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Engineered Gene Drives for Plant Pest Management

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INTRODUCTION

When Gregor John Mendel tracked pea-plant characteristics over successive generations in the nineteenth century, his landmark study revealed key insights into the fundamental mechanisms governing genetic inheritance. In this typical scenario of genetic inheritance, both maternal and paternal copies of a gene have an equal probability of being inherited and gave us the concept of random segregation and independent assortment. However, inheritance does not always proceed so fairly, and in some cases the odds of a particular copy of a gene being transmitted to the next generation can be heavily skewed. According to Collins (2018), gene drives are systems of biased inheritance that enhance the likelihood a sequence of DNA passes between generations through sexual reproduction and potentially throughout a local population and ultimately all connected populations of a species.

Broadly speaking, molecular mechanism of all gene drivesare classified into three types based on their ability to typically induce biased inheritance patterns, their spread, and their likelihood that the gene drive organisms (GDOs) will develop resistance in response to the drive cassette (Champer *et al.,* 2016). On the basis of occurrence, there exist two types of gene drives – natural and engineered. There are four types of gene drive occurring in nature: homing endonuclease genes (HEGs), segregation distorters, transposons, and heritable microbes (Miglani, 2019). Transposons are inherited in a non-Mendelian pattern (Conklin, 2019). Conner and Jacobs (2019) illustrate the presence of a naturally present conditional gene drive active in *Brassica napus* populations. Plants heterozygous for an allele conferring herbicide resistance at a single locus exhibited Mendelian inheritance. The first report on natural gene drives was given by Burt (2003) who suggested to use site-specific genetic elements as tools for control and genetic

engineering of natural populations.Site-specific selfish genes exploit host functions to copy themselves into a defined target DNA sequence, and include HEGs, group II introns and some long interspersed elements (LINE)-like transposable elements. If such genes can be engineered to target new host sequences, then they can be used to manipulate natural populations, even if the number of individuals released is a small fraction of the entire population.

Initially coined to describe the process of stimulating biased inheritance of particular genes to alter entire population of a species, the term gene drive is now increasingly used to describe the actual synthetic genetic element designed to increase in frequency over time in a population (Conklin, 2019). Such gene drives are popularly known as "engineered gene drives". Scientists are investigating how populations might be altered by adding, disrupting, or editing genes or suppressed by propagating traits that reduce reproductive capacity (Ouagrham-Gormley *et al.*, 2016) by using genome-editing technology.

CHARACTERISTICS OF GENE DRIVES

Homing, evolutionary stability, reversibility, and wide applicability are some important characteristics of gene drive. Homing genes have ability to copy themselves onto the opposite chromosomes that does not contain gene drive alleles. The copying process is termed 'homing', while the endonuclease-containing cassette that is copied is referred to as a 'gene drive' or simply a 'drive'. Burt (2003) was first to propose gene drives based on site-specific HEGs. A key feature of this construct is that it is evolutionarily stable in the sense that the mutant forms are most likely to arise as it spreads through a population will be selected against and lost. A further attractive feature of the proposed construct is that it is fully reversible. If one targets a gene that, when knocked out, is strongly deleterious, then there will be strong selection in favour of resistant alleles sequences that are functional, but are not recognized and cut by theHEG. One can engineer resistant alleles by, for example, using the degenerate property of the genetic code to create a DNA sequence that codes for the same amino acid sequence but differed in nucleotide sequence from the target. The logic of the approach requires that the knock outs be largely recessive, and that homing occurs either at meiosis,or in such a way that the fitness of heterozygous zygotes is not impaired but they produce a predominance of HEG meiotic products

In sexual reproduction, each of the two alleles of any gene is transmitted to 50% of offspring. Gene drives are genetic elements that circumvent this rule, significantly increasing the probability that the offspring will inherit the allele containing a gene drive element rather than a wild-type allele. Because of this, a gene drive can spread through a population even if it carries a fitness cost to the organism, as individuals with a gene drive element will produce more offspring with the gene drive allele than without it (Champer *et al.*, 2016).

MECHANISM OF GENE DRIVE

At the molecular level, endonuclease gene drives work by cutting chromosomes that do not encode the drive at a specific site, inducing the cell to repair the damage by copying the drive sequence onto the damaged chromosome. This is derived from genome-editing techniques and similarly relies on the fact that double-

stranded breaks (DSBs) are most frequently repaired by homologous recombination (HR) if a template is present, and less often by non-homologous end-joining (NHEJ). The cell then has two copies of the drive sequence. To achieve this behaviour, endonuclease gene drives consist of two nestedelements:first, HE that cuts the target sequence in recipient cells, and second, a template sequence used by the DNA repair machinery after the target sequence is cut. To achieve the self-propagating nature of gene drives, this repair template contains at least the endonuclease sequence. Because the template must be used to repair a DSB at the cutting site, its sides are homologous to the sequences that are adjacent to the cutting site in the host genome. By targeting the gene drive to a gene coding sequence, this gene will be inactivated; additional sequences can be introduced in the gene drive to encode new functions. As a result, the gene drive insertion in the genome will re-occur in each organism that inherits one copy of the modification and one copy of the wild-type gene. If the gene drive is already present in the egg cell (e.g., when received from one parent), all the gametes of the individual will carry the gene drive (instead of 50% in the case of a normal gene). Figure 1 shows the whole molecular mechanism that occurs during gene drive (Esvelt *et al.*,2014).

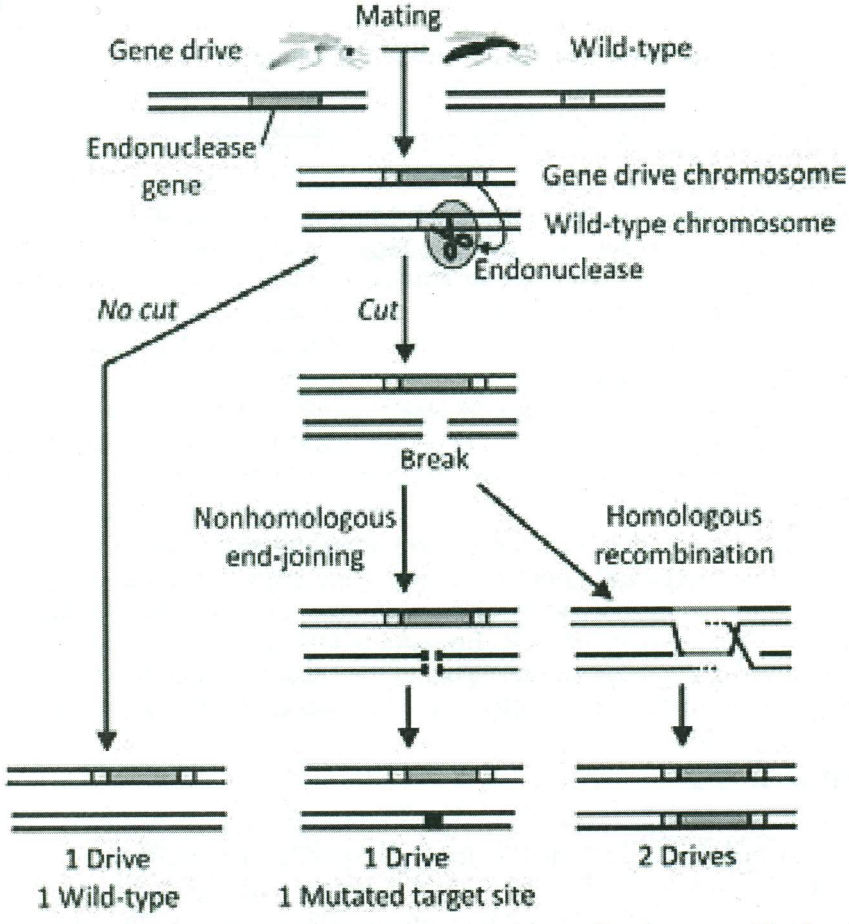

Figure 1 : Molecular mechanism behind gene drive [Redrawn with thanks from Esvelt *et al.* (2014). Creative Commons Attribution]

Gene drives, systems of biased inheritance that enhance the likelihood a sequence of DNA passes between generations through sexual reproduction and potentially throughout a local population and ultimately all connected populations of a species. In these cases, cells with only one copy of the gene drive, known as heterozygotes, will now have two copies and become homozygotes for the gene in question. If this occurs in germ line cells then 100% of offspring will inherit the drive and any associated genes, as opposed to the expected 50% if the organism remained heterozygous (Ouagrham-Gormley *et al.*, 2016). This process repeats within the population until the gene spreads at a rate far above that predicted by classical Mendelian inheritance. This inheritance pattern can result in rapid proliferation of the gene through a population, even overcoming some negative selection and in some cases very strong selection, for example where the trait being driven is female infertility.

ENGINEERED GENE DRIVES

Engineered gene drives are those that use artificial endonuclease genes or tools for gene drive process in which homing process was carried out using genome-editing tool clustered regularly interspaced short palindromic repeats (CRISPR) and the associated RNA-guided endonucleases such as Cas9 or Cpf1) or transcription activator-like effector nucleases (TALENs) (Miglani, 2019). Gene drives have potential to spread desirable genes throughout wild populations. Depending on mode of action, there are two types of gene drives – standard drives and suppression drives. Standard drivesspread genomic changes and associated traits through populations. Burt's orig-inal study proposed using them to drive the spread of other transgenes or to disrupt existing genes (Burt, 2014). The gene drive copying step can take place immediately upon fertilization or occur only in germline cells that are immediate precursors to sperm or eggs, leaving most of the organism's somatic cells with only one copy of the drive.Suppression drivesreduce the size of the targeted population. Austin Burt outlined an elegant strategy involving the use of gene drives to disrupt genes that cause infertility or lethality only when both copies are lost. These 'genetic load' drives would spread rapidly through minimally impaired heterozygotes when rare, and eventu-ally cause the population to crash or even become extinct due to the accumulated load of recessive mutations.

Burt (2003) and Sinkins and Gould (2006) have explained the mechanism by which HEGs increase in frequency within a population. A specific HEG is typically found inserted between two specific sequences of DNA within the genome. The HEG codes for the production of an enzyme that recognizes these two specific coding sequences when they are not interrupted by the presence of an HEG. In individuals that carry the HEG on only one of two homologous chromosomes, the enzyme catalyzes a break within the DNA sequence of the chromosome that lacks the HEG (step 1), which is then naturally repaired using the HEG within the homologue as a template (step 2). Basic construct for controlling natural populations by using HEGs has three essential features: (1) A HEG is engineered to recognize and cut a sequence in the middle of an essential gene, and the HEG is inserted into the middle of its own recognition sequence, simultaneously disrupting the gene and protecting the chromosome from being cut. Naturally occurring HEGs do not usually disrupt the function of the host gene because they are associated with self-splicing group I introns or inteins (Chevalier and Stoddard 2001), but the engineered element would not have these features. (2) The target gene

is chosen such that the knockout mutation has little phenotypic effect in the heterozygous state, but is severely deleterious when homozygous (i.e., the knockout is recessive). (3) The HEG is under the control of a meiosis-specific promoter, so that heterozygous zygotes develop normally, but transmit the HEG to a disproportionate fraction of their gametes.CRISPR-Cas9 nuclease or TALENs can be used to selectively disrupt the coding sequence of these candidate genes and analyze reproductive phenotypes to validate the suitability of these genes as homing targets.

CRISPR-Cas9 system is less expensive, less time consuming, specific in action and efficient enough to cut and edit multiple genes in a single experiment (Miglani, 2017; Wu *et al.*, 2019). The enzyme is active in a wide variety of organisms and is also quite spe-cific, cutting only protospacersthatarenearlyidenticaltothe spacer sequence of the guide RNA (gRNA). Moreover, methodsthat allow Cas9 to bind but not cut enable the expression of target genes to be regulated by selectively recruiting regulatory proteins attached to Cas9 or the gRNA. Various steps involved in RNA-guided gene drive are presented in Figure 2.

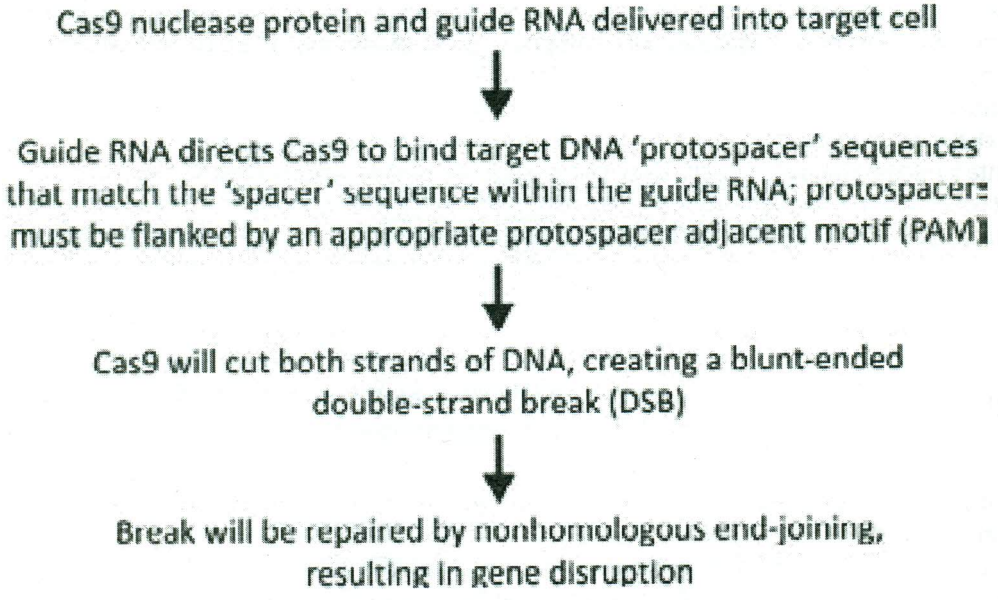

Figure 2 : Steps in RNA-guided gene drive

To generate a CRISPR-Cas9 gene drive, a founder population is first created by targeted insertion of a drive cassette into a specified locus. This is achieved by transformation of the organism of interest with a plasmid encoding the Cas9 nuclease and the Cas9-targeting gRNA, both flanked by regions of DNA corresponding to the genomic sequences flanking the Cas9 target site. Following targeted cleavage of the locus of interest by Cas9, the drive construct is integrated at high frequency into the disrupted locus via HR, generating heterozygotes. The remaining wild-type allele is then targeted and cleaved via gRNA and Cas9 expressed from the drive allele, leading to its conversion to a drive allele and homozygosity for the drive(Drury *et al.*, 2017).

Gene Drive Organisms

Experiments with GDOs are aimed at designing creatures that automatically spread their engineered genes across whole habitats and ecosystems. They could, it is claimed, make some of our key agricultural pests extinct, reduce the need for pesticides and speed up plant breeding programmes. According to some of their proponents, gene drives could even be compatible with non-genetically modified organisms (GMOs) and organic farming (ETC Group, 2018). While gene drive developers claim that there may be ways to effectively contain GDOs in the future, these hypothetical claims and assumptions need to be rigorously examined and tested. In the meantime, precaution and justice requires a moratorium on any releases. Strict laboratory handling and containment rules for all gene drive research must be internationally agreed and put into practice before further research can proceed even in the lab. At present, it appears possible to develop new GDOs without them being subject to any specific biosafety regulations (Esvelt *et al.*,2014).

GDOs are organisms that are supposed to reliably force one or more genetic traits onto future generations of their own species. The term for gene drives used by French scientists, 'Forcage Genetique' (genetic forcer) makes the intention clear: to force a human-crafted genetic change through an entire population or even an entire species. If they work, and that is not guaranteed at present, GDOs could accelerate the distribution of corporate-engineered genes from the lab to the rest of the living world at dizzying speed and in a potentially irreversible process. GDOs are organisms containing engineered gene drives. They are designed, over time, to replace non-GDO organisms of the same species in a population via an uncontrolled chain reaction. This ability may make them a far more dangerous biohazard than GMOs (ETC Group, 2018).

Gene drive is most efficient in populations with a short generation time as effects on the population will be seen most rapidly, therefore insects and lab model organisms are effective targets whereas traits would take much longer to spread in longer-lived species. Disease vectors such as malarial mosquitoes might be engineered to resist pathogen acquisition or eliminated with a suppression drive. Wild populations that serve as reservoirs for human viruses could be immunized using Cas9, RNAi machinery, or elite controller antibodies carried by a gene drive. Reversal and immunization drives could help ensure that all transgenes are safe and controlled. Drives might quickly spread protective genes through threatened or soon-to-be-threatened species such as amphibians facing the expansion of chytrid fungus (Rosenblum *et al.*, 2010). Invasive species might be locally controlled or eradicated without directly affecting others. Sensitizing drives could improve the sustainability and safety of pesticides and herbicides. Gene drives could test ecological hypotheses concerning gene flow, sex ratios, speciation, and evolution. Technical requirements for these applications vary with the drive type required.

PHASED TESTING PATHWAY

A number of criteria must be met for gene drives to be responsibly developed. A step-by-step approach can guide research from the laboratory to the field. To help guide gene drive research, the committee adapted and expanded upon the *phased testing pathway* outlined by the World Health Organization (WHO) for the testing of genetically modified mosquitoes (WHO, 2014). A phased testing pathway is a step-wise

approach to guide the preparation for and conduct of research that begins in the laboratory and continues through, if applicable, environmental monitoring. The idealized pathway for research on a gene-drive modified organism includes five steps: Research Preparation (phase 0), Laboratory-Based Research (phase 1), Field-Based Research (phase 2), Staged Environmental Release (phase 3), and Post-Release Surveillance (phase 4). Although the overall goal is for unidirectional movement from early to later phases, the pathway includes a set of feedback loops, to encourage repetition and refinement of studies based on new findings and data generated during the course of research. Each of the nine important pest organisms have a reality-meter as a rough indication of how far the technology has progressed towards release in the wild (ETC Group, 2018).

PEST MANAGEMENT USING ENGINEERED GENE DRIVE

RNA-guided engineered nuclease, CRISPR-Cas9, has potential to serve as a general method for spreading altered traits through wild populations over many generations (Esvelt *et al.*,2014). The ability to edit populations of sexual species would offer substantial benefits to humanity and the environment. For example, RNA-guided gene drives could potentially prevent the spread of insect-borne disease, curtailing the spread of vector-borne infectious diseases, support agriculture by reversing pesticide and herbicide resistance in insects and weeds, and control damaging populations of environmentally and economically invasive species.

The evolution of resistance to pesticides and herbicides is a major problem for agriculture. It is been assumed that resistant populations will remain resistant unless the relevant alleles impose a substantial fitness cost in the absence of pesti-cide or herbicide. It has been proposed that RNA-guided sensitizing drives may replace resistant alleles with their ancestral equivalents to restore vulnera-bility. For example, sensitizing drives can poten-tially reverse the mutations allowing the western corn rootworm, *Diabrolica virgifera*LeConte,to resist *Bt* toxins (Gassmann *et al.*,2014) or horseweed and pigweed to resist the herbicide glyphosate (Gaines *et al.*,2010), which is currently essential to more sustainable no-till agriculture.

The widespread planting of crops genetically engineered to produce insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (*Bt*) places intense selective pressure on pest populations to evolve resistance (Gassmann *et al.*,2014). Western corn rootworm, *D. virgifera*, is a key pest of maize, and in continuous maize fields it is often managed through planting of *Bt* maize. During 2009 and 2010, fields were identified in Iowa in which western corn rootworm imposed severe injury to maize producing *Bt* toxin Cry3Bb1. Subsequent bioassays revealed Cry3Bb1 resistance in these populations. Gassmann *et al.*,(2014) reported that, during 2011, injury to *Bt* maize in the field expanded to include mCry3A maize in addition to Cry3Bb1 maize and that laboratory analysis of western corn rootworm from these fields found resistance to Cry3Bb1 and mCry3A and cross-resistance between these toxins. Resistance to *Bt* maize has persisted in Iowa, with both the number of *Bt* fields identified with severe root injury and the ability of western corn rootworm populations to survive on Cry3Bb1 maize increasing between 2009 and 2011. Additionally, *Bt* maize targeting western corn rootworm does not produce a high dose of *Bt* toxin, and the magnitude of resistance associated with feeding injury was less than that seen in a high-dose *Bt* crop. These first cases of resistance by western

corn rootworm highlight the vulnerability of *Bt* maize to further evolution of resistance from this pest and, more broadly, point to the potential of insects to develop resistance rapidly when *Bt* crops do not achieve a high dose of *Bt* toxin.

Glyphosate resistance has evolved in *Amaranthus palmeri* populations within glyphosate-resistant cotton fields reported in Georgia. Evolution of glyphosate resistance in weed population was due to *EPSPS* gene amplification and increased *EPSPS* expression. High copy number of the gene and their location throughout the genome has been observed. Amplification was suggested to have originated via a transposon or an RNA-mediated mechanism (Gaines *et al.*, 2010).

Oye *et al.,* (2014) recommend various steps toward integrated management of environmental and security risks. Before any primary drive is released in the field, the efficacy of specific reversal drives should be evaluated. Research should assess the extent to which the residual presence of gRNAs and/or Cas9 after reversal might affect the phenotype or fitness of a population and the feasibility of reaching individual organisms altered by an initial drive. Long-term studies should be conducted to evaluate the effects of gene drive use on genetic diversity in target populations. Even if genome-level changes can be reversed, any population reduced in numbers will have reduced genetic diversity and could be more vulnerable to natural or anthropogenic pressures. Genome-editing applications may similarly have lasting effects on populations owing to compensatory adaptations or other changes.

Courtier-Orgogozo *et al.,* (2017) discuss another potential application of CRISPR based gene drive, namely the control of pest species to increase crop production. However, the gene drive based pest control strategies should receive more attention from policymakers and the public given their enormous potential impact on the environment, their easy accessibility, and the current dearth of regulations.

The presence and prevalence of drives should be monitored by targeted amplification or meta-genomic sequencing of environmental samples. Because effects will mainly depend on the species and genomic change rather than the drive mechanism, candidate gene drives should be evaluated on a case-by-case basis (James *et al.*, 2018). To assess potentially harmful uses of drives, multidisciplinary teams of experts should be challenged to develop scenarios on deliberate misuse. Integrated benefit-risk assessments informed by the actions recommended above should be conducted to determine whether and how to proceed with proposed gene drive applications. Such assessments should be conducted with sensitivity to variations in uncertainty across cases and to reductions in uncertainty over time.

The same properties that will enable the applications of gene drive technology may also pose a risk if GDOs that are equipped with gene drive cassettes are unintentionally released into the environment (van der Vlugt *et al.*, 2018). Although several groups of scientists and regulators have started to address these safety concerns, there are currently no dedicated guidelines published on the required risk assessment and minimal control measures applicable to GDOs in contained use. To fill this gap, these workers describe a fundamental approach to assessing the risks of these organisms while handled in a contained laboratory environment. Based on the likelihood that an adverse effect will arise from the handling of a GDO and the severity of this effect, three risk classes for contained use activities are presented. Finally, specific minimum

requirements regarding physical measures and working practices are proposed according to the presented risk classes and tailored to activities with rodents, insects, and fungi, which are most likely to be used for gene drive applications in the near future.

Risk assessment and management of GDOs have three components: (a) characteristics of the GDO to be assessed, (b) risk assessment, and (c) risk management. In risk assessment, likelihood of occurrence of potential adverse effects is classified as high, medium, low and negligible. Similarly, severity of potential adverse effects are classified as negligible, low, medium, and high. An additional layer of physical containment, restricted access, a monitoring plan, and identification of animals are recommended for a risk class 2 activity. These measures should prevent or enable the detection of an unintentional release while still being proportionate to a non-permanent establishment of the GDO in the environment. The risk assessment framework presented by van der Vlugt *et al.,* (2018) describes the first structured methodology specifically aimed at assisting in the assessment of GDOs in contained use. It thus addresses the demand from several countries to have a consistent approach to the assessment of the biological risks of gene drive technology as any unintentional release may have international consequences. Likewise, a consistent approach of a GDO risk assessment as well as guidance material is necessary for both users and risk assessors to facilitate the development of the technology while ensuring that risks are appropriately managed.

Present Scenario

Shelton *et al.,* (2008) used gene-drive technology to manage *Plutellaxylostella* Linnaeus(Diamond back moth) that causes crop damage, leaf wilt and abnormal whitening in *B. oleracea* (Broccoli), *B. oleracea* var. botrytis (Cauliflower), *B. oleracea* var. gemmifera (Brussels sprouts). Shukla and Palli (2013) used *Medea* elements of gene-drive technology to manage*Tribolium castaneum* (Red flour beetle) that causes Red rust disease in *Sorghum bicolor* (Sorghum), *Triticum aestivum*(Wheat), and *Zea mays* (Maize).

US Citrus Research and Development Foundation (2016) has been trying to introduce GDSs into the *Diaphorina citri* Kuwayama (Asian citrus psyllid) that would make the psyllid unable to transmit Huanglongbing (HLB) or citrus greening disease in *Citrus reticulata* (Citrus). Leaf shredding and/orpremature drop of fruits are caused by*Spodoptera littoralis* (Noctucid moth) in *Gossypium arboreum* (Tree Cotton), *G. hirusitum*(Upland Cotton), *Solanum lycopersicum* (Tomato), *Nicotiana tabacum* (Tobacco) and *Z. mays* (Maize). Koutroumpa *et al.,* (2016) knocked-out the olfactory receptor co-receptor *Orco* gene to investigate its function in Lepidoptera olfaction; 89.6% of the injectedindividuals carried *Orco* mutations 70% of which transmitted them to the next generation.

Irregular ripening of *S. lycopersicum*(Tomato), White stalk of *B. oleracea*(Broccoli) and *B. oleracea* var. botrytis (Cauliflower), White stem of poinsettia(*Euphorbia pulcherrima*), Light root of carrots,*Daucus carota* (Carrot), severe cosmetic damage in *B. oleracea* var. capitata (Cabbage),fruit rot diseasein *Cucumis melo* (Melon), older leaves developing a red colour in *C. sativus*(Cucumber), Squash silver leaf disorder in *Cucurbita pepo*(Pumpkin), leaf curl in *G. arboreum*, *G. hirusitum* and *Ipomoea batatas* (Sweet potato) are caused by *Bemisia tabaci* Gennadius (Silverleaf whitefly). Scott *et al.,* (2017) observed that *T. castaneum* possesses naturally occurring selfish genetic elements (*Medea* elements) which provide more stable and reliable gene-drive mechanism than Cas9-based systems.

Efforts are underway to clarify this element's mode of action, and to develop its use for pest control. *Nilaparvata lugens*Lugens (Brown plant hopper) causes Grassy stunt in rice (*Oryza sativa*). Scott *et al.,* (2017) are using *Medea* element as a prime target in their experiments for the use of gene-drive technology.

Fruitfly,*Ceratitis capitata* (Medfly) causes premature ripening and rotting in*Magnifera indica* (Mango), *Malus pumila* (Apple), and *Prunus armeniaca* (Apricot). For production of gene-drive organisms, target is eye pigmentation gene *white eye* (w^e). Meccariello *et al.,* (2017) performed experiments for successful adaptation of CRISPR-Cas9-based gene disruption in the medfly, which assists progress towards novel genetic strategies for control of pest insects, such as gene drive.

Spotted wing Fruit fly, *Drosophila suzukii* Matsumara, causes rotting of fruits in *Prunus persica* (Peach), *Prunus avium* (Cherry), and *Prunus* Subg. *Prunus* (Plum). Buchman *et al.,* (2018) developed a *D. suzukii* synthetic *Medea* gene-drive system which has been shown capable of biasing Mendelian inheritance rates with up to 100% efficiency. Table 1 summmarizes above-mentioned examples where engineered gene-drive technology has been actually used or experiments are in progress for insect-pest management in agriculture.

Table 1: Examples showing use of gene-drive technology in plant pest management

Name of pest	Host plant	Disease	Experiment	Phase of experiment	Reference
Plutella xylostella L. (Diamond back moth)	*Brassica oleracea* (Broccoli) *Brassica oleracea var. botrytis* (Cauliflower) *Brassica oleracea var. gemmifera* (Brussels sprouts)	Crop damage, leaf wilt and abnormal whitening	Field· cage experiments performed Usedsterile insect technique to target pests	Phase-2 (Field-based trials)	Shelton *et al.,*(2008)
Tribolium castaneum (Red flour beetle)	*Sorghum bicolor* (Sorghum) *Triticum aestivum* (Wheat) *Zea mays* (Maize),	Red rust	Medea elements provide more stable and reliable gene-drive mechanism than Cas9-based systems	Phase-0 (Research preparation)	Shukla and Palli (2013)
Diaphorina citri Kuwayama (Asian citrus psyllid)	*Citrus reticulata* (Citrus)	Huanglongbing (HLB) citrus greening disease	US Citrus Research Board trying tointroduce gene drive systems into the Asian citrus psyllid that would make the aphid unable to transmit citrus greening disease	Phase-0 (Research preparation)	Citrus Research and Development Foundation (2016)
Spodoptera littoralis	*Gossypium arboreum*	Leaf shredding in cotton,	Knocked-out the olfactory	Phase-1 (Lab-based	Koutroumpa *et al.,* (2016)

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Name of pest	Host plant	Disease	Experiment	Phase of experiment	Reference
(Noctucid moth)	(Cotton *Gossypium hirusitum* (Cotton) *Solanum lycopersicum* (Tomato) *Nicotiana tabacum* (Tobacco) *Zea mays* (Maize)	Premature drop of fruits in tomato	receptor co-receptor *Orco* gene to investigate its function in Lepidoptera olfaction. 89.6% of the injected individuals carried *Orco* mutations70% of which transmitted them to the next generation	research)	
Bemisia tab aci Gennedius (Silverleaf whitefly)	*Brassica oleracea var. botrytis* (Cauliflower) *Brassica oleracea var. capitata* (Cabbage) *Brassica oleracea* (Broccoli) *Cucumis melo* (Melon) *Cucumis sativus* (Cucumber) *Cucurbita pepo* (Pumpkin) *Daucus carota* (Carrot) *Gossypium arboreum* (Cotton) *Gossypium hirusitum* (Cotton) *Ipomoea batatas* (Sweet potato) *Solanum lycopersicum* (Tomato)	Silver leaf of squash; Irregular ripening of tomato; White stalk of broccoli and cauliflower; White stem of poinsettia; Light root of carrots	*T. castaneum* possesses naturally occurring selfish genetic elements (*Medea* elements) which provide more stable and reliable gene-drive mechanism than Cas9-based systems	Phase-0 (Research preparation)	Scott *et al.*, (2017)
*Nilaparvata lugens*Lugens (Brown plant hopper)	*Oryza sativa* (Rice)	Grassy stunt of rice	Used as a prime target for the use of gene drives	Phase-0 (Research preparation)	Scott *et al.*, (2017)
Ceratitis capitata (Medfly)	*Magnifera indica* (Mango)*Malus pumila* (Apple) *Prunus armeniaca* (Apricot)	Premature ripening and rotting	Target eye pigmentation gene *white eye* (*we*). Successful adaptation of CRISPR-Cas9-	Phase-1 (Lab-based research)	Meccariello *et al.*, (2017)

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Name of pest	Host plant	Disease	Experiment	Phase of experiment	Reference
			based gene disruption in the medfly, which assists progress towards novel genetic strategies for control of pest insects, such as gene drive		
Drosophila suzukii Matsumara (Fruit fly)	*Prunus persica* (Peach) *Prunusavium* (Cherry) *Prunus* Subg. *Prunus* (Plum)	Rotting of fruits	Creation of a *D. suzukii* synthetic *Medea* gene drive system. Capable of biasing Mendelian inheritancerates with up to 100% efficiency	Phase-1 (Lab-based research)	Buchman *et al.*, (2018)

Open Field Trials

Two leading gene drive teams (Target Malaria for the UK and GBIRD, based in North Carolina) have proceeded towards building GDOs and are preparing for open field trials, including steps to select test sites in Australia, New Zealand, Burkina Faso, Uganda, Mali and Ghana, and to create government and community acceptance of the use of gene drives in key testing sites (http://www.nogeoingegneria.com/news-eng/australia-and-new-zealand-to-be-test-sites-for-gm-insect-trials-courtesy-of-darpa/). Mosquitoes containing gene drives are being proposed for malaria control in Africa. DARPA is revealed to be funding a high profile UK team of researchers targeting African communities with gene drive mosquitos. This funding was not previously made public. While claiming potential health benefits, any application of such powerful technologies should be subject to the highest standards of transparency and disclosure. Next few years are going to be critical and we are going to have to take the fight outside the laboratory. Genetic-engineering approaches are being developed to manipulate the inheritance pattern of a gene copy such that it will spread through a population more rapidly than would be expected by normal Mendelian inheritance, generating what is called a gene drive and leading to Super-Mendelian inheritance.

Open field trials by Dr Tony Shelton of Cornell University are in progress for *P.xylostella*(Diamond back moth), a pest of *B. oleracea* (Broccoli), *B. oleracea* var. botrytis (Cauliflower)and *B. oleracea* var. gemmifera (Brussels sprouts) (https://shelton.entomology.cornell.edu/diamondbackmoth/diamondback-moth-project-at-cornell-university-faq/).

The discovery of CRISPR-Cas9 meant that Burt's proposal was closer to reality via the creation of a self-perpetuating gene drive that could be deployed in potentially any genomic location in any eukaryotic species (Esvelt *et al.*,2014; Gantz and Beir, 2015).

Grunwald *et al.,* (2019) described the Cas 9 mediated Super Mendelian Inheritance in the mammals utilizing an active genetic "CopyCat" element embedded in the mouse *Tyrosinase* gene to detect genotype conversions after Cas9 activity is induced in the embryo and in the germline. Since the 2.8 kb insert disrupts the *Tyr* open reading frame, *TyrCopyCat* is a functionally null allele that is propagated by Mendelian inheritance in absence of Cas9. To test whether it is possible to observe super-Mendelian inheritance of the *TyrCopyCat* allele they crossed mice that carry the *TyrCopyCat* element to transgenic mice that produce Cas9.

Such a self-propagating nature of global GDSs renders the technology uniquely suited to address large-scale ecological problems, but self-limiting nature of local GDSs make the technology suitable for altering the local breeding populations. 'Daisy drive' systems offer a way to alter the traits of only local populations in a temporary manner. Because they can exactly duplicate the activity of any global CRISPR-based drive at a local level, daisy drives may enable safe field trials. It is a powerful form of local drive based on CRISPR-mediated homing in which the drive components are separated into an interdependent daisy-chain. Despite these promising theoretical results, current technological limitations preclude the safe use of daisy drive elements (Noble *et al.*,2017).

LIMITATIONS OF GENE DRIVE TECHNOLOGY

Given their potentially widespread availability, it will be essential to develop a comprehensive understanding of the fundamental limitations of genetic-drive systems. First and most important, gene drives require many generations to spread through populations. Once transgenic organisms bearing the gene drive are constructed in the laboratory, they must be released into the wild to mate with wild-type individuals in order to begin the process of spreading the drive through the wild population. The total time required to spread to all members depends on the number of drive-carrying individuals that are released, the generation time of the organism, the efficiency of homing, the impact of the drive on individual fitness, and the dynamics of mating and gene flow in the population, but in general it will take several dozen generations (Burt, 2003). Thus, drives will spread very quickly in fast-reproducing species but only slowly in long-lived organisms. A newly released drive will typically take dozens of generations to affect a substantial proportion of a target population, unless drive-containing organisms are released in numbers constituting a substantial fraction of the population. The process may require only a year or less for some invertebrates, but centuries for organisms with long generation times.

Second, gene drives cannot affect species that exclusively practice asexual reproduction through clonal division or self-fertilization. This category includes all viruses and bacteria as well as most unicellular organisms (Esvelt *et al.*, 2014) . Highly efficient standard drives might be able to slowly spread through populations that employ a mix of sexual and asexual reproduction, such as certain plants, but drives intended to suppress the population would presumably force target organisms to reproduce asexually in order to avoid suppression.

Third, drive-mediated genome alterations are not permanent on an evolutionary timescale (Esvelt *et al.*, 2014). While gene drives can spread traits through populations even if they are costly to each individual organism, harmful traits will eventually be

outcompeted by more fit alleles after the drive has gone to fixation. Highly deleterious traits may be eliminated even more quickly, with non-functional versions appearing in large numbers even before the drive and its cargo can spread to all members of the population. Even when the trait is perfectly linked to the drive mechanism, the selection pressure favouring the continued function of Cas9 and the gRNAs will relax once the drive reaches fixation. Maintaining deleterious traits within a population indefinitely is likely to require scheduled releases of new RNA-guided gene drives to periodically overwrite the broken versions in the environment.

Fourth, our knowledge of the risk management and containment issues associated with gene drives is largely due to the efforts of researchers focused on mosquito-borne illnesses (Benedict *et al.*, 2008). Frameworks for evaluating ecological consequences are similarly focused on mosquitoes (David *et al.*, 2013) and the few other organisms for which alternative genetic biocontrol methods have been considered. While these examples provide an invaluable starting point for investigations of RNA-guided gene drives targeting other organisms, studies examining the particular drive, population, and associated ecosystem in question will be needed.

Fast-forward to the age of genomics and we appear to be on the threshold of genetic methods that will enable us to spread genes that disrupt the ability of pests to transmit pathogens or bias their sex ratio so that virtually all offspring are male. Of course, a population with few females is bound for extinction. There are typically two components to these manipulations. First, there must be a way to alter or disable a gene to alter the individual pest's biology. Then, there needs to be a way to link it to a gene or to genetic systems to drive it into the population (Esvelt *et al.*, 2014).

Gene drive may escape the target location in which it was to be inserted with high stability, also DNA flanking the gene drive cassette may ride along the gene drive system to the further generation offspring and have undesired ecological impact. They usually reduce the reproductive fitness of the carrier animal due to the presence of the payload gene transmitted with the gene drive system. The creation of first generation of individuals is a bit critical task to accomplish a gene drive inserted into the host nuclear genome with high stability and with less chances in the species to develop resistance against it in a few generations. Also, it takesdozens of generations to affect a substantial proportion of a target population. Determining which species is a pest for which population is a critical phase because a pest to one population could be a pollinator to another (Esvelt *et al.*, 2014; ETC group, 2018;). The necessity to have the gene drive research approved from various regulatory agencies itself is a barrier to the thriving field of gene drive such as (Committee on Gene Drive Research in Non-Human Organisms, 2016):

IS GENE DRIVE TECHNOLOGY SAFE?

However, drives may present environmental and security challenges as well as benefits. Transforming the genetics of an entire population may have unintended consequences on environment (Saplakoglu, 2017). Extensive testing should precede any release of gene-edited individuals into the environment for transforming the entire (for example, mosquito) population. It is very difficult to predict how gene drive would affect population dynamics and ecosystems. In some cases, the purpose of gene drives would be to reduce population sizes of an organism, which could influence processes like

pollination and transmission of parasites. In other cases, gene drives can be used to weed out disease by driving the population that carries that disease to extinction. Eliminating an organism (one plant species, for example) or reducing its numbers greatly can cause the proliferation of others, and this leads to a series of changes in the ecosystem. If modified mosquitoes are released in Town A, the mosquitoes may not have any problem flying to Town B, even though Town B is not interested in having them. They will go there anyway. We need to understand the system well enough so that we can take ethical concerns into account as we make decisions.

The Food and Drug Administration (FDA) approved the release of genetically modified mosquitoes, altered to control the Zika virus, in certain areas of Florida(https://www.the guardian.com/world/2016/aug/05/florida-genetically-modified-mosquitoes-zika).The advantage of these other technologies is that they are effective only as long as modified male mosquitoes are released. When the manipulation of the population is stopped, it bounces back to normal levels. One has a control over the system that is yet to be demonstrated for gene drives where once you alter the genes in these populations, they just keep changing.

REGULATORY ASPECT OF GENE DRIVES

Regulatory Challenges

Responsibility for regulating animal applications of drives in the United States rests with the FDA. An FDA guidance issued in 2009 states that genetically engineered DNA constructs intended to affect the structure and function of an animal, regardless of their use, meet the criteria for veterinary medicines and are regulated as such. Developers are required to demonstrate that such constructs are safe for the animal. Approval of new veterinary medicines is to be based on the traditional FDA criterion "that it is safe and effective for its intended use". It is unclear whether these requirements can be reconciled with projected uses of drives, including suppression of invasive species. Nor is it clear how this guidance would apply to insects. The application of existing U.S. Department of Agriculture (USDA) and U.S. Environmental Protection Agency (EPA) regulations governing GMOs to gene drives is also ambiguous, with jurisdictional overlaps across the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the Toxic Substances Control Act, and the Animal and Plant Health Inspection Service (APHIS) (Bar Yam *et al.*, 2012).

Existing international conventions cover international movements of gene drives, but do not define standards for assessing effects, estimating damages, or mitigating harms. International movements of living modified organisms are treated under the 2003 Cartagena Protocol on Biosafety (CPB), ratified by 167 nations not including the United States and Canada. Article 17 of the Protocol obligates parties to notify an International Biosafety Clearinghouse and affected nations of releases that may lead to movement of living modified organisms with adverse effects on biological diversity or human health. Other provisions empower nations to use border measures to limit international movements, but these measures are not likely to control diffusion of drives. The 2010 Nagoya-Kuala Lumpur Supplementary Protocol calls on Parties to adopt a process to define rules governing liability and redress for damage from international movements. Neither the process nor rules have been defined (CBD, 2003).

The draft U.S. Government Policy on Dual Use Research of Concern (DURC) combines a broad definition of concerns with a narrow definition of scope of oversight, the latter focusing on experiments of concern on listed pathogens and toxins (www.federalregister.gov/articles). The listed-agent-toxins approach is also used in the U.S. Select Agent Rule, USDA Select Agents/Toxins, and Commerce Department export control regulations. Drives do not fall within the scope of required oversight of DURC and other listed-agent-toxin-based policies.

Filling the Regulatory Gaps

Oye *et al.,* (2014) recommend adopting a function-based approach that defines risk in terms of the ability to influence any key biological component the loss of which would be sufficient to cause harm to humans or other species of interest. The agents and targets of concern with a functional approach could include DNA, RNA, proteins, metabolites, and any packages thereof. Thus, suppression drives would be covered because they would cause loss of reproductive capability in an animal population, whereas an experimental reversal drive that could only spread through engineered laboratory populations could be freely developed. Steps taken to mitigate environmental concerns will address security concerns and *vice versa*. Regulatory authority for each proposed RNA-guided gene drive should be granted to the agency with the expertise to evaluate the application in question. All relevant data should be made publicly available and, ideally, subjected to peer review (Reeves *et al.*,2014).

The criteria that are presently in use for regulating GDOs are the same as are applied to GMOs. This seems to be logically unacceptable as the natural selection pressure is not prevalent in case of GDOs to the extent existing for GMOs. Thus it would be desirable to formulate separate regulations for controlling research on GDOs (ETC Group, 2018).While gene drive experiments have raced ahead at a breath-taking pace, the regulatory reforms and defined standards would be required for the international public support to utilize these drive systems in the wild (Oye *et al.*, 2014). There are compelling arguments in favour of eliminating insect-borne human diseases, developing and supporting more sustainable agricultural models, and controlling environmentally damaging invasive species. At the same time, there are valid concerns regarding our ability to accurately predict the ecological and human consequences of these interventions. By bringing these possibilities before the scientific community and the public prior to their realization in the laboratory, scientists hope to initiate transparent, inclusive, and well-informed discussions concerning the responsible evaluation and application of these nascent technologies (Esvelt *et al.*,2014). A group of scientists working on framing guidelines for regulating work involving GDOs includes Kenneth A Oye, Kevin Esvelt, Evan Appleton, Flaminia Catteruccia, George M. Church, Todd Kuiken, Shlomiya Lightfoot, Julie McNamara, Andrea Smidler, and James P Collins.

KEY QUESTIONS IN GENE DRIVES

Kuiken *et al.,* (2014) and Drinkwater *et al.,* (2014) have examined key questions concerning effects of development and use of gene drives in varied species and contexts. The first question concerns targeted wild organisms. Scientists have minimal experience engineering biological systems for evolutionary robustness. Drive-induced traits and altered population dynamics must be carefully evaluated with explicit attention to stability. For example, a drive may move through only part of a population before a

mutation inactivates the engineered trait. In some cases, preferred phenotypes might be maintained as long as new drives encoding updates are periodically released. The effects of a strategy dependent on repeatedly releasing drives to alter a population should be thoroughly assessed before use.

Second question deals with non-targeted wild organisms. In theory, precision drives could limit alterations to targeted populations, but the reliability of these methods in preventing spread to non-target or related populations will require assessment (Drinkwater *et al.*, 2014). To what extent and over what period of time might crossbreeding or lateral gene transfer allow a drive to move beyond target populations? Might it subsequently evolve to regain drive capabilities in populations not originally targeted? There may also be unintended ecological side effects. Contained field trials should be performed before releasing organisms bearing a drive that spreads the trait.

Next point of concern is about crops and livestock. A technology capable of editing mosquito populations to block disease transmission could also be used to alter populations of agricultural plants or livestock by actors intent on doing harm ((Drinkwater *et al.*, 2014)). However, doing so surreptitiously would be difficult because many drive-containing organisms must be released to alter populations within a reasonable time span. Moreover, drives are unlikely to spread undetected in contract seed production farms and animal breeding facilities that test for the presence of transgenes. It would thus be difficult to use drives to affect food supplies in the United States and other countries that rely on commercial seed production and artificial insemination. Developing countries that do not use centralized seed production and artificial insemination could be more vulnerable.

Lastly, gene drives will be ineffective at altering human populations because of our long generation times (Drinkwater *et al.*, 2014). Furthermore, whole-genome sequencing could be used to detect the presence of drives. Drives are thus not a viable method for altering human populations. Rare individuals might experience an allergic reaction to peptides in the Cas9 protein if exposed to an affected organism. Thus, toxicological studies should be conducted to confirm that proposed drive components are safe.

Safeguards to Gene Drives

Given the potential for gene drives to alter entire wild populations and therefore ecosystems, the development of this technology must include robust safeguards and methods of control. There are three strategies to safeguard the gene drives, i.e., chemical, temporal and genetic approaches.*Chemical approaches* to population control might utilize "sensitizing drives" to render target organisms vulnerable to a particular molecule using one of three strategies. First, a sensitizing drive might reverse known mutations that confer resistance to existing pesticides or herbicides. Second, it might carry a prodrug-converting enzyme (Schellmann *et al.*, 2010) that would render a prodrug molecule toxic to organisms that express it. *Temporal approaches* to controlling populations would deliberately limit the lifetime of a suppression drive by rendering its effects evolutionarily unstable. For example, a male-determining or female-specific sterility gene carried by a standard drive on an autosome would suppress the target population, but the effect would be short-lived because any drive that acquired a loss-of-function mutation in the cargo gene would be strongly favoured by natural selection

due to its ability to produce fertile female offspring. Notably, turning existing female specific sterility lines (Fu *et al.*,2010; Labbe *et al.*, 2012) into unstable drives may increase their effectiveness. *Genetic approaches* to population control might initiate suppression only when two distinct "interacting drives" encounter one another. For example, a cross between standard drives A and B might produce sterile females and fertile males that pass on the "sterile daughter" trait when crossed with females of any type. Scattering A- and B-carrying individuals throughout an existing population would produce many tiny pockets dominated by either A or B and very few organisms in between due to the infertility of AB females. Because each drive would spread from a small number of initially released individuals scattered over a wide area, this strategy may be capable of large-scale population suppression, but its effectiveness and resolution will depend on the average distance between released A and B individuals (Esvelt *et al.*, 2014).

Immunizing drives might protect specific sub-populations from the effects of full-scale suppression drives released elsewhere (Esvelt *et al.*, 2014) . Assuming some degree of gene flow, the immunized population will eventually replace the suppressed population, though this might be delayed if crossing the two drives generates a sterile-daughter effect as described above. Due to the comparatively uncontrolled spread of both drive types through the wild-type population, this method would only be suited to large geographic areas or subpopulations with limited gene flow. For example, immunization might be used to protect the native population of a species while suppressing or eradicating populations on other continents.

For containment strategies and management of ecological risks, there exist ecological and molecular containments. Ecological containment involves building and testing gene drives in geographic areas that do not harbour native populations of the target species. For example, most gene drive studies involving tropical malarial mosquitoes have been conducted in temperate regions in which the mosquitoes cannot survive or find mates. Molecular containmentensures that the basic requirements for driveare not met when mated with wild-type organisms. Truedrives must cut the homologous wild-type sequence andcopy both the gene encoding Cas9 and the gRNAs.Experiments that cut transgenic sequences absent fromwild populations and copy either the gene encoding Cas9or the gRNAs - but not both - should be quite safe.Ecological or molecular containment should allow basicresearch into gene drive effectiveness and optimization tobe pursued with negligible risk (Esvelt *et al.*, 2014).

FUTURE PROSPECTS OF GENE DRIVE TECHNOLOGY

As gene drive is a new emerging technology and it has efficiency to solve many ecological problems like it can help in eradication of major pests, for example, whitefly (*B. tabaci*)in cotton. In previous years whitefly caused large number of losses in cotton in Punjab (India) due to environmental conditions and also due to resistance evolution by whitefly (Naveen *et al.*, 2017). Gene drive also may help to control vector borne diseases like dengue, malaria and chickengunya. Chickengunya is the major disease now a days. Many people died because of this disease in 2016, so gene drive may provide cure for this vector-borne disease. Gene drive may help in sustainable pest management in plants as there was example of resistance evolution in case of weed *A. pameri*. Gene-editing technology can be harnessed to produce 'guided gene drives' (Esvelt *et al.*, 2014) which could be deployed to modify the genetic traits of wild organisms. In essence, this

would bestow unprecedented powers to genetic engineers and allow them to restructure entire ecosystems to suit human specifications. Perhaps editing technologies and guided gene drives will be used in the future to control or eliminate scourges like malaria by modifying mosquito vector populations.

Assessing the environmental risks associated with such manipulations will be challenging. Although (in principle) a second guided gene drive might be employed to reverse a previously released gene drive producing undesirable impacts, undoing any consequential ecological damage may be impossible (Oye *et al.*, 2014). As a way out it has been suggested to target only recently evolved pests, such as *D. suzukii*, for gene drive experiments to reduce ecological impact of GDOs (Esvelt *et al.*, 2014; ETC Group, 2018).

Given their potential to alter entire ecosystems (Oye *et al.*,2014; Akbari *et al.*, 2015), it may be advantageous for GDSs to be either completely removable from a population by the release of wild-type organisms (in the case of high-threshold gene drives) or convertible to a neu-tral configuration by the construction of a second-generation gene drive, which is frequently denoted as a 'reversal' gene drive. Of note, despite their name, rever-sal gene drives do not restore the original modification to the wild-type; rather, they induce further changes that may undo a phenotypic alteration caused by the initial gene drive. The properties discussed above all need to be considered when evaluating the type of gene drive that is best suited for a particular application and assessing context-dependent risks (Esvelt *et al.*,2014).

Increasing Efficiency

Gantz and Bier (2015) describing a CRISPR-Cas-based method for converting heterozygous mutations into homozygous mutations in the fruit fly,*Drosophila melanogaster* Meigen demonstrated that using their approach a mutation can be spread to the next generation with an incredible 97% efficiency. This allows for what is called a gene drive: biasing the way certain genes are inherited that eventually leads to alteration in the genome of the whole population. What's the promise? An ease and ability to introduce genes into the populations of disease spreading organisms, such as, for instance, malaria carrying mosquitoes. The amount of regulation imposed upon crop varieties made through genome editing will impact the cost of their development and how quickly they make it into the food supply.

Evaluating Suitability

Gene drive technology is being explored as a potentially durable and cost-effective strategy for controlling the transmission of deadly and debilitating vector-borne diseases that affect millions of people worldwide, such as Zika virus and malaria. Suitability of gene drive is being evaluated for various potential applications in conservation biology, including a highly specific and humane method for eliminating invasive species from sensitive ecosystems (Emerson *et al.*, 2017).

Manipulating Regulation

Scientists well recognize that gene drives need regulation as every product of gene drive research will not be desirable. A covert coalition is lobbying for relaxed regulations around gene drive technology with help from a well-funded public relations firm, Emerging Ag, by attempting to manipulate the United Nations Convention on Biological

Diversity (CBD) (Cohen, 2017). Bill and Melinda Gates Foundation paid this agriculture and biotechnology PR firm $1.6 million for activities on gene drives (http://genedrivefiles.synbiowatch.org/2017/12/01/gates_foundation_pr/;https://www.independentsciencenews.org/news/gates-foundation-hired-pr-firm-to-manipulate-un-over-gene-drives/). Ad Hoc Technical Expert Group (AHTEG) is linked to the process of regulation of gene drive technology. AHTEG, convened by the UN Convention on Biological Diversity (CBD) is addressing the issues around so-called gene drives, a highly controversial genetic extinction technology with potential applications for agricultural, conservation or military use (https://corporateeurope.org/food-and-agriculture/2017/12/gene-drive-files-reveal-covert-lobbying-tactics-influence-un-expert).

US Defense Advanced Research Projects Agency (DARPA) is the top funder and influencer behind a controversial genetic extinction technology known as "gene drives" (http://www. synbiowatch.org/2017/12/the-gene-drive-files/?lores) pumping $100 million into the field. "Emerging Ag," a private PR firm funded by the Gates Foundation is working behind the scenes to stack key UN advisory processes with gene drive-friendly scientists. Emerging Ag has also been collaborating with a biotechnology lobby group Public Research and Regulation Initiative (PRRI), who run a similar co-ordination. National government representatives of Canada, U.S., UK, Brazil and the Netherlands were being remotely assisted by PRRI during closed door discussions.

Population suppression and population alteration are the potential uses of gene drives which can be used to deal with a number of problems related to human health, environment and agriculture (Esvelt *et al.*,2014). Meiotic suppression drives have the ability to spread without limit and may incur a substantial risk of extinction therefore, alternative gene drive types might be used to grant finer control over the extent of suppression. These drives involve chemical approaches, temporal approaches and genetic approaches. Also, ecological containment and molecularcontainment ensure that the basic requirements for drive are not met (Esvelt *et al.*,2014).

Due to the discovery of GDSs in insects by using molecular scissors (e.g., engineered site-specific nucleases), the last couple of years have seen a profound rise in excitement about the many possible uses of GDSs. GDSsare capable of altering the traits of wild populations and associated ecosystems. A gene drive biases the transmission of a particular allele of a gene such that it is inherited at a greater frequency than by random assortment (Committee on Gene Drive Research in Non-Human Organisms, 2016). A consequence of gene drives is an increased frequency of specific genetic elements or alleles and their accelerated spread throughout populations over successive generations (Conner and Jacobs, 2019).

CONCLUSIONS

There is currently sufficient knowledge to begin constructing ecological risk assessments for some potential gene-drive modified organisms, including mosquitoes and mice. In some other cases, it may be possible to extrapolate from research and risk analyses of other modified organisms and non-indigenous species. However, laboratory studies and confined field tests (or studies that mimic field tests) represent the best approaches to reduce uncertainty in an ecological risk assessment, and are likely to be of greatest use to risk assessors.

The potentially widespread implications of RNA-guided gene drives demand a thoughtful and collected response. Numerous practical difficulties must be overcome before gene drives will be in a position to address any of the suggested applications. Many of our proposals and predictions are likely to fall short simply because biological systems are complex and difficult to engineer. Even so, the current rate of scientific advancement related to Cas9 and the many outcomes accessible using the simplest of gene drives suggest that molecular biologists will soon be able to edit the genomes of wild populations, reverse or update those changes in response to field observations, and perhaps even engage in targeted population suppression.

There are compelling arguments in favour of eliminating insect-borne human diseases, developing and supporting more sustainable agricultural models, and controlling environmentally damaging invasive species. At the same time, there are valid concerns regarding our ability to accurately predict the ecological and human consequences of these interventions. By bringing these possibilities before the scientific community and the public prior to their realization in the laboratory, we hope to initiate transparent, inclusive, and well-informed discussions concerning the responsible evaluation and application of these nascent technologies (Marshall and Hay, 2014).

On the basis of present scenario (see Table 1 for summary of various examples) of application of gene-drive technology in pest management in agriculture, it will be pertinent to mentioned here that gene drive is a developing technology which still requires refinement and answer many important questions.Hoping that in near future the limitations of this technology will be overcome, farmer-related and regulatory issues resolved, CRISPR-Cas9-based gene drive may become a major biotechnological approach in management of diverse pest populations to enhance crop yield.

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Plant diseases are a threat to world's agricultural and general food security. Significant yield losses due to the attack of pathogen occur in most of the agricultural and horticultural crop species. The indiscriminate use of chemicals for the management of plant diseases has affected humans and their environment and insect pests remains to be one of the major limiting factors in sustaining the productivity of various crops. The situation demands judicious blending of conventional, unconventional and frontier technology for the management of plant diseases. 'Biotechnology' with its promise to revolutionize agriculture around the world and in India, has opened an existing frontier in agriculture.

The present book **" Biotechnology for Plant Disease Diagnostics and Management"** incorporates review articles on varied aspects of diagnosis and management of plant diseases using biotechnological approaches. This volume contains 10 chapters written by eminent scholar in the field incorporating recent development in the subject. Topics covered in the book are : Biotechnological approaches for the management of plant diseases; Involvement of non-host resistance genes in disease resistance plausible for future crop improvement; Engineered gene drives for plant pest management; biotechnology in management of plant diseases; Biotechnological applications in management of bacterial and fungal plant diseases; Advanced technologies for plant disease detection; Role of biotechnology in detection and prevention of plant diseases; Biotechnological techniques for management of post harvest bio-deterioration of stored food commodities; Role of quorum sensing in plant pathogenesis.

I am sure the information given in the book will prove helpful to those interested in Plant disease management using latest biotechnological techniques for many years to come and generate interest in an upcoming budding scientist in the field.

Prof. (Dr.) P.C. Trivedi, Former Vice Chancellor, D.D.U. Gorakhpur University, Gorakhpur and Dr. R.M.L. Avadh University, Faizabad (U.P.) has over four decades of experience in teaching and research in the Department of Botany, University of Rajasthan, Jaipur. He has authored and edited 170 books to his credit. He has published more than 350 research/ review articles in journals of repute; guided 44 Ph.D. students; Completed 21 major research projects funded by national agencies; visited 15 countries by invitation for academic purpose. He is a fellow of nine academies. He was awarded more than 25 prestigious awards and honours for his research contribution including Birbal Sahni Gold Medal by Indian Botanical Society; B.P.Pal Gold Medal by Indian Science Congress, Kolkata (Given by PM of India); Life Time Achievement Award, I.P.S. Recognition Award etc. Presently he is a President of Mendelian Association of India and served as President of many prestigious academies including Indian Botanical society, Indian Science Congress Association (Plant Science); Indian Phytopathology Society (West zone), Society for Promotion of plant sciences etc. To honour his contribution Indian Botanical Society instituted a medal in his name "Dr. P.C.Trivedi Medal for Editorial Excellence" from 2019 onwards.

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