GENOME EDITING AND ITS APPLICATIONS FOR INSECT PEST CONTROL: CURSE OR BLESSING?

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SUMMARY

Gene and genome editing are described as cutting-edge research tools with the potential to tackle urgent global challenges in the management of agricultural pests and human disease vectors such as mosquitoes. The field is defined by the chances and challenges to interlink the disciplines of insect genomics, molecular biology, and pest control together with the need for clear risk assessment, policy development and public approval of the application of such novel technologies. The goal is to generate innovative and sustainable pest control solutions applied in the best interest for the environment and human society. Here, starting from available genome editing technologies, the current strategies and applications for insect pest control are discussed, including approaches to overcome the evolution of resistance alleles and other potential pitfalls to be expected from selective pressures resulting from gene drive applications. They are supplemented by views on regulatory, policy and ethical considerations that in our opinion will be necessary to define how the different tools can be used in the future in a safe and responsible way.

Key Words: CRISPR-Cas9, gene editing, gene drive, genome engineering, GMO, transgenic, insect genomics, engineered nucleases, meganucleases, zinc finger nucleases, TALEN, homing endonucleases, regulations, risk assessment, ethics

1. INTRODUCTION

Europe faces serious problems related to insect pests in two key areas: health and agriculture. The European Centre for Disease Prevention and Control has warned European authorities of the increased risk of local transmission cycles and epidemics of mosquito-borne infectious diseases (ECDC 2018). Locally transmitted cases of malaria, for instance, have already been reported in Greece, while in other regions in the world the disease claims nearly half a million lives annually. Similar threats are emerging with viral diseases such as chikungunya and viral haemorrhagic fevers including Hantaan, dengue, and yellow fever. European authorities have responded to this new threat with recommendations for vector control measures, as well as increased financing for relevant research, demonstrating the interest and concern of the European Union (EU).

Additionally, endemic and invasive agricultural insect pests cause tremendous losses in agricultural yield and revenues. Worldwide, insects cause up to 40% loss of potential crop yields, and the European Environment Agency reported the presence of over 10 000 invasive pest species for Europe in 2017 (EEA 2017). Significant damage caused by these pests affect the economy and negatively impact biodiversity. Invasive species directly cost Europe billions of Euros per year. Additionally, heavy reliance on pesticides to control them generates significant unquantified costs. These are costs associated with environmental pollution, e.g. soil and water. Other costs arise from resistant pests and include those required to develop alternative new effective pesticides. Also, loss in biodiversity, reintroducing biodiversity, and the impact on public health from pesticide residues or direct exposure to pesticides add to the expenses.

For these reasons, innovative pest management methods and strategies are urgently needed. Numerous stakeholders have expressed this urgency to prevent total crop losses and combat diseases transferred by mosquito vectors, but, at the same time, demand clear information about the risks and benefits of such novel technologies.

Novel vector and pest control solutions based on genome editing tools have the potential to improve the lives of millions of people, both by offering adequate protection against insect-borne diseases and by preventing crop and livestock damages caused by invasive agricultural pests. By using these novel technologies, different approaches are now possible that were only fiction in the past. While they sound promising, their possible side effects have to be considered and detailed evaluations prepared concerning the applicability of such new systems. Moreover, societal questions need to be answered like ‘Should we use some of the technologies at all?’ In the end, it is a combination of science, technology, ethics, policy, and communication that will define the feasibility and applicability of one or the other technology.

In this chapter, we want to review the technological genome editing options that are available and have been developed in the field so far. We also discuss their ‘curse or blessing’, together with considerations for their safe and responsible application for insect pest control tactics and strategies that do not pose risks and are friendly to the environment. The views presented here are the personal opinion of the authors on this important topic, without the claim for completeness or being the only or best possible solution. More considerations on the evaluation of gene editing and gene drive technologies are presented in the chapters by Liebert (this volume) and Nielsen (this volume).

2. AVAILABLE GENOME EDITING TECHNOLOGIES

Genome editing technologies can be divided into three categories: homologous recombination, engineered nucleases, and the CRISPR/Cas system.

2.1. *Homologous Recombination*

The early genome editing trials were performed by *homologous recombination* (HR), based on observations from the yeast *Saccharomyces cerevisiae* Meyen ex E.C. Hansen, where HR occurs at a high frequency. A specific sequence can be inserted into the genome at a defined position by flanking the sequence with homology arms identical to the sequences left and right of the selected insertion position. The construct is then injected into cells and inserted into the genome by recombination of the homology arms with the corresponding genomic sequences. However, the recombination rate in most cell types is meagre. In higher plants, it is estimated to be in the range of 0.01-0.1% (Puchta 2002; Hanin and Paszkowski 2003; Reiss 2003). In mammalian cells such as mouse embryonic stem cells it can be 1% or higher, but it can as well be as low as one in more than a million events (Vasquez et al. 2001). Moreover, the integration often occurs at unspecific sites, leading to off-targeting with a frequency of one in 10^2 to 10^4 treated cells (Vasquez et al. 2001). Design of the homology arms (sequence and length) seems crucial for the off-target rate in human cells, as the genome contains a lot of repetitive elements such as LINEs and SINEs (Long and Short Interspersed Nuclear Elements), which comprise 36% of the human genome. The longer the arms, the higher the probability of including such repetitive elements, which can cause recombination at unspecific sites (Ishii et al. 2014).

2.2. *Engineered Nucleases*

A more efficient way to edit genomes was established with the use of *engineered nucleases*. These nucleases act like molecular scissors, inducing double strand breaks at specific genomic positions that are then repaired by one of the two cellular repair pathways: non-homologous end joining (NHEJ) or homology-directed repair (HDR).

Three different nuclease families have been engineered for genome editing: meganucleases, zinc finger nucleases (ZFNs), and transcription activator-like effector-based nucleases (TALEN).

Meganucleases are predominantly found in microbes, and it is almost impossible to find a natural meganuclease targeting the specified sequence. Therefore, scientists applied different strategies, including random mutagenesis and high throughput screening, fusion of different nucleases, as well as rational design, to modify the binding specificity of the enzymes to expand the rather limited choice of target sequences (Sussman et al. 2004; Arnould et al. 2006; Rosen et al. 2006). With a recognition sequence of 14–40 nucleotides, meganucleases have a high target site specificity.

The ZFNs and TALENs are fusion proteins consisting of a non-specific DNA cutting enzyme, the restriction endonuclease FokI, which is linked to a zinc finger (ZF) or transcription activator-like effector (TALE) domain. These peptides recognize specific DNA sequences and thereby confer sequence specificity to the endonuclease. Like meganucleases, the choice of naturally occurring target sites of ZFs and TALEs is limited and can be extended by protein engineering to theoretically bind nearly any desired sequence.

Targeted genome editing including TALENs was named the 2011 method of the year by Nature Methods (Becker 2012). Protein engineering, however, is time consuming, cumbersome and costly. Therefore, while ZFNs and TALENs were successfully applied to modify different insect genomes (Bozas et al. 2009; Takasu et al. 2010; Liu et al. 2012; Aryan et al. 2013; Smidler et al. 2013; Takasu et al. 2013), a widespread use of these endonucleases was prevented by the need for a new engineered protein for each new genomic target site.

2.3. *The CRISPR-Cas System*

The discovery of *Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)* has tremendously advanced the field of genome editing. CRISPR sequences were first discovered in bacteria and archaea in the 1990s and were identified as a prokaryotic equivalent to the eukaryotic acquired immune system. Upon infection of a bacterial or archaeal cell with a pathogen, the cell incorporates a short sequence of the foreign DNA (e.g. virus or plasmid) into its genome. Such foreign DNA sequences are collected in clusters and separated by short repeat sequences. Small clusters of Cas (CRISPR associated) genes are located next to the repeat-spacer arrays (for comprehensive explanations and illustrations see Horvath and Barrangou 2010; Marraffini and Sontheimer 2010; Bhaya et al. 2011). Upon reinfection, the arrays are transcribed and processed by one family of Cas proteins into short CRISPR RNAs (crRNA), which are bound by another class of Cas proteins.

The crRNAs then guide these Cas proteins to the foreign DNA for degradation by endonucleolytic cleavage in a mechanism similar to RNA interference in higher eukaryotes (Marraffini and Sontheimer 2010). The target site specificity of the Cas endonuclease is determined by its bound crRNA. Therefore, Cas proteins can be programmed to target nearly any genomic site by adjusting the crRNA sequence. As the crRNA sequence adjustment is easy and cost-effective, CRISPR-Cas is an ideal and versatile tool for genome editing and has essentially abolished the use of ZFNs and TALENs.

The Cas proteins are categorized into two classes. The class I systems use multiple Cas proteins for the degradation of foreign nucleic acids, whereas the class II systems consist of one single protein. Therefore, the class II systems are more suitable for research and application purposes. The most used nuclease for CRISPR genome editing is the multifunctional class II protein *Cas9*. In addition to the crRNA, *Cas9* requires a transactivating CRISPR RNA (tracrRNA) to function. To streamline its application in the laboratory, scientist fused the crRNA and tracrRNA into one single-guide RNA (sgRNA) (Jinek et al. 2012). CRISPR-Cas can be used to knock out existing genes making use of the cell's NHEJ repair pathway or to introduce new DNA sequences including whole genes and transgene constructs by adding a repair template containing the respective sequence information and homology regions to the *Cas9* target site. The cell's HDR pathway then uses the repair template to repair the Cas-induced double-strand break.

Since its first application for genome editing in 2012/2013, more class II Cas proteins suitable for genome editing purposes have been identified (Zetsche et al. 2015; Abudayyeh et al. 2016; Yang et al. 2016), and the system is continuously being adjusted and optimized for different applications and purposes. These efforts include modifications to decrease the off-target rate of the CRISPR-Cas system (Fu et al. 2014; Kleinstiver et al. 2016; Nowak et al. 2016).

Shortly after its first application for genome editing, CRISPR-Cas was used in *Drosophila melanogaster* Meigen (Ren et al. 2013; Yu et al. 2013; Bassett et al. 2014). Within just four years, it was subsequently applied to several other insect species including the lepidopterans *Bombyx mori* L. (Wang et al. 2013; Ma et al. 2014), *Spodoptera litura* (F.) (Bi et al. 2016), and *Danaus plexippus* (L.) (Markert et al. 2016), the orthopteran *Gryllus bimaculatus* (De Geer) (Awata et al. 2015), and importantly also to several dipteran vector and pest species, namely *Aedes aegypti* (L.) (Kistler et al. 2015), *Anopheles gambiae* (Giles) (Hammond et al. 2016), *Ceratitis capitata* (Wiedemann) (Meccariello et al. 2017; Aumann et al. 2018) and *Drosophila suzukii* (Matsumura) (Li and Scott 2016; Kalajdzic and Schetelig 2017; Li and Handler 2017), as well as in the non-pest dipteran, *Musca domestica* L. (Heinze et al. 2017).

The CRISPR-Cas genome editing system also transformed gene drive research and development. Gene drives are genetic drive mechanisms that can be used to spread a genetic trait through a population by biasing its inheritance beyond the Mendelian inheritance rate of 50%. Gene drives were initially designed using naturally occurring selfish genetic elements like *Medea* (maternal-effect dominant embryonic arrest) (Chen et al. 2007; Buchman et al. 2018) or homing endonucleases

(HE) (Burt 2003; Windbichler et al. 2011). HE recognize and cut short DNA sequences. These sequences are only located on chromosomes different from the one on which the HE is located, and additionally on the homologous chromosome exactly at the location of the HE. In a heterozygous individual, after introduction of a double-strand break by the HE on the homologous chromosome, the cell's repair mechanism uses the chromosome containing the HE to repair the break, thereby copying the HE gene onto the homologous chromosome and converting the heterozygote into a homozygote. This process is called *homing*.

The CRISPR-Cas system can be programmed to act like an HE by targeting its genomic integration site on the homologous chromosome using a respective homing guide RNA. Identical to the HE, the induced cut is repaired using the allele containing the CRISPR construct as a template, thereby copying it onto the homologous chromosome. Thus, in contrast to genome editing via CRISPR, where only the CRISPR-induced molecular changes, but not the CRISPR components themselves are passed on to the next generation, in CRISPR gene drives also the genes coding for the CRISPR components are incorporated into the genome and passed on to the offspring. With the inherent programmable target site specificity of the CRISPR-Cas system, nearly any genomic position can be selected for the placement of the CRISPR homing construct. If homing occurs in the germline, then theoretically all the offspring will carry the CRISPR construct instead of only 50% like in normal Mendelian inheritance.

The potential use of these genome editing technologies, especially of CRISPR-Cas gene drives for insect pest control, is discussed below.

3. CURRENT STRATEGIES AND APPLICATIONS INVOLVING GENOME EDITING FOR INSECT PEST CONTROL

A promising and proven, sustainable and species-specific method to manage insect pest populations is the Sterile Insect Technique (SIT) (Dyck et al. 2021). It is based on the mass-production and release of sterilized males of the target species in the affected area. The sterile males mate with wild females in the field, which will not result in viable offspring, thereby reducing the wild population size of the pest in the next generation. By repeated releases, the population can be decreased to a manageable level. The SIT is most successfully applied in area-wide programmes against several pest species of agricultural importance, including the Mediterranean fruit fly, the Mexican fruit fly, *Anastrepha ludens* Loew, or the New World screwworm, *Cochliomyia hominivorax* (Coquerel).

The effectiveness of such programmes can in some cases be increased with the establishment of so-called sexing systems to eliminate females to allow male-only releases. Male-only releases are desirable for some agricultural pests and are a prerequisite for vector insects such as mosquitoes. In both cases, early elimination of females enables more cost-effective mass-rearing and release.

Most importantly, the efficiency of male-only releases is superior due to the lack of undesired mating between sterilized males and sterilized females (Franz et al. 2021).

In mosquito control programmes, the release of only male insects is essential as it precludes the risk of increasing the number of disease-transmitting individuals by releasing females. Therefore, mass-reared males and females must be separated at large-scale or females be eliminated at some point during the production process. This sexing of the insects (needed up to one billion male insects per week) is a significant bottleneck for the application of the SIT to new insect species. There is, for example, no effective sexing system to date for any of the vector mosquitoes.

For recent field trials with transgenic *Ae. aegypti* mosquitoes carrying a conditional lethal RIDL system (Release of Insects carrying a Dominant Lethal) that kills the offspring in the late larval stage (Thomas et al. 2000; Phuc et al. 2007), male and female pupae were separated mechanically by hand, resulting in the production of 0.5-1.5 million males per week and a female contamination of the released insects of less than 1% (Carvalho et al. 2015). This labour-intensive and time-consuming method, however, does not allow large-scale programmes beyond the field trial scale. Therefore, coordinated international research efforts are ongoing to establish sexing systems in different *Anopheles* and *Aedes* species (Gilles et al. 2014; Bourtzis and Tu 2018). Genome editing, combined with available classical genetics or transgenic technologies, can help to solve these and other issues related to insect pest control.

3.1. Unravelling Sex Determination Pathways in Insects

Genome editing is being used in basic research of insect pests, for example, to uncover gene functions and thereby better understand the target insect's biology and physiology. One major point of interest concerning sexing is the elucidation of the sex determination pathways in pest species to identify the responsible gene(s) for male/female development. CRISPR technology was used to knock out the candidate gene for the male-determining factor *Nix* in *Ae. aegypti* (Hall et al. 2015). Knockout of *Nix* resulted in feminized males, whereas ectopic expression resulted in masculinized females, identifying *Nix* as necessary and sufficient for determining maleness in the yellow fever mosquito.

Similarly, the Cas9-mediated knockout of the candidate M-factor gene *Mdmd* in *M. domestica* confirmed the key role of this gene for male gonadal and germline development in the house fly (Sharma et al. 2017). Once the sex determination pathways are understood for pest species of interest, the knockout or overexpression of the sex determination genes via CRISPR-Cas could be used to create female lethality, or for the conversion of females into phenotypic males to create strains for large-scale sexing (Meccariello et al. 2019).

3.2. Site-directed Mutagenesis in Pest Insects to Enable Population Control

For several economically important insect species, transgenic insect strains have been established to demonstrate the ability to generate male-only populations for control programmes. The first strains consisted of transgenic conditional female-lethal systems in agricultural and livestock pests like *C. capitata* (Fu et al. 2007; Ogaugwu et al. 2013), *Anastrepha suspensa* Loew (Schetelig and Handler 2012b), *A. ludens* (Schetelig et al. 2016), and *L. cuprina* (Yan et al. 2017). In these systems, under permissive conditions, a lethal gene is explicitly expressed in females to kill them at an early embryonic stage via apoptosis (Schetelig and Handler 2012b; Ogaugwu et al. 2013; Schetelig et al. 2016; Yan et al. 2017), or later in development by the ubiquitous accumulation of tTA (Fu et al. 2007), resulting in a 100% male cohort for release. Similar systems have been created expressing the lethal cassette in both sexes to produce genetic sterility (Gong et al. 2005; Schetelig et al. 2009; Schetelig and Handler 2012a). A release of these insects would result in biologically fertile matings, but the offspring would die between the early embryonic (Schetelig et al. 2009; Schetelig and Handler 2012a) and the late larval stage (Gong et al. 2005). All the transgenic systems described here, and many others, have commonly been introduced into the insect genomes via transposable elements, which integrate into the genome randomly. Integration at an unfavourable genomic site, however, can have adverse effects on insect fitness due to insertional mutagenesis, as well as on transgene expression levels due to nearby regulatory genomic elements. Genome editing technologies now allow inserting the transgene construct at specific, characterized genomic positions, thus avoiding harmful side effects of random integrations and improving the quality of transgenic strains for pest control.

Genome editing technologies furthermore offer the possibility of recreating a genetic trait from one species in another, which is not possible with any other technology with similar efficiency and ease. Mediterranean fruit fly SIT programmes, for example, rely on a conditional, temperature sensitive lethal (*tsI*) mutation, obtained by classical mutagenesis and breeding, to eliminate female embryos during mass-rearing via heat shock, resulting in the release of only sterile males (Franz et al. 2021). It has been tried to generate such *tsI* strains in other insect pests via classical mutagenesis (Ndo et al. 2018), or to link other selectable markers to one sex via radiation-induced translocations. Most successful have been pupal colour markers in the Mediterranean fruit fly and the Mexican fruit fly, as well as dieldrin resistance in *An. gambiae* (Curtis et al. 1976), *An. arabiensis* (Curtis 1978) and *Anopheles albimanus* Wiedemann (Seawright et al. 1978). In *An. arabiensis*, classical mutagenesis was used again years later to induce insecticide resistance in males as a tool for female elimination (Yamada et al. 2012). All these sexing tools have been developed either by classical mutagenesis or selection of a naturally occurring phenotype and linking it to one sex. The underlying mutations and mechanism of the corresponding phenotype in most cases are unknown, although it would be extremely

valuable to know. Once such a mutation created by classical mutagenesis (e.g. the conditional *tsl* in the Mediterranean fruit fly) is identified, CRISPR-Cas genome editing could be used to precisely edit the genome of another pest species to create an identical mutation in the respective gene. The mutation created in this way would not be different from the mutations induced by the approved classical mutagenesis techniques, not involving any transgene or foreign DNA (but usually rather small deletions or single nucleotide changes).

Li and Handler (2017) recently used CRISPR-Cas to create point mutations into the transformer sex determination gene in the fruit pest *D. suzukii*, thereby recreating the temperature-sensitive *D. melanogaster transformer-2* mutations (*tra-2^{ts1}* and *tra-2^{ts2}*) in a proof-of-principle experiment. The CRISPR-Cas system was also used to create an X-shredder in *An. gambiae* mosquitoes to achieve female elimination. The Cas9 is expressed gonadally to target X-chromosomal ribosomal RNA sequences, thereby destroying the X chromosome in X gametes, resulting in predominantly Y gametes and therefore predominantly male offspring (Galizi et al. 2016). The Cas9-based X-shredder is based on the original idea from Galizi et al. (2014) using the endonuclease I-PpoI to cut X-chromosomal ribosomal RNA sequences.

Besides the important topic of sexing, genome editing could be used to improve any other aspect of insect pest control programmes, for example, to enhance insect fitness to overcome deficits induced in the insects by the mass-rearing process such as low competitiveness and short life span. Alternatively, males could be modified to improve mate-seeking success, all of which could improve the efficacy of SIT control programmes.

3.3. Gene Drive Systems for Population Suppression or Replacement

Finally, genome editing technologies like CRISPR also open a new path to insect pest control via gene drives. Gene drives could be used for pest control in two ways, via insect population suppression or population replacement. Like other approaches based on the release of sterile insects, population suppression via gene drives results in population size reduction in the next generation. However, population suppression drives do not use reproductive or genetic sterility in the classical sense, as this would prevent the spread of the trait into the population, as all the offspring carrying the trait would die before reproduction. Therefore, the genetic trait conferring population size reduction is linked to a gene drive component. Upon a one-time release of a seed population, the gene drive component drives the trait into the population with each successive generation, abolishing the need of repeated mass-releases. A population suppression gene drive could, for example, be a genetic modification killing females and resulting in only male offspring. These males will carry the “sterility” construct and pass it on to their sons upon mating with wild type females, thereby decreasing the population size further with each generation as no females are produced, until the population collapses, at which point the gene drive construct would disappear.

Another approach could be a modification that reduces female fertility. Such a system has been developed in the vector mosquito *An. gambiae* by targeting different putative female fertility genes. The gene drive components were designed to home in the germline of both sexes to ensure that all female offspring is affected (Hammond et al. 2016). In a different project, female sterility was achieved by targeting a female-specific exon of the sex determination gene *doublesex* in *An. gambiae*. Disruption of this exon by Cas9 did not affect male development but resulted in females with an intersex phenotype that were completely sterile (Kyrou et al. 2018). Most gene drive research has been performed in *Anopheles* mosquitoes so far, where it might be applied first in a control programme. There is only one report in an agricultural pest, *D. suzukii*, showing the functionality of a synthetic *Medea* gene drive system that could be used to spread a cargo gene into the population, for example for population suppression purposes (Buchman et al. 2018).

Population replacement gene drives mostly make sense for vector insects. The idea is to replace the wild type population by insects that are refractory to the infection with the pathogen, thereby interrupting the disease transmission cycle. In the first CRISPR-based gene drive in mosquitoes, Gantz et al. (2015) developed a drive system in which the Cas9 homing construct is expressed in the male and female germline of *An. stephensi*. The construct further contains previously identified dual anti-pathogen effector genes conferring resistance to *Plasmodium* infection, which are expressed somatically (Isaacs et al. 2011, 2012).

Gene drive technology holds great promise to solve problems caused by harmful insects, and the initial enthusiasm expressed in view of the options for gene drive design that opened up with the discovery of CRISPR was huge. It triggered statements that there would be the first mosquito gene drives out in field trials within less than two years. This enthusiasm was dampened, however, when the technology hit a sudden roadblock, making it very clear that we have to understand much more about drive mechanisms and their potential pitfalls before releases could be considered.

Two predominant issues arose in laboratory experiments: first, loss of function due to the evolution of resistant alleles (Hammond et al. 2017; Marshall et al. 2017; Unckless et al. 2017; KaramiNejadRanjbar et al. 2018), which is mostly a problem for homing-based CRISPR-Cas gene drives (i.e. mutations evolving at the homing gRNA target site), and second, failure of the drive due to population genetic diversity (Drury et al. 2017; Buchman et al. 2018) (i.e. sequence variation at the gene drive target site throughout a population; this affects CRISPR-based as well as other gene drives). Evolution of resistance alleles increases with the selective pressure put onto the gene drive-carrying insect by the drive itself or a linked genetic construct and is inverse proportional to the conservation level of the target sequence (see Section 7.3.1. for approaches being followed to overcome the technological pitfalls identified). The CRISPR-Cas technology with its versatility will be instrumental to overcome the identified evolutionary pitfalls, and in developing new drive systems to study and improve this technology for safe applications in the future.

4. REGULATORY CONSIDERATIONS OF GENOME EDITING

Genome editing technologies did not play a significant role in the development of genetically modified organisms (GMO) for agriculture, the food industry or other applications before the discovery of CRISPR and its potential for large-scale and high throughput, affordable genome editing. Therefore, questions of how organisms resulting from genome editing should be regulated were not relevant. This changed with the introduction of CRISPR, triggering large-scale research not only in crop optimization but also in modifying farm animals to be leaner, to develop faster, have longer wool, or a differently coloured coat. It raised the urgent question of how such products should be evaluated and regulated for bringing them to the market, and how currently existing regulations apply to genome editing. Should genome-edited products be classified and treated as GMO or not?

In the European Union (EU), the deliberate release of GMO is regulated by the 2001 EU directive (2001/18/EC). The directive specifies the procedures required in the EU for the evaluation and authorization of GMO releases. While the directive covers all kinds of GMOs, it so far has been applied only for the regulation of genetically modified (GM) crops. This selective implementation has been described as the ‘plant paradigm’. The 2001 directive states that an organism is characterized as GMO if its genetic material has been altered in a way that could not have occurred naturally by mating or recombination. Therefore, also organisms developed by non-transgenic methods can be classified as GMO. At the same time, however, conventional mutagenesis techniques like radiation or chemical mutagenesis, that were considered safe in 2001, are exempt from the GMO directive as long as they do not involve recombinant DNA (the mutagenesis exemption). Genomic modifications created by genome editing technologies can be changes on the level of whole genes (including the insertion of transgenic constructs) but can also be the introduction of small mutations (insertions or deletions of a few base pairs) all the way down to single nucleotide changes, as they could also occur by classical mutagenesis and breeding techniques. Therefore, from a rational and scientific point of view, such small mutations induced by genome editing technologies should be treated similarly to mutations induced by classical mutagenesis.

To clarify if and to which extent the rules of the 2001 directive would apply to the new genome editing technologies, the French Council of State sent an inquiry to the European Court of Justice (ECJ) to interpret the directive for the new technologies. The ECJ’s answer was provided in the form of a complex ‘Advocate General’s opinion in Case C-528/16’ statement, released in January 2018. It principally stated that changes induced by genome editing that could also have occurred by conventional mutagenesis should be regulated in the same way. However, scientifically unexpected, the court ruled in July 2018 against the Advocate General’s advice and stated that all products resulting from genome editing are subject to the directive and are to be treated as GMOs.

This decision raises different questions and uncertainty for the application of the new technologies. Interestingly, however, from a scientific point of view, it also puts the regulations for the established and safe methods in question. In an open letter to the Federal Ministry of Education and Research in Germany, the German Life Sciences Association stated that the inquiry was answered by the judges according to the legal conditions, but was not based on scientific facts available from EU authorities (the Scientific Advice Mechanism (SAM) and the European Food Safety Authority (EFSA)) as well as from a large number of scientists worldwide.

This EU decision follows years of complicated communication attempts in the area of regulation of GM organisms and products, which is still not consistent between countries. Regulation of GMO in the EU considers and evaluates the *process*, not the *product*. Therefore, two products with identical traits developed by different technologies are regulated differently. The recent court ruling on genome-edited organisms is a perfect representative of this evaluation approach. Genome editing as a process would be regulated, not the resulting product, meaning that all genome-edited products would be regulated without exception, even if the same trait could have been obtained by approved technologies without regulatory requirements. Moreover, in the EU, only the potential risks of the GMO are considered, while the prospective benefits are not considered.

The USA essentially takes the opposite approach. There, only the product is evaluated, independent of the method used to create it (Global Legal Research Center 2014). Consequently, the USA does not regulate any products that could as well be the result of traditional mutagenesis or breeding techniques, summarized by the United States Department of Agriculture (USDA) in the following statement on agricultural products on its website:

“Under its biotechnology regulations, USDA does not currently regulate or have any plans to regulate plants that could otherwise have been developed through traditional breeding techniques as long as they are developed without the use of a plant pest as the donor or vector and they are not themselves plant pests” (USDA 2018).

Thus, small deletions or single nucleotide substitutions are not regulated in the USA. Even the introduction of larger nucleotide sequences that could have also occurred by cross-breeding is not regulated. In a March 2018 press release, the USDA specifically stated that this includes changes made by genome editing technologies. This approach to product evaluation resulted in the recent clearing for commercialization of a mushroom edited by CRISPR-Cas (Waltz 2016). The clearance was given without the regulation for GMOs, as only a few base pairs in the polyphenol oxidase gene were removed to enhance the shelf-life. Another significant difference in the USA approach for risk assessment compared to the EU is that also potential benefits of the commercial GMO are considered.

The existence of globally diverse approaches to the evaluation and regulation of GMO not only causes problems in international trade, as products will be classified differently in different countries, but also pose an enormous challenge for the release of modified insects. With their big range of motion and dispersal, insects will not stop at borders. Therefore, especially for insects carrying a drive mechanism with the potential to spread through whole populations, a common international ground for evaluation and regulation has to be found.

5. THE IMPORTANCE OF INFORMED DECISION-MAKING ON GENOME EDITING TECHNOLOGIES

The long ongoing GM crop debate in the EU essentially led to a moratorium in the EU on GM crops from 1998 to 2010, which in 2003 triggered a case filing by the USA and other countries with the World Trade Organization (WTO) against the EU. The ban on GM crops combined with unfavourable and sometimes biased news coverage created fear and insecurity in the EU, resulting in strong opposition against GM technologies by the public. This stalled the corresponding research and caused the industry in the EU to step by step pull out of GM crop research and production, e.g. Bayer Crop Science and BASF closing their GM crop research in Germany between 2004 and 2011.

With the most recent ruling by the ECOJ that also classifies all genome-edited products as GMO, the required checks and controls needed to develop such products for the market would be too expensive for research institutes and small companies. Moreover, the classification of all genome-edited organisms as GMO will probably lead again to strong opposition and general rejection by the public, adding another hurdle for the marketing of genome-edited products. Therefore, in our opinion, decisions like the one by the ECOJ equal a moratorium on genome-edited products in the EU with far-reaching consequences. Again, it will incapacitate a whole biotechnology industry sector that will flourish in countries with less restrictive regulation. It will prevent innovation and the development of new technologies in the EU and will cause biotechnology companies to withdraw their respective departments from the EU market, which in turn will prevent the creation of or destroy jobs. Also, in the international biotechnology sector, there is concern that the EU will continue to lag behind countries such as the USA, where the regulations are more favourable for GMOs and the GM crop market. The ECOJ decision on genome-edited organisms will also influence trade markets, as occurred already during the EU GM moratorium, which experienced in the early 2000s negative impacts on the agricultural export revenue of countries such as the USA, Canada, and Argentina (Disdier and Fontagne 2010).

Regarding pest control programmes intending the area-wide use of genome-edited insects, this could be most problematic for agricultural pests that feed inside fruits/crops like the Mediterranean fruit fly. Marketing of such crops in the EU might be restricted by the control measures for GMO contamination in food, for which the EU tolerance levels are as low as 0.9%. For a polyphagous insect like the Mediterranean fruit fly, which feeds on many different fruits, multiple agricultural products in the treatment area might be affected by the EU import restrictions (Max-Planck-Gesellschaft 2017). Use of genome-edited insects for pest control will therefore require the establishment of a definition of insect contamination in food and a decision on tolerance levels. This general framework will be critical for exporting countries to decide if the trade-off between the possible import restrictions and concomitant loss of markets on one side, and the reduced crop production costs as well as more abundant harvests on the other side, are worth the use of genome-edited insects for pest control.

Besides the import restrictions, however, genome-edited insects might not be regulated by the 2001 EU directive on the release of GMO if the insects are 100% sterile. Sterile insects do not fit the definition of an organism and therefore aren't a GMO either. Thus, 100% sterilized mosquitoes released as part of a SIT programme seem not to be regulated in the EU (HCB 2017). The release of radiation-sterilized *Ae. albopictus* mosquitoes is carried out in Italy and Germany as part of SIT mosquito suppression trials. To our knowledge, in Germany, these releases did not require prior authorization and risk assessment. Thus, one could speculate that scientists might be allowed to use genome editing, for example, to develop sexing strains for a pest species, allowing to remove female insects during rearing and to release the corresponding males after (100%) sterilisation by irradiation.

6. ETHICAL ASPECTS OF GENOME EDITING AND GMO RELEASES

The CRSIPR/Cas system allows genome editing with an ease and effectiveness that appeared to be some way in the future just a few years ago. Now that “everything” seems feasible, the always present question of what is socially, environmentally and also ethically responsible to do is coming into the focus of discussions. As we are no experts on social sciences and bioethics, we want to just briefly touch on some questions that have been raised in different panels and newsgroups.

A major topic evolves around the moral aspect of genome editing. This involves the fundamental questions if we are allowed to edit an organism's genome at all, and in case of area-wide pest management programmes, if we have the right to eliminate invasive or native populations or even a species as a whole. Both questions have been raised before but became more prominent with the discovery of the possibilities that opened up by CRISPR-Cas. While these questions are important to discuss, it is also important to consider the impact that currently applied strategies and technologies have.

The classical mutagenesis and breeding techniques, for example, that have been broadly applied for decades and are widely accepted and mostly haven't been questioned, can randomly change the genome of an organism at multiple positions, and the induced changes on the molecular level are commonly not known. What can be achieved with CRISPR genome editing, depending on the modification, would not be different from what has been done for decades, but can be done now in a less random process.

Similarly, the use of insecticides has the potential to eliminate a pest population in the targeted area, with the side-effect of not only eliminating the target species but also affecting many other beneficial insects, besides the environmental impact. With the recent alarming news on the decline of overall insect numbers and biodiversity (Hallmann et al. 2017), the extensive use of insecticides has come once more into the focus of widespread criticism and public concern.

Another important point for ethical discussion deals with questions concerning constitutional rights, individual expectations, democratic decisions, and also questions about how we can balance the potential elimination of a species against the decreased burden for the human population (e.g. the decrease of infectious diseases, or reduced insecticide use and crop losses due to agricultural pests). The application of GM insects for pest control directly affects the people in the target area by releasing the insects into their air space. Constitutionally everyone has the personal right to decide on things affecting one's own life. Moreover, different groups involved in and affected by the release of genome-edited insects (scientists, companies, authorities, producers, the public) will be guided by a variety of (contradicting) motivations concerning the release. Different perceptions of what is a desirable future and fear of new technological developments due to lack of understanding further complicate the situation.

Thus, it will be essential to identify and involve all the different stakeholders at an early stage of a project. An open and honest discussion of the limits of a technology and of the scientific knowledge, as well as public education will be crucial to build trust in science, in involved organizations, and in the decision-making process. Open discussion forums, where representatives of different stakeholders meet, could help to promote dialogue and the mutual understanding of goals, motivations, expectations and concerns.

Finally, the involvement of the public should not be limited to information and education campaigns. It should also include the collection of concerns of the educated public for discussion and consideration in the decision-making process.

7. POTENTIAL RISKS, CHANCES, AND CHALLENGES OF GENOME EDITING IN INSECT PEST CONTROL

7.1. Are Genome Editing Technologies for Insect Pest Control a Curse?

In general, genome editing could be used to create insect strains for population suppression or replacement approaches. Strains for population suppression could contain a trait that for example allows sexing (female elimination during rearing), and the strain would then be used in a SIT approach (i.e. radiation sterilisation and subsequent repeated mass-releases of the sterile males). On the other hand, they could contain a gene drive construct in combination with a trait allowing population reduction, for example by targeting female fertility genes. Such strains would then be used in a one-time limited release and the trait for population reduction would be driven through the population until a critical population density threshold is reached where effective reproduction is impaired. At this point the population would collapse in the targeted region and the genetic trait would disappear from the environment.

The situation would be different for the release of population replacement gene drives, as these genetic elements are designed to remain in the environment. Such genome-edited insects typically would have the gene drive component in their genome combined with a genetic modification that for example makes a mosquito population refractory to pathogen infection. The goal of such a construct would be to drive the immunity against the pathogen through the mosquito population until all or the majority of the insects are immune, which would interrupt the pathogen transmission cycle between the human host and the mosquito.

7.1.1. Use of Genome Edited Insects for Population Suppression in SIT-like Approaches

From a scientific point of view, the risk of using genome-edited insects for population suppression in SIT-like approaches is comparable to strains developed by classical mutagenesis approaches. The genome-edited trait disappears from the environment with the death of each released generation, as these insects cannot reproduce.

For both genome modifying technologies, genome editing and classical mutagenesis, a random mutation could arise in the generated strains that inactivates the trait. In the example of a genomic modification that kills specifically females for sexing purposes, a revertant mutation could occur at the modification site that allows females to survive again. Studies in *D. melanogaster* showed that such revertant mutations could occur at a frequency of 10^{-7} or less (Chovnick et al. 1971; Handler 2016). Therefore, at a mass-rearing scale of up to $1-3 \times 10^9$ insects per week, a few revertant insects are expected. Depending on the numbers, this could result in an efficiency concern, the release of females, or the contamination of the wild population with a marker. Nevertheless, all of those insects can be sterilized before release and would therefore not interfere with the overall success of the programme.

Critics might articulate concerns about off-target effects caused by genome editing technologies (for a review on CRISPR off-target effects see Zhang et al. 2015), resulting in additional mutations at other positions in the genome than the intended one. However, classical mutagenesis using chemicals or radiation also causes multiple unknown mutations and chromosome breaks in the genome in addition to the one causing the selected trait. In the case of classical mutagenesis this fact is accepted and not considered as a risk or potential problem, and the respective organisms can be deployed without regulations.

7.1.2. Self-perpetuating Gene Drive Systems Used for Population Suppression

In the case of a self-perpetuating gene drive systems used for population suppression, the success of a programme depends, besides the stability of the population reduction trait, on the stability of the gene drive. Resistance development against the drive would abolish the spread of the population reduction trait in the population, going back to normal Mendelian inheritance. This would not have any harmful consequences except that the suppression approach will not work anymore, and the modified insects will decline until completely gone, unless the mutation not only inactivates the drive but also lends a selective advantage over unmodified insects. However, even then, the non-functional insect is very unlikely to pose a threat to the environment or human health. For further population reduction, a new drive system would have to be developed.

7.1.3. Gene Drive Systems Used for Population Replacement

In the case of population replacement drives, potential resistance development on several levels could impair the success of the approach. First, the mosquito could develop a resistance against the drive, stopping the spread of the immunity trait and resulting in its eventual loss. Second, the pathogen could develop resistance against the immunity trait. In this case, the trait would further spread through the population due to the gene drive component, but it would not have the immunizing effect anymore.

The selection of resistant alleles, in general, is to be expected in each approach that puts a selective or survival pressure on a population of an organism. Such pressure would be applied to the target insect in case of population suppression approaches, and on the pathogen in replacement approaches spreading immunity through a mosquito population. This resistance development mechanism, however, is in no way connected to the use of genome editing technologies. It is already happening globally with the observed pathogen resistance developing against antivirals, antibiotics, or anti-malarial drugs, with the increasing resistance of insects against insecticides, or with behavioural resilience, for example the change in biting behaviour of mosquitoes from night to day to avoid bed nets (Liu 2015; Thomsen et al. 2017). It will always be an arms race, and we have to be aware that every human activity applying survival pressure on an organism will select for an evading reaction.

7.1.4. Risk of Overestimating the Understanding of Gene Functions

Genome editing experiments in different mammals have shown that there is a risk of overestimating our understanding of gene functions. It is often limited to one or two single functions, when instead genes often are part of large regulatory networks or have different functions throughout development. Modification of their expression can lead to unforeseen and unwanted consequences besides the desired effect. A variety of genome editing studies have been performed to knock out the *Myostatin* (*MSTN*) gene in different mammals. *MSTN* controls muscle growth, and existing knockout mutants show higher muscle mass and leaner meat. However, while resulting in the desired higher muscle mass, the *MSTN* knockout also caused unwanted side effects like additional thoracic vertebra in piglets (Qian et al. 2015), or rabbits with enlarged tongues and severe health problems like high rates of stillbirth and early stage death (Guo et al. 2016).

These results show that despite an increasing number of sequenced genomes, we still don't know much about gene functions. Therefore, careful studies will be needed for genome editing approaches aiming at population replacement, which will involve editing of specific genes to produce a certain phenotype – envisioned beneficial – that should stably persist in the target population without side-effects. This is of less concern when genome editing is used for population suppression applications, for example in scenarios where:

- A conditional female-killing mutation like the Mediterranean fruit fly *tsl* is recreated in another species to establish a sexing system
- A sex determination gene is knocked out to produce single-sex offspring for population reduction purposes, or
- A transgene construct is introduced into a specific genomic position previously characterized not to be disadvantageous to the insect.

In these scenarios, the quality of sterilized males in terms of mating performance, reproduction, and life span is the main characteristic to evaluate the usability of any strain.

7.1.5. Other General Risks

There are other general risks associated with area-wide pest or vector management approaches. These are, again, not specific to the use of genome edited insects, but apply to any control approach. In case of vector-borne diseases, the local eradication of a disease maintained over several generations can pose a risk to the human population in case of the reintroduction of the disease. Since the pathogen would not have challenged the human immune system for several generations, a reintroduction of the disease into that population could result in severe outcomes of the infection.

For population suppression approaches targeting endogenous species, the consequences of local eradication for the ecosystem are mostly unknown, as the species' role in the ecosystem is often not well studied. Therefore, possible consequences on the food chain, on competing species, and the possible long-term consequences for the human population, can only be guessed.

One concern in mosquito elimination approaches, for example, is that the niche opened by the (local) elimination of the target species could be filled quickly by another vector species that might transmit the same or even other diseases.

7.2. Are Genome Editing Technologies for Insect Pest Control a Blessing?

Currently the most used strategy to fight agricultural pests and vector insects is the application of insecticides. While they can be very effective in achieving rapid suppression in local applications, insecticides have many disadvantages. A major concern is their lack of species-specificity and the concomitant negative impact on many non-target insect species, or potentially even representing a risk to the environment. Furthermore, increasing resistance to insecticides is being observed in a rapidly growing number of pest insects worldwide, requiring an increase in application doses or the combination of different insecticides to still have an effect. This, however, is a dead end as it will lead to even stronger impacts on the environment and ultimately to complete resistance and loss of function of existing substances. Excessive use of insecticides in combination with other factors has already caused a dramatic decrease in the overall insect mass in Europe (Hallmann et al. 2017) and has led to the drastic decline of beneficial insects like pollinators as described in newspaper articles for some areas of China. An additional limitation of insecticides is that they cannot be applied on an area-wide basis due to public opposition. As a result, remote pest breeding sites are not accessible for treatment, thereby representing a constant, untouchable reservoir for the resurgence of the pest population after or despite of insecticide treatment.

Area-wide control programmes based on the release of modified insects are a promising strategy for the sustainable and species-specific pest control without the adverse side effects of insecticides. 'Modified' in this context can mean anything from sterilized by radiation for pest control using the SIT approach, all the way up to genetically modified insects being, for example, refractory to pathogen infection and carrying a gene drive construct for population replacement. Any method based on the release of modified insects has the substantial advantage of being highly specific to the target species and therefore not negatively impacting other non-target organisms, even those closely related. They also do not have toxic or other adverse side effects for the environment. Methods based on the release of modified insects are therefore environmentally safe and sustainable. Moreover, they allow an area-wide treatment against the whole population of the insect pest, and the mobility of the released insects also allows reaching remote habitats or protected areas that are inaccessible or not open for other approaches like the spraying of insecticides.

Approaches based on the release of modified insects also allow reaching the next generation of pest insects, like the drought-resistant eggs of *Aedes* mosquitoes, that are not affected by insecticide spraying and thus lead to a resurgence of the population in the next rainy season. The presence of modified insects throughout the egg hatching period, in contrast, would intercept the newly emerging generation and thus prevent a resurgence of the wild population.

Currently, the application of the SIT against new pest species is limited by a few bottlenecks that can be overcome. Besides a suitable mass-rearing system, an efficient sexing system that can be applied at large-scale is essential. With genome editing technologies one can envision the generation of such systems. For example, once suitable mutations obtained by classical mutagenesis in one target species are uncovered, genome editing could be used to reproduce these mutations in homologous genes in other insect pests.

One prominent example would be the above-mentioned *tsI* mutation in the Mediterranean fruit fly that specifically kills female embryos upon heat shock, allowing 100% sexing of this pest species at a large scale, which has been the key for Mediterranean fruit fly SIT programmes since more than 25 years and could allow building sexing systems in other pest insects (Robinson 2002; Franz et al. 2021). This mutation is an excellent example of a classically generated mutation that, once identified, can pave the way for similar technologies to be built in other pest insects.

Genome editing technologies are moreover the key to any approach intending to spread, via the use of a gene drive, sterility or pathogen refractoriness in a population without continuous mass-releases of the modified insects. Such strategies can only be pursued with the use of genome editing. The use of a gene drive to spread a lethality or sterility trait into a population for suppression approaches would have the advantage that optimally a one-time release of a seed population would be sufficient to suppress a population compared to continuous mass-releases required for the SIT approach.

Genome editing technologies will therefore be able to support and advance environment-friendly and sustainable pest control methods on various levels, and will thus help protect beneficial insect species, the biological diversity, and healthy ecosystems.

7.3. Challenges and Possible Solutions for Genome Editing Strategies

Genome editing technologies face multiple challenges that have to be understood and evaluated before they will gain the status of being acceptable and safe for use in insect pest control.

7.3.1. Technological Challenges

One of the challenges is the development and selection of resistant alleles observed with different (mostly CRISPR homing) gene drive approaches (Hammond et al. 2017; Reed 2017; Unckless et al. 2017; KaramiNejadRanjbar et al. 2018). While the drives in general work with a satisfying efficiency in laboratory experiments, the evolution of resistance alleles that are not recognized anymore by the gRNA of the CRISPR-Cas system was generally observed with CRISPR homing drives within just a few generations (Champer et al. 2018), resulting in the inactivation of the drive. Much effort is therefore being invested into the development of new drive strategies and improvement of the existing systems. Approaches include the use of multiple gRNAs, the expression of the drive exclusively in the male germline, or the targeting of highly conserved genes (Kyrou et al. 2018).

The rationale of the first approach is that a mutation in one of the gRNA target sites will not be able to inactivate the drive as the other target sites are still functional. A simultaneous mutation of multiple target sites is much less likely (even though it is not impossible), thereby preventing or at least strongly postponing drive inactivation (Prowse et al. 2017). Activating the drive only in the male germline should limit resistance-allele formation post-fertilization, as the sperm should transmit only low amounts of the Cas endonuclease into the embryo, thereby preventing mutagenic events in the embryo. Targeting highly conserved genes like the sex determination gene *doublesex* for homing has been effective in building a functional gene drive system in the laboratory (Kyrou et al. 2018). It can prevent resistance allele formation as mutations in highly conserved sequences would likely be deleterious to the organism, and the resistance alleles would not persist (Esvelt et al. 2014; Champer et al. 2016; Noble et al. 2017; Champer et al. 2018; Nash et al. 2018).

Other proposed systems reduce resistance potential in so-called daisy-chain drives and toxin-antidote systems (Champer et al. 2016; Noble et al. 2019). Current studies and simulations seem to indicate, however, that resistance evolution will remain an issue that can't be completely suppressed, just slowed down, and might require the combination of different strategies to design successful drives (Callaway 2017; Champer et al. 2018). A drive will be successful if it can spread through the whole population before the first resistance allele formation occurs.

Besides the development of resistance to gene drives, any genome editing approach can be affected by the appearance of spontaneous inactivating or revertant mutations due to the natural mutation rate in the genomes of insects. Most of such inactivating mutations would be point mutations, deletions, or changes by recombination events or moving transposons. With a mutation frequency of 10^{-5} to 10^{-7} per base pair, depending on the mutation event (Chovnick et al. 1971; Tobari and Kojima 1972; Bender et al. 1983; Neel 1983; Woodruff et al. 1983; Handler 2016), the occurrence of multiple mutations is to be expected as mass-rearing is scaled up towards 10^9 insects per week.

This phenomenon is not specific to genome editing technologies, however. It is a concern for any genetic modification used in insect mass-rearing approaches, including transgenic technologies or modifications induced by classical mutagenesis. Thus, already now, safeguard technology is used for strain maintenance like the filter rearing system used for ongoing fruit fly SIT programmes, to prevent the occurrence and persistence of inactivating mutations in the release population (Caceres 2005; Franz et al. 2021). Furthermore, besides such filter rearing systems, the safe use of transgenic insects for large-scale programmes will require backup systems. In case of failure of one system due to a random mutation, the other will serve as a safeguard to either preserve the strain function or prevent the strain from surviving in the wild due to the inactivating mutation (Eckermann et al. 2014; Handler 2016).

7.3.2. Ecologic and Economic Challenges

The success of any area-wide control programme involving, but not limited to, genome-edited insects will also depend on understanding the pest insect's population ecology. Population suppression, as well as replacement, strategies will be influenced to a different extent by factors like the dynamics within the population, insect migration behaviour and range, the geographic situation, environmental factors influencing the target population, as well as density threshold levels for successful reproduction or effective disease transmission, but also the adaptive or evasive potential of the target species. For a successful insect pest control programme with regard to environmental and human safety, it will additionally be essential to understand the insect's role in the food chain and its interaction or competition with other species.

Critics of population elimination approaches claim that the target pest could be replaced by a competing pest, causing the same or other problems. However, except for some rare cases, such as a very strong and stable gene drive that in theory should be capable of eliminating a whole population, most strategies for population control will only lead to a reduction in population size. Even if potentially they can lead to elimination in large areas, they won't have the potential for global eradication of a species, and a resurgence of the targeted species is expected as soon as the treatment is stopped.

The commercial applicability of genome-edited insects also depends on the crop and the pest complex threatening this crop. If one major pest species threatens the crop, the use of a species-specific strategy involving genome-edited insects is a practical approach. However, in a situation where a crop is equally affected by two or more pest species, the specific suppression or elimination of one of them could lead to population increases of the remaining pest species. In this case, any species-specific insect release strategy would also need to simultaneously address the other pest(s), but such an approach may be less economically viable.

7.3.3. Regulatory Challenges

The release of genome-edited insects for area-wide pest control programmes will face significant challenges concerning existing diverging regulations for genome-edited organisms. As discussed above in Section 5, there is no uniform international approach to the evaluation, risk assessment and the release of GM and genome-edited organisms, with every country pursuing an individual approach to regulation. Consequently, one country might allow the release of a genome-edited insect after a thorough evaluation of the product for stability and safety, while a neighbouring country might generally prohibit the release of any genome-edited insect. This will pose a problem for releasing countries and their agricultural trade, as the insects will not stop at their country's borders, and they would not be able to guarantee the confinement of the genome-edited insects within their territory, even if the insects are released far from the border (Reeves and Phillipson 2017). While this is already a difficult situation for non-disseminating approaches such as GM crops and insects, it would practically require inter-country regulation of any strategy relying on gene drives which have the potential and the purpose to spread.

Besides the regulations concerning the release of genome-edited insects, some food safety regulations will pose a challenge for the use of these insects in agricultural pest control. This will be most relevant for insects that feed inside of the fruits/crops. Such crops could be contaminated with the genome-edited insects, at least for the time of the releases, which could restrict marketing of these products in other countries, leading to the possible (temporary) loss or shifting of markets (Max-Planck-Gesellschaft 2017). In this case, the producing country would have to decide if the application of the genome-edited insects is economically worthwhile. It will also influence the approval processes for new genome-edited insect strains by the governments and regulatory bodies. How regulatory authorities in different countries will classify genome-edited insect material in agricultural products will, therefore, have a strong impact on international trade.

7.3.4. Identification and Involvement of Stakeholders

Area-wide pest management approaches involving the release of genome-edited insects will affect many different stakeholders. Identifying these stakeholders and their interests, and involving them in the planning and decision-making process, will be crucial for the success of any genome-edited insect deployment; it will also strongly impact the future of pest management projects. We summarize some important points here and refer the interested reader to the more in depths analyses performed by Gould (2008) and Baltzegar et al. (2018) in relation to the deployment of genome-edited insects in agriculture and human health.

Ecological, economic, regulatory, and social contexts need to be understood in depth in a case-by-case assessment to be able to determine all the groups and subgroups that will benefit or be negatively affected by each intervention with genome-edited insects. Regarding the ecological impact of the genome-edited insect

release, different groups might have to be involved in the process on this level, including for example nature conservation organizations, ecologists, entomologists, farmer associations, public health workers, as well as environmental activists or NGOs opposing or not the use of GM or genome-edited organisms.

A central question for the identification of stakeholders will be who pays for the programme and who profits from it. Moreover, are the ones paying also the ones profiting from the deployment? Considering the case of agricultural pest control, who will benefit from the genome-edited insect releases will strongly depend on the production systems, but also on the crop(s) and the pest complexes threatening these crops. It can be assumed that a conventional farmer will mostly profit from the release due to the lower pest burden, resulting in lower costs for insecticide treatments and higher quality harvests with better yields and access to more profitable markets.

In contrast, for the organic farmers, the situation is complex. While they will also profit from the reduced pest insect burden, they might not be allowed to sell their products as they may contain residues of genome-edited material (for example in the form of insect larvae feeding inside the product). In many countries, there is a zero tolerance for GM material in organic food. Depending on how genome-edited material in organic food will be classified and regulated by individual authorities, the use of genome-edited insects might have a negative impact on organic farmers as their product might not be marketable anymore.

Thus, in case of a financing model that involves the producers in the application area, all would pay but not all would benefit. On the other hand, due to the insects' dispersal capacities, neighbouring producers might also profit from the decreased insect burden without paying, for example, if their production areas are located in proximity to a local programme.

Potential sales problems due to food contamination with genome-edited material will not be restricted to local markets but also affect international agricultural trade, where the different tolerance levels for GM/genome-edited material in food will affect the accessibility of markets. The negative impact will therefore not stop at the level of the producers, but it will also affect all downstream links in the trade chain and therefore many more stakeholders (Max-Planck-Gesellschaft 2017). On the one hand, the use of genome-edited insects might close some markets for certain production types (like organic farming), at least for the period of the release. On the other hand, the use of genome-edited insects could also lead to the reopening of markets, if the pest control measure results in the elimination of a quarantine pest, whose presence in the product would have prevented the export or required expensive additional post-harvest measures such as phytosanitary treatments.

Finally, a strong group of stakeholders that needs to be involved is the public, as the insects will be released in their airspace. Involvement of the public in the past has been handled very diversely. The first releases of GM insects were carried out with at best unidirectional public information campaigns but without any possibility to influence the decisions. This caused protest and considerable mistrust against compa-

nies and organizations promoting releases of GMOs (Subbaraman 2011; Baltzegar et al. 2018). Such experiences, step by step, initiated an important reconsideration and change in behaviour towards the public by scientists and organizations. In 2015, the release of GM diamondback moths was halted in New York, USA by the responsible authorities due to public disapproval despite the regulatory approval for the release (Boor 2015; USDA/APHIS 2015; Baltzegar et al. 2018). Similarly, the release of genetically modified mosquitoes by Oxitec Ltd. has been opposed in the target area in the Florida Keys, USA causing Oxitec to finally withdraw the application with the US Environmental Protection Agency (US-EPA) (Klingener 2018).

In Brazil, a major public awareness and engagement campaign was successfully conducted before initiating the release of transgenic mosquitoes in an urban area, emphasizing participatory action and a community-based programme (Capurro et al. 2016). Reconsideration of public involvement goes as far as claims from scientists to make biotechnology research that will affect “everyone” completely transparent from the beginning to build trust (Esvelt 2016, 2017). Certainly, for the success of a programme and its safe, economically worthwhile, and socially and environmentally responsible application, representatives of all relevant stakeholder groups should be identified and involved early in the decision-making process.

8. RECOMMENDATIONS FOR THE SAFE APPLICATION OF GENETICALLY ENGINEERED INSECTS FOR PEST CONTROL

8.1. Evaluation of the Product, not the Technology

From a scientific point of view, the evaluation process of a genome-edited insect should focus on the product, not the technology that was used to create the product. A technology like genome editing can be used to create a variety of genetic modifications, as discussed above, from point mutations and small insertions or deletions, to the introduction of whole transgene constructs. These modifications can also be achieved with other technologies like classical mutagenesis or transgenic approaches, although with different mechanisms. Therefore, the primary evaluation criteria for the product should be the introduced genetic trait, its properties, the projected consequences and benefits, as well as the potential risks in case of field deployment. In a second step, the evaluation should also consider the molecular mechanisms of the technology used, to be able to investigate potential side effects that are characteristic for each technology, including classical mutagenesis and breeding approaches.

A thorough product evaluation in our opinion should include the following points (without claim for completeness):

- Introduced genetic trait and its phenotypic properties – does it fulfil the aspired task?
- Stability of the genetic modification over time, and potential reasons for the trait to fail
- Consequences of a failure of the trait
- Molecular mechanism of the technology and its potential for side effects
- Are off-target mutations present and do the identified changes to the genome have consequences for the trait and its stability, for the genomic stability of the product in the environment upon release, or for the biological quality of the insect?
- Projected benefits of the release of the modified insect
- Potential risks of the deployment of the modified insect into the environment
- Benefits and risks (if any) of current pest control strategies
- Weighting of the benefits and risks of the new strategy against those of the current control strategy and against no intervention
- Possible ecological consequences (e.g. species reduction or elimination).

8.2. *Product Evaluation and Risk Assessment*

All the above points should be considered in the risk assessment of the product. For the best possible decision concerning a positive impact on society as well as nature, however, a thorough scientific product evaluation and risk assessment will not be enough. It will be crucial to have a wholistic decision-making process that will identify and involve representatives of all possible stakeholders and openly discuss their motivation, expectations and concerns early in the process.

The involvement of as many perspectives and sources of knowledge as possible from the start of the decision-making process will allow a more comprehensive process, that will also have a stronger democratic legitimization (Hartley et al. 2016).

One important point will be the honest, transparent and open discussion of the limits and gaps of the technology and the scientific knowledge and the consequences of a release. This will help to build trust in the scientific process and counteract the hype that can surround new biotechnologies (Caulfield and Condit 2012; Hartley et al. 2016). The 2016 publication by Hartley, based on the discussions of an international and interdisciplinary workshop entitled "Responsible Risk? Achieving Good Governance of Agricultural Biotechnology" held in Norway in November 2015, identifies five points that should be implemented in the scientifically and socially responsible development and application of agricultural biotechnology products: (1) commitment to candour, (2) recognition of underlying values and assumptions, (3) involvement of a broad range of knowledge and actors, (4) consideration of a range of alternatives, and (5) preparedness to respond (Hartley et al. 2016). In the end it should be a common decision of all stakeholders, if the expected positive impact of the product on society and nature is worth taking the risk, even if small.

9. CONCLUSIONS

Genome editing has a high potential for improvement of diverse human life situations. It could be the solution to control many vector-borne diseases, or safely and species-specifically control devastating agricultural pests, to reduce the burden on the human population worldwide. Nevertheless, it has to be studied thoroughly to the point where the technology and its potential drawbacks and side effects are very well understood, current technological roadblocks have been overcome, and the products are evaluated carefully according to well-defined regulations.

Regulations for genome-edited insects and other genome-edited products should be in the best interest of society based on scientific data acquired, rather than being based on an opinion from groups that are not independent. Therefore, also the underlying interests of institutions funding genome editing research should be critically analysed before they are allowed to give “public” views at any stage in the decision-making process. False information policies lead to the stalling of academic as well as industrial research, and in the case of Germany killing important innovation and economic development that will instead move to more open and innovation-friendly countries.

Genome editing is still in its infancy, and the consequences of tampering with gene function are not yet well understood, even when editing genes that researchers thought to be well studied. The gene networks are far more complex than so far assumed and meddling with them at this stage can have unforeseen consequences. While this lack of full understanding urges progressing with caution, it poses at the same time a strong demand for more research to understand the mechanisms involved, learn about side effects, and re-engineer the technology accordingly to make genome editing the safest possible technology. What would be counterproductive is a substantial restriction or even shutdown of the research due to uninformed or even misinformed fears and false information, which prevents better understanding and improvement of gene editing technologies.

In no way this should be a charter to do anything that is possible, but to base decision-making of every new technology on transparent science, facts, and comparing them to existing technologies to allow decisions like “are they an improvement or not?” This train of thought has been used in plant breeding for a long time, where only seeds with proven improvements over the existing ones are allowed on the market.

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