissues, including the length and concentration of the dsRNA, environmental conditions, tissue characteristics and physiological response (e.g. degradation of dsRNA) of target weeds to exogenous RNA. Uptake of dsRNA into cells through leaves [17•], roots [18,19] and flower buds [20] has been achieved in the laboratory. Spray-on methods are the most practical technique for weed management, but spray-induced gene silencing has only recently been demonstrated in plants [17•,21•].

Large-scale production of dsRNA

One of the critical considerations to translate the RNAi effect for field-level weed management would be the large-scale production of dsRNA. *In vitro* synthesized dsRNA constructs are inexpensive to use for laboratory research, but do not scale economically for levels that would be required for use of RNAi as herbicides. Attempts are being made (e.g. viral-based, yeast-based and bacteriophage-based RNA replication system) to enable mass production of dsRNA at a cheaper cost [22,23]. Self-perpetuating viral vectors carrying dsRNA that can transmit among plants can be engineered to silence genes (engineered biocontrol agent). However, the spread of dsRNA among populations will be impacted by considerations we cover under 'gene drive' below.

Gene drive

Gene drives are selfish genetic elements that advantage their own transmission through sexual reproduction and can potentially spread through entire populations [24]. The spread of gene drives through a population is mainly governed by the relative strength of the drive (i.e. deviation from Mendelian inheritance) compared to any fitness cost associated with the drive [25]. The spread of modified traits through a population can occur even in the presence of a fitness cost to the organism, as individuals with a gene drive element can produce more gametes and subsequently more offspring with the 'drive allele' than without it.

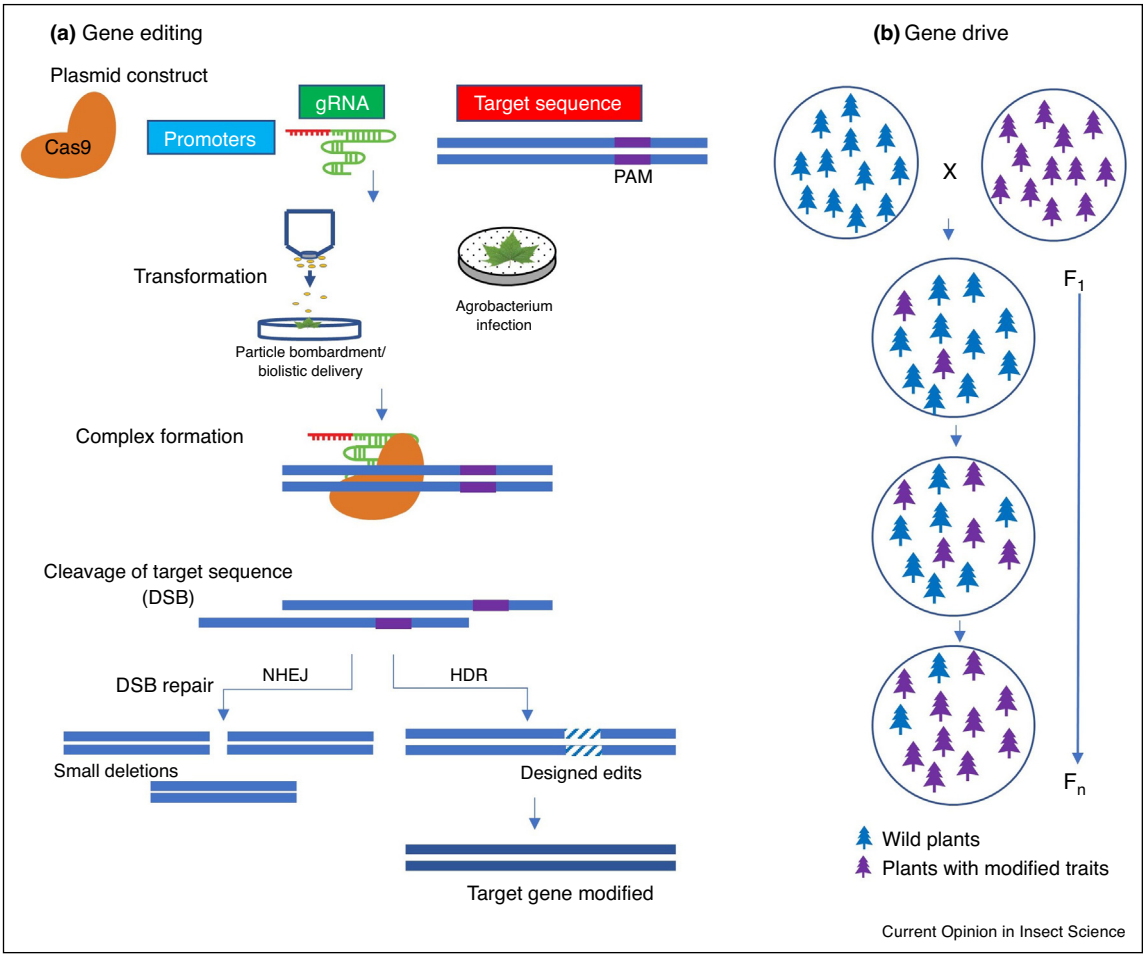
Gene drives can occur naturally [26,27••] or can be engineered as briefly described below (Figure 2). A variety of engineered drive systems (e.g. meiotic drive, underdominance and homing endonucleases) have been described. Among these, homing-based drives are being developed in several systems given the flexibility with tools available for genome editing. For example, Engineered Zinc-Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs) and Clustered Regularly Interspaced Palindromic Repeat (CRISPR)-associated Cas proteins can introduce precise genome modification [28]. RNA-guided CRISPR-Cas9 has to-date offered greater potential than ZFNs and TALENs and remain the method of choice for genome engineering, primarily due to easier construct development, effective target recognition, suitability for multiplexing which enables simultaneous targeting of multiple genes and the ability to delete large chromosome or gene clusters [28,29,30•].

We discuss below drive-specific constraints such as drive efficiency, resistance alleles and repair mechanisms, in addition to plant ecological traits that may affect the spread of drive alleles. For gene drives that rely on endonucleases, key constraints of genome editing also need to be considered to design effective constructs for efficient cleavage of target sites and to select appropriate transformation systems. Strategies to improve genome editing are discussed at length in literature [31,32] and are also summarized below.

Gene editing considerations for endonuclease-based drives

Gene editing of laboratory strains to be released into wild is affected by guide-RNA (gRNA) design for site selection, CRISPR-Cas9 construct, transformation system, delivery mechanisms and off-target editing. Several computational and web-based tools (e.g. E-CRISPR, CRISPRscan, CHOPCHOP, CRISPR-PLANT, CRISPRdirect, Cas-OFFinder, VARSCOT) are available to assist with scanning genomes, selecting unique targets, designing and constructing Cas9/gRNA, predicting gRNA efficiency and off-target editing [33–36]. Among

Figure 2



Schematic representation of endonuclease gene editing and gene drive workflows. **(a)** In CRISPR-mediated editing, constructs encoding sequence-specific Cas nucleases (e.g. Cas9) directed by specific guide RNA (gRNA) generate double-strand breaks (DSBs) at target loci. Then the DSB is repaired by either non-homologous end joining (NHEJ), which modifies the broken DNA ends and ligates them together, or by homology directed repair (HDR), which uses an undamaged DNA template to repair DSB. The repair through HDR enables precise genomic manipulations and can therefore be used to generate targeted gene replacement, knockout, insertion or editing at precise sites within the genome. **(b)** Such modified genes can be driven into populations through gene drive. Ideally, alleles conferring negative traits would perpetuate to spread to subsequent generations until population suppression or replacement of wild plants is achieved.

these, CRISPR-P, CGAT, CRISPR-PLANT and CRISPR-Local are specifically developed for plants and can be adapted for weeds [37,38]. There are customized CRISPR-Cas9 systems (e.g. meristem-specific, germline-specific, viral-based and DNA-free systems) and transformation systems (e.g. *Agrobacterium*-mediated transformation, particle bombardment) successfully used in crop plants that could be adapted in related weed species [32]. Similarly, protospacer adjacent motif (PAM) next to the 3′ end of target sequence, longer PAM and optimal Cas9/gRNA concentrations are thought to reduce the risk of off-target mutations [31,39].

Homing efficiency

By definition, a successful gene drive relies on the efficiency of the drive mechanism which, in the case of a nuclease-based drive, is reliant on the homing efficiency. Cleavage and homing should occur at appreciable frequencies in germline, not in somatic cells, for the drive alleles to spread among populations [40]. In engineered drives, constructs or cargos cannot impose fitness costs which outweigh the homing rate. In wild populations, genomic sequence variation (e.g. polymorphic sequences) may affect the targeting and cleavage and eventually affect the homing efficiency [41] so a population level understanding of genetic variation at the target sites is essential.

Resistant alleles

Alleles resistant to endonucleases-based gene drives can emerge through error-prone copying and during double strand break (DSB) repair [42••]. Resistant alleles resulting from these are likely to spread rapidly through

populations because of strong selection to escape the deleterious effects of the drive [43,44], which in turn can affect the drive depending on their fitness effects.

Targeting highly conserved sites in genes, genes mediating traits favoured by natural selection, promoters enabling high germline drive conversion rate and gRNA multiplexing have been recommended to limit the emergence of resistant alleles [30•,43,45] but this is yet to be empirically tested [46,47]. In weed management, recurrent release of drive alleles may be required to counteract and eventually eliminate the emergent resistance alleles.

Repair mechanisms

Non-homologous end joining (NHEJ) and homology-directed repair (HDR) are two competitive repair mechanisms involved in repairing DSB in DNA [48,49]. NHEJ is thought to be the dominant repair mechanism in plants and can be more error-prone in plants than in other organisms [48,49]. HDR is more precise than NHEJ and is an absolute requirement for functionality of nuclease-based gene drives [33,35]. Suppression of NHEJ through gene silencing, inhibitors and enhancers, enriching cells with HDR alleles and synchronizing Cas9 delivery with the cell cycle may increase the frequency of HDR [50]. However, these strategies are proposed for mammalian cells and hence validation is required in plants. The relative frequency of HDR or NHEJ in weeds is not known. However, these processes are highly conserved in plants [51], and hence knowledge from model/crop plants can be translated into weeds.

Reproductive biology of weeds

Gene drive is limited by mode of reproduction, with limited applications in fully self-compatible and asexually reproducing weed species [24]. For example, autogamous species (e.g. *Polygonum* spp., *Chenopodium* spp., *Conyza canadensis*, *Senecio vulgaris*, *Cirsium vulgare*) and apomictic weed species (e.g. *Chondrilla juncea*) may not be good targets for gene drives. Cross-fertilization and self-incompatibility are common across weed species (e.g. *Lupinus arboreus*, *Cyperus rotundus*, *Cynodon dactylon*, *Eichhornia crassipes*) and, in many cases is essential for reproduction (e.g. *Lantana camara*). These modes of reproduction should make such weeds good targets for control using gene drives.

For the same reasons, in mixed-mating species, higher rates of outcrossing will be required to promote the spread of drive alleles [52]. Spread of the drive may be greater in a fully outcrossing species relative to species with complex and specialized pollination biology that might impact the outcrossing rate. The relative influence of other reproductive traits (e.g. dioecy, protandry, protogyny, dichogamy) on outcrossing rates and gene drive efficiency also needs further elucidation.

Generation time, spatial ecology, fecundity and seed bank

Drive alleles are predicted to spread faster in weeds with shorter development times or higher reproductive rates [2]. Annuals with multiple generations in a year would be ideal, enabling rapid fixation of traits [2]. Likewise, outcrossing perennials with incessant reproduction also can be targeted to limit the emergence of fit progeny. However, annuals with multiple generations in a year carrying resistant alleles are also likely to allow for the faster emergence of drive-resistant alleles.

Regarding spatial distribution, drive alleles may spread effectively in a monoculture of large populations because of greater chance of outcrossing with 'drive' individuals, whereas targeted release of drive alleles may be required for spatially heterogeneous or isolated small populations. These predictions are difficult to validate under field conditions because of regulatory restrictions with genetic technologies, but simulation models can be used to predict and validate most of these assumptions [53].

Fecundity, propagule pressure and seedbank are also likely to significantly affect the spread and efficacy of gene drives. Species with high fecundity, seedling recruitment and seed dispersal will enable spread of drive alleles provided the release rate is above the threshold to cross-fertilize with most of the existing wild individuals. Likewise, a seedbank will have significant impact on penetration of the drive alleles in the population. In species with a short-lived or a moderately sized seedbank, a few generations may be enough to replace the population emerging from the seedbank. However, it can take more generations for drive alleles to spread in species with a long-lived seedbank or the frequency of drive alleles simply may not reach the threshold to achieve population suppression or replacement [27••]. Hence, prolific seed producers with poor seedbanks or with poor seed longevity (e.g. *Lychnis alba*, *Setaria glauca*) may be more suitable targets. Seed dormancy may also affect the spread of gene drives if seeds with drive alleles become dormant. In species with seasonal seedling emergence, the release of the drive allele should be made such that emergence and reproduction coincide with wild-type individuals.

Knowledge gaps in the deployment of gene technologies in weed management

In addition to above considerations specific to RNAi and gene drive, fundamental gaps in genetic knowledge also affect the development of gene technologies. The majority of weed species lack high quality genomic resources (e.g. an annotated genome and transcriptome) which will be a constraint to develop genetic tools for weed management. An annotated genome will help identify the physical location of DNA sequences, coding regions, noncoding sequences and open reading frames that are

important for gene silencing and to determine specificity in gene editing [31]. Transcriptome profiles with functionally characterized genes (and traits) will be crucial to target the genes underlying the trait of interest for both RNAi- and gene drive-based control strategies. While using model species can aid in this process, appropriate plant models are still lacking for important weed taxa. For homing-based gene drives, transformation systems and site-specific integration of gene drive modules will also be critical with the latter being poorly developed even in model systems [33].

Risk considerations

Unintended consequences of gene drive include spread of drive to non-target species through inter-specific hybridization. Hence, the risks with outcrossing species such as *Raphanus raphanistrum* and *Sorghum halapense*, that have been reported to hybridize with their crop congeners, are very high. In invasive species management, risks of a drive system altering or suppressing the native range population of invasive species through intra-specific hybridization are high especially in drive systems that are likely to be highly invasive (e.g. CRISPR-based) [30•,44].

Removing the drive system, or at least the effector gene, is important in the event of unintended consequences. A 'reversal drive' to counteract the first gene drive, a daisy-chain drive that will not spread indefinitely, a precision drive targeting a unique trait of a population and an immunizing drives which block the spread of other gene drive have been discussed as mitigation strategies [30•,54], but their feasibility in different contexts has yet to be established. Marshall & Akbari [55] proposed that threshold-independent drives that can spread independent of their frequency are highly risky compared to threshold-dependent and self-limiting drives since the latter only spread when they are released above a critical frequency and are easier to contain to their target populations. Risks associated with other gene drives such as chromosomal rearrangement and engineered underdominant constructs are lower since they are less likely to spread to non-target populations. The broader ecological context in which a gene drive is deployed needs to also be explicitly considered, as is the risk of the drive spreading from the deployed range to the native range of the target organism [1]. Alleles specific to the target population maybe targeted to avoid the gene drive effect on non-target (e.g. native range) populations [56].

Risks associated with RNAi silencing are relatively low compared to gene drive. Potential risks include off-target silencing of homologues genes in non-target plants and dissipation of topically applied RNAi into soil and its possible non-target silencing of functional genes in interacting organisms. *Prima facie* these risks are like those posed by existing chemical controls, but RNAi has the added benefit of (some) sequence specificity. In weed species for which classical biological control attempts are in place, the interactions between RNAi application and the biocontrol agents need to be assessed.

Gene drive or gene silencing

The choice between gene silencing versus gene drive will be influenced by the features of each of these methods in the context of the biology of the target organisms (Table 1). Almost any trait (including those mediated by housekeeping genes) can be targeted for RNAi. However, for gene drive, the targeted traits should not reduce fitness and the individual with gene drive alleles should be able to reproduce with wild individuals; these features are important for the modified trait to reach fixation within the target population. Manipulating herbicide resistance traits need not reduce fitness. Hence, weeds can reproduce as normal and inherit drive alleles which ultimately produces potential for conditional lethality via herbicide applications. Likewise, a trait that contributes to weediness (e.g. seed longevity), or reproduction (e.g. female sterility) can be targeted using gene drive since such modifications would not be lethal, but still have a desirable weed management outcome. Generally, gene

Table 1

Comparison of RNA-interference gene silencing versus gene drive. For integrated management, it may be possible to integrate RNAi with bioherbicides and gene drive with classical biocontrol provided there is a synergy, social acceptance and regulatory framework to achieve this

	RNAi	Gene-drive
Heritability	Non-heritable mostly; possibly heritable if the effect is epigenetic	Heritable
Sustainability	Augmentative/ repeated application is necessary	One-off[a]
Regulatory concerns[b]	Low	High to very high
Weed targets	Possibly in weeds of crops, and isolated populations of environmental weeds	For cropping as well as for landscape level management
Difficulty with achieving population suppression	Comparatively easier with stable dsRNA, efficient delivery and repeated applications	Complex - several factors can affect the expression of modified traits in lab adapted plants versus wild plants
Delivery mechanisms	Exogenous application of dsRNA specific to genes of interest. Topical application in cropping systems is possible	Release of transgenic individuals into the wild
Effect of mode of reproduction of weed species	Species with all modes of reproduction	Sexually reproducing and outcrossing species

[a] Dependent on the drive efficiency.
[b] Varies among countries.

drive is more likely to work in species with high outcrossing rates. Therefore, in highly selfing species RNAi can be considered. Further, gene drive is more complex than silencing, and release rate of drive alleles, stability of the drive alleles in subsequent generations and number of generations required for the drive allele to spread among population need to be modelled to engineer a successful gene drive.

Integrating gene technologies with other existing biocontrol options may be possible to maximize the impact of weed management tactics. For instance, it may be possible to integrate RNAi with bioherbicides and gene drive with classical biocontrol. Further, delivery of 'drive alleles' or dsRNA into population might be achieved through releasing genetically engineered biocontrol agents. Using insects (as vectors of plant viruses) to deliver desired traits (integrated into plant viruses) into plant populations has been proposed already [57]. It is possible that the aforementioned gene technologies interact antagonistically with existing management tools. For instance, plants with a gene drive alleles or silenced genes may indirectly affect the life history traits of biocontrol agents by rendering the plant nutritionally unsuitable; such aspects need to be assessed before any integration. All efforts to integrate genetic technologies with other forms of biological control need to be made under the appropriate regulatory frameworks with social acceptance of such integrative approaches. It would be prudent to ensure that any attempts at integration do not disrupt the social license currently utlized by beneficial technologies like classical biological control.

Conclusions

Gene technologies have significant potential, on their own or as integrated tools, to assist in the development of efficient and effective weed management solutions. Developing such solutions will require us to overcome various technical challenges (e.g. stability of RNAi construct and Cas9/sgRNA, drive conversion efficiency and practical applications) to ensure we effectively influence key weed traits in a manner to mitigate their impacts to agriculture and the environment. Additional key decisions will include the deployment of the most appropriate technologies (e.g. gene silencing, gene drives) in different contexts and against different targets to ensure the apt use of these approaches. It is important to pay heed to the fact that development and deployment of gene technologies are regulated under appropriate legislative frameworks in different countries and are subject to varying degrees of intellectual property (IP) considerations (e.g. license agreements using the Cas9 nuclease exclude use in gene drives). All this work also needs to be done with appropriate levels of public and stakeholder engagement to ensure that they are developed in a responsible manner and have the desired levels of social acceptance [58]. Navigating all these aspects to balance the risks, costs and benefits of these genetic approaches will be crucial to the safe and sustainable use of these technologies in weed management.

Declarations of interest

None.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Webber BL, Raghu S, Edwards OR: **Opinion: is CRISPR-based gene drive a biocontrol silver bullet or global conservation threat?** *Proc Natl Acad Sci U S A* 2015, **112**:10565-10567.

2. Neve P: **Gene drive systems: do they have a place in agricultural weed management?** *Pest Manag Sci* 2018, **74**:2671-2679.

3. Baulcombe D: **RNA silencing in plants**. *Nature* 2004, **431**:356.

4. Guo Q, Liu Q, Smith NA, Liang G, Wang M-B: **RNA silencing in plants: mechanisms, technologies and applications in horticultural crops**. *Curr Genomics* 2016, **17**:476-489.

5. Agrawal N, Dasaradhi P, Mohmmed A, Malhotra P, Bhatnagar RK, Mukherjee SK: **RNA interference: biology, mechanism, and applications**. *Microbiol Mol Biol Rev* 2003, **67**:657-685.

6. Echeverri CJ, Perrimon N: **High-throughput RNAi screening in cultured cells: a user's guide**. *Nat Revi Genet* 2006, **7**:373.

7. Watson J, Murison J, Grigor M, Havukkala I, Munro G, Abernethy N, Webster G: *Targeted Delivery of RNA Interference Molecules*. Google Patents; 2005.

8. Wesley SV, Helliwell CA, Smith NA, Wang M, Rouse DT, Liu Q, Gooding PS, Singh SP, Abbott D, Stoutjesdijk PA: **Construct design for efficient, effective and high-throughput gene silencing in plants**. *Plant J* 2001, **27**:581-590.

9. Helliwell C, Waterhouse P: **Constructs and methods for high-throughput gene silencing in plants**. *Methods* 2003, **30**:289-295.

10. Isshiki M, Kodama H: **Plant RNAi and crop improvement**. *Molecular Techniques in Crop Improvement*. Springer; 2010:653-673.

11. •• Kodama H, Komamine A: *RNAi and Plant Gene Function Analysis*. Springer; 2011
Authors summarized successful attempts of application of RNAi in plants and provided information about protocols (e.g. promoters, computational tools, transformation systems, delivery) successfully used in crop species that can be adapted to related weed species.

12. Horiguchi G: **RNA silencing in plants: a shortcut to functional analysis**. *Differentiation* 2004, **72**:65-73.

13. Zaidi SS, Mansoor S: **Viral vectors for plant genome engineering**. *Front Plant Sci* 2017, **8**:539.

14. Butterfield GL, Lajoie MJ, Gustafson HH, Sellers DL, Nattermann U, Ellis D, Bale JB, Ke S, Lenz GH, Yehdego A: **Evolution of a designed protein assembly encapsulating its own RNA genome**. *Nature* 2017, **552**:415.

15. Joga MR, Zotti MJ, Smagghe G, Christiaens O: **RNAi efficiency, systemic properties, and novel delivery methods for pest insect control: what we know so far**. *Front Physiol* 2016, **7**:553.

16. Numata K, Ohtani M, Yoshizumi T, Demura T, Kodama Y: **Local gene silencing in plants via synthetic ds RNA and carrier peptide**. *Plant Biotechnol J* 2014, **12**:1027-1034.

17. • Mitter N, Worrall EA, Robinson KE, Li P, Jain RG, Taochy C, Fletcher SJ, Carroll BJ, Lu GM, Xu ZP: **Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses**. *Nat Plants* 2017, **3**:16207

Using clay nanoparticles as carriers of dsRNA, authors demonstrated improved stability and sustained release of dsRNA inducing resistance to plant viruses. This is a significant improvement in the development of spray-on RNAi techniques.

18. Jiang L, Ding L, He B, Shen J, Xu Z, Yin M, Zhang X: **Systemic gene silencing in plants triggered by fluorescent nanoparticle-delivered double-stranded RNA**. *Nanoscale* 2014, **6**:9965-9969.

19. Li H, Guan R, Guo H, Miao X: **New insights into an RNAi approach for plant defence against piercing-sucking and stem-borer insect pests**. *Plant Cell Environ* 2015, **38**:2277-2285.

20. Lau S-E, Schwarzacher T, Othman RY, Harikrishna JA: **dsRNA silencing of an R2R3-MYB transcription factor affects flower cell shape in a Dendrobium hybrid**. *BMC Plant Biol* 2015, **15**:194.

21. • Koch A, Biedenkopf D, Furch A, Weber L, Rossbach O, Abdellatef E, Linicus L, Johannsmeier J, Jelonek L, Goesmann A: **An RNAi-based control of Fusarium graminearum infections through spraying of long dsRNAs involves a plant passage and is controlled by the fungal silencing machinery**. *PLoS Pathog* 2016, **12**:e1005901

Authors demonstrated spraying of a long noncoding dsRNA inhibiting the growth of *Fusarium graminearum* fungi. This research provides insights on developing RNAi-based strategies for spray-induced gene silencing.

22. Aalto AP, Sarin LP, Van Dijk AA, Saarma M, Poranen MM, Arumäe U, Bamford DH: **Large-scale production of dsRNA and siRNA pools for RNA interference utilizing bacteriophage ϕ6 RNA-dependent RNA polymerase**. *RNA* 2007, **13**:422-429.

23. Niehl A, Soininen M, Poranen MM, Heinlein M: **Synthetic biology approach for plant protection using ds RNA**. *Plant Biotechnol J* 2018, **16**:1679-1687.

24. Burt A: **Site-specific selfish genes as tools for the control and genetic engineering of natural populations**. *Proc R Soc Lond Ser B Biol Sci* 2003, **270**:921-928.

25. Sandler L, Novitski E: **Meiotic drive as an evolutionary force**. *Am Nat* 1957, **91**:105-110.

26. Lindholm AK, Dyer KA, Firman RC, Fishman L, Forstmeier W, Holman L, Johannesson H, Knief U, Kokko H, Larracuente AM: **The ecology and evolutionary dynamics of meiotic drive**. *Trends Ecol Evol* 2016, **31**:315-326.

27. •• Barrett LG, Legros M, Kumaran N, Glassop D, Raghu S, Gardiner DM: **Gene drives in plants: opportunities and challenges for weed control and engineered resilience**. *Proc R Soc B* 2019, **286**:20191515

Authors discussed natural gene drives occurring in plants. Discussed opportunities and challenges associated with natural gene drives for their utilization in weed population management.

28. Jinek M, East A, Cheng A, Lin S, Ma E, Doudna J: **RNA-programmed genome editing in human cells**. *eLife* 2013, **2**: e00471.

29. Gaj T, Gersbach CA, Barbas CF III: **ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering**. *Trends Biotechnol* 2013, **31**:397-405.

30. • Esvelt KM, Smidler AL, Catteruccia F, Church GM: **Emerging technology: concerning RNA-guided gene drives for the alteration of wild populations**. *eLife* 2014, **3**:e03401

Authors discussed underlying mechanism of engineered and endonuclease gene drives and their limitations. In addition, summarized Cas9 nuclease genome editing attempts and complexities associated with gene editing.

31. Hahn F, Nekrasov V: **CRISPR/Cas precision: do we need to worry about off-targeting in plants?** *Plant Cell Rep* 2019, **38**:437-441.

32. Mao Y, Botella JR, Zhu J-K: **Heritability of targeted gene modifications induced by plant-optimized CRISPR systems**. *Cell Mol Life Sci* 2017, **74**:1075-1093.

33. Belhaj K, Chaparro-Garcia A, Kamoun S, Patron NJ, Nekrasov V: **Editing plant genomes with CRISPR/Cas9**. *Curr Opin Biotechnol* 2015, **32**:76-84.

34. Cui Y, Xu J, Cheng M, Liao X, Peng S: **Review of CRISPR/Cas9 sgRNA design tools**. *Interdiscip Sci* 2018, **10**:455-465.

35. Peng R, Lin G, Li J: **Potential pitfalls of CRISPR/Cas9-mediated genome editing**. *FEBS J* 2016, **283**:1218-1231.

36. Wilson LO, Hetzel S, Pockrandt C, Reinert K, Bauer DC: **VARSCOT: variant-aware detection and scoring enables sensitive and personalized off-target detection for CRISPR-Cas9**. *BMC Biotechnol* 2019, **19**:40.

37. Lei Y, Lu L, Liu H-Y, Li S, Xing F, Chen L-L: **CRISPR-P: a web tool for synthetic single-guide RNA design of CRISPR-system in plants**. *Mol Plant* 2014, **7**:1494-1496.

38. Sun J, Liu H, Liu J, Cheng S, Peng Y, Zhang Q, Yan J, Liu H-J, Chen L-L: **CRISPR-local: a local single-guide RNA (sgRNA) design tool for non-reference plant genomes**. *Bioinformatics* 2018, **35**:2501-2503.

39. Wu X, Kriz AJ, Sharp PA: **Target specificity of the CRISPR-Cas9 system**. *Quant Biol* 2014, **2**:59-70.

40. Prowse TA, Cassey P, Ross JV, Pfitzner C, Wittmann TA, Thomas P: **Dodging silver bullets: good CRISPR gene-drive design is critical for eradicating exotic vertebrates**. *Proc R Soc B Biol Sci* 2017, **284**:20170799.

41. Oberhofer G, Ivy T, Hay BA: **Behavior of homing endonuclease gene drives targeting genes required for viability or female fertility with multiplexed guide RNAs**. *Proc Natl Acad Sci U S A* 2018, **115**:E9343-E9352.

42. •• Unckless RL, Clark AG, Messer PW: **Evolution of resistance against CRISPR/Cas9 gene drive**. *Genetics* 2017, **205**:827-841

Modeling the dynamics of CRISPR/Cas9 gene drive, authors calculated probability of occurrence of resistant alleles due to mutation of wild type alleles and DNA repair. This framework provides insights and strategies to develop gene drives with limited resistance potential.

43. Marshall JM, Buchman A, Akbari OS: **Overcoming evolved resistance to population-suppressing homing-based gene drives**. *Sci Rep* 2017, **7**:3776.

44. Noble C, Olejarz J, Esvelt KM, Church GM, Nowak MA: **Evolutionary dynamics of CRISPR gene drives**. *Sci Adv* 2017, **3**: e1601964.

45. Champer SE, Oh SY, Liu C, Wen Z, Clark AG, Messer PW, Champer J: **Computational and experimental performance of CRISPR homing gene drive strategies with multiplexed gRNAs**. *bioRxiv* 2019:p679902.

46. Kyrou K, Hammond AM, Galizi R, Kranjc N, Burt A, Beaghton AK, Nolan T, Crisanti A: **A CRISPR–Cas9 gene drive targeting doublesex causes complete population suppression in caged *Anopheles gambiae* mosquitoes**. *Nat Biotechnol* 2018, **36**:1062.

47. Champer J, Liu J, Oh SY, Reeves R, Luthra A, Oakes N, Clark AG, Messer PW: **Reducing resistance allele formation in CRISPR gene drive**. *Proc Natl Acad Sci U S A* 2018, **115**:5522-5527.

48. Tuteja N, Singh MB, Misra MK, Bhalla PL, Tuteja R: **Molecular mechanisms of DNA damage and repair: progress in plants**. *Crit Rev Biochem Mol Biol* 2001, **36**:337-397.

49. Manova V, Gruszka D: **DNA damage and repair in plants–from models to crops**. *Front Plant Sci* 2015, **6**:885.

50. Danner E, Bashir S, Yumlu S, Wurst W, Wefers B, Kühn R: **Control of gene editing by manipulation of DNA repair mechanisms**. *Mamm Genome* 2017, **28**:262-274.

51. Yoshiyama K, Sakaguchi K, Kimura S: **DNA damage response in plants: conserved and variable response compared to animals**. *Biology* 2013, **2**:1338-1356.

52. Bull JJ, Remien CH, Krone SM: **Gene-drive-mediated extinction is thwarted by evolution of sib mating**. *bioRxiv* 2019:p558924.

53. Champer J, Kim I, Champer SE, Clark AG, Messer PW: **Suppression gene drive in continuous space can result in unstable persistence of both drive and wild-type alleles**. *bioRxiv* 2019:p769810.

54. Noble C, Min J, Olejarz J, Buchthal J, Chavez A, Smidler AL, DeBenedictis EA, Church GM, Nowak MA, Esvelt KM: **Daisy-chain gene drives for the alteration of local populations**. *Proc Natl Acad Sci U S A* 2019, **116**:8275-8282.

55. Marshall JM, Akbari OS: **Can CRISPR-based gene drive be confined in the wild? A question for molecular and population biology**. *ACS Chem Biol* 2018, **13**:424-430.

56. Sudweeks J, Hollingsworth B, Blondel DV, Campbell KJ, Dhole S, Eisemann JD, Edwards O, Godwin J, Howald GR, Oh K: **Locally fixed alleles: a method to localize gene drive to island populations**. *bioRxiv* 2019:p509364.

57. Reeves RG, Voeneky S, Caetano-Anollés D, Beck F, Boëte C: **Agricultural research, or a new bioweapon system?** *Science* 2018, **362**:35-37.

58. Emerson C, James S, Littler K, Randazzo FF: **Principles for gene drive research**. *Science* 2017, **358**:1135-1136.