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Review Article

Dynamic timelines required for development of new insect genetic pest control technologies

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With 2 figures

Abstract: Modern tools in genetic engineering are enabling the development of diverse and exciting strategies for the genetic control of pest insects. This technological development takes time. Communicating where along the developmental timeline a nascent technology exists, and how long the technology might take to reach the next stage, is important for managing expectations between key stakeholders. Importantly, while the key stages in technology development can be standardized with different metrics or stage-gates, the residence time that different technologies will require at each stage is not consistent across technologies. In this perspective, we use the Technology Readiness Level (TRLs) metric for genetic pest control. We give examples of how different genetic pest control strategies used in insects have predictable and unique challenges at different stages of technology maturation.

Keywords: pgSIT; SSIMS; HEGD; RIDL; non-persistent; *Drosophila suzukii*; sterile insect technique (SIT); Technology Readiness Level (TRLs)

1 Introduction

"Time flies like an arrow. Fruit flies like a banana." - Anthony Oettinger

Genetic pest control has the potential to transform pest management efforts in the coming decades. Here we use the term "genetic pest control" to refer to the strategy to rationally manipulate the genetic content of a pest organism to turn it into an agent that will impact wild populations of the same species. Release of the modified pest control agent into a population of wildtype organisms allows the agent to interbreed and pass on its modified genetic material to its offspring, with the end goal of population suppression or replacement.

We emphasize rational manipulation of the control agent's genome in our definition to distinguish from techniques that use random mutagenesis of the control agent, such as X-ray or gamma-irradiation. This distinction is not meant to diminish the impact that random mutagenesis approaches have had in pest management. Indeed, traditional sterile insect technique (SIT) (Knipling 1959), is arguably the most powerful pest eradication strategy to be deployed, as exemplified in the New World screwworm eradication program (Knipling 1960). Traditional SIT programs are the proverbial shoulders on which rational genetic pest control stands. We adopted a narrow definition of genetic pest control for this perspective to focus on technologies for which the path to full implementation is not as well established.

There are dozens of approaches to achieve genetic pest control (Alphey 2014). Some methods, such as release of insects with dominant lethality (RIDL) (Alphey et al. 2014; Thomas et al. 2000) or sex-sorting incompatible male system (SSIMS) (Upadhyay et al. 2022), result in death of all hybrid offspring. Other tactics, such as female-specific RIDL (Alphey et al. 2014; Fu et al. 2007), trojan males, or the daughterless carp (Teem et al. 2014) approach result in a bias in the sex ratio in subsequent generations. Gene drives direct the super-Mendelian inheritance of a selfish genetic element to spread a desired trait into a population (Burt & Crisanti 2018). Recently described genetic pest control approaches can even target the parental generation, by spreading toxic proteins to wild females through seminal fluid (Beach & Maselko 2025). Lastly, there are technologies that span the

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boundary between random and rational genetic manipulation. For example, transgenic embryonic sexing systems (TESS) are rationally designed systems that can be used alone or with traditional SIT to enhance its effectiveness (Concha et al. 2020; Schetelig et al. 2021). Our short perspective is not intended to comprehensively review genetic pest control approaches, as others have recently done (Wang et al. 2021; Yan et al. 2023). We instead seek to defend the claim that different genetic pest control methods are expected to have unique bottlenecks at predictable points along a defined path to technological maturation.

Genetic pest control approaches have been developed or are currently in development for a wide array of target pest organisms, including human disease vectors, agricultural pests, or invasive species. Specific development projects are in various stages of maturity. Without a standardized and widely used stage-gating process, communicating the maturity of a new technology can be problematic. In the drug development sphere, the investigative new drug (IND) and clinical trial progression facilitate this communication. Stating that a candidate drug is pre-IND, IND, or in a Phase I/II/III Clinical Trial immediately gives stakeholders a sense of its maturity and the likely road ahead (Barrett 2022). Similarly, the National Aeronautics and Space Administration (NASA) developed Technology Readiness Levels (TRLs) in the 1970s and 1980s to classify and communicate the maturity or readiness of a new technology for deployment in the real-world (i.e., to assess whether a technology was fit for purpose) (Héder 2017). Such standards also function to mitigate risk. No equivalent stage-gating method currently exists for the development of genetic pest control.

2 Adapting NASA's Technology Readiness Levels (TRLs) for genetic pest control of insects

Elsewhere we have made a detailed argument for the adoption and adaptation of TRLs for non-persistent genetic pest control (Badger et al. 2025), but provide a high-level summary focused on insect pests here. TRLs describe the 9-step maturation of a technology from the ideation stage (TRL1) to demonstration in "flight proven" systems (TRL 9). TRLs are driven by performance history and facilitate comparisons among approaches with respect to a specific application, implementation, and operational environment (Kimmel et al. 2020). The narrow-sense purpose of genetic pest control is to reliably affect a change in the population density or genetic structure of a wild pest species. How quickly and how extensively these changes are expected to occur are important considerations about the viability of an approach, but these issues might be viewed as ancillary and solved through parallel technological advancements, for example, in rearing, sex-sorting, or releasing the modified pests. Our proposed TRLs take a hybrid approach, with early TRLs focusing on the success of genetic manipulations at cellular, individual, and small-population scales. Later TRLs address the functionality of the modified agents in operational settings. We briefly summarize the proposed TRLs for genetic pest control agents in Fig. 1. While previous work focused on aquatic invasive species (Badger et al. 2025) and this paper uses insect examples, implementation of a TRL scale will be useful for any genetic pest control technology development.

Early stage, TRL1–3, research is focused on designing the genetic pest control system, characterizing its component genetic parts, and demonstrating proof-of-concept in a model laboratory organism. We use the term "system" or "genetic system" to describe the composite of all genetic components that are brought together to achieve the desired genetic control results (e.g., Fig. 2). TRL4 is an important milestone where the model system is translated to the target pest organism for which real-world applications exist (e.g., from *Drosophila melanogaster* to the agricultural pest *Drosophila suzukii*), but the work is still confined to a laboratory environment. Mid stage, TRL5–7, research is aimed at testing the pest control system in increasingly real-world applications, from small-scale field trials to large-scale field trials and eventually beta-testing that includes all aspects of manufacturing, shipping, and assessment of efficacy of the pest control campaign. Finally, late stage, TRL8–9, research is focused on iterative refinement of the technology to ensure it remains effective and economical compared to alternative control techniques. Badger et al. (2025) provide a more detailed description of TRLs as they apply to genetic pest control and place existing technologies for the control of aquatic invasive species into the TRL framework.

In this perspective, we use the TRL framework to illustrate an important aspect of new technology development. Different technologies, in this case different strategies for insect genetic pest control, can remain in each TRL for various durations. We call these durations the residence times in each TRL. In other words, each method might experience a bottleneck at a different stage of technological development. Moreover, these bottlenecks can be somewhat predictable based on the underlying design of a particular genetic pest control system. Foresight and consideration of the expected residence times in each TRL can allow key stakeholders in the development of genetic pest control, including technologists, regulators, and funders, to take a long view of technology development and avoid the traps of chasing after short-term gains.

3 Case studies of four alternative strategies for genetic pest control

To provide a more tangible example of our argument about residence times in different TRLs, we will use four insect genetic pest control strategies that have been described in the

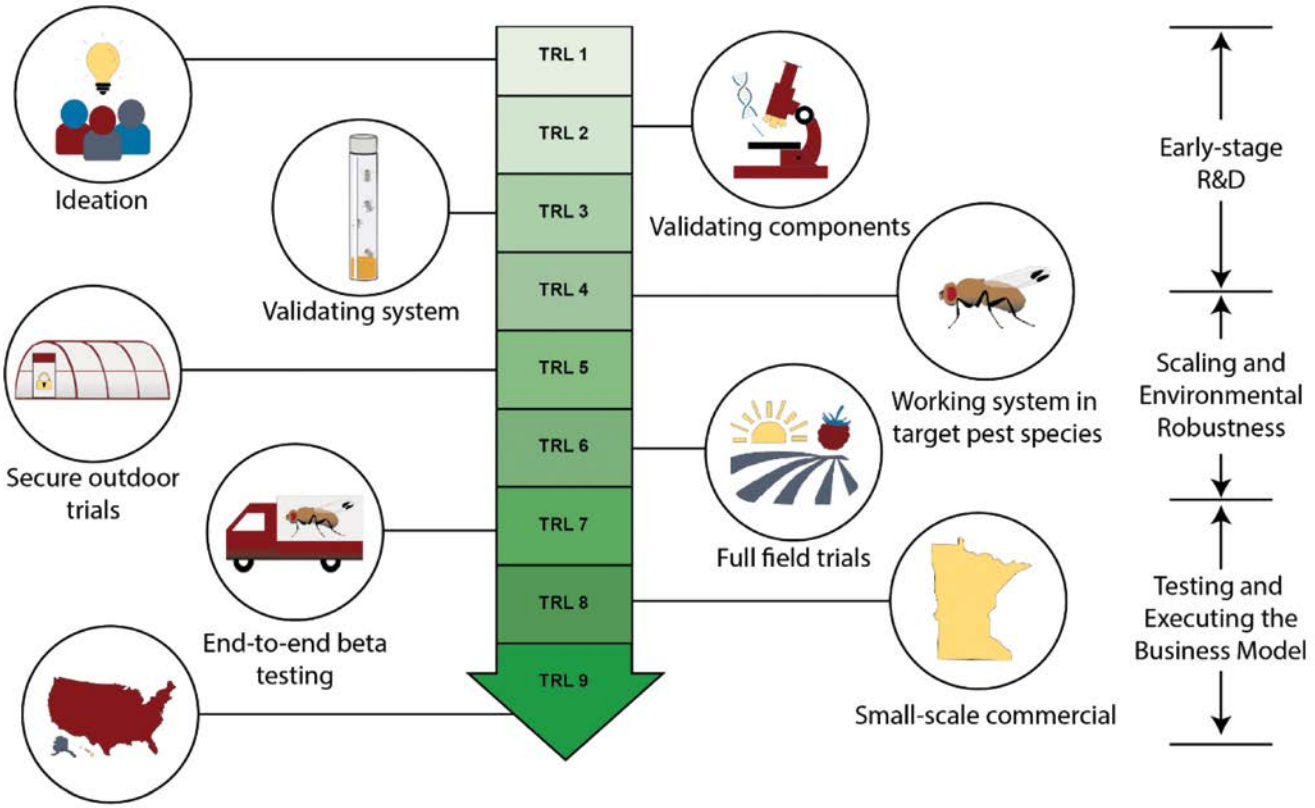

Fig. 1. Summary of technology readiness levels (TRLs) for genetic pest control development. The TRLs are discussed in more depth elsewhere (13) (Badger et al., 2025), but roughly correspond to ideation (TRL 1), validating behavior of component parts (TRL 2), validating behavior of the complete system (TRL 3), validating in an applied organism that is ready for research related to scaling (TRL 4), secure/contained field trials with applied organisms (TRL 5), full-scale field trials (TRL 6), beta-testing of the end-to-end supply chain to customer (TRL 7), small-scale commercial deployment (TRL 8), and full-scale commercial deployment (TRL 9).

literature (Fig. 2): precision-guided sterile insect technique (pgSIT) (Kandul et al. 2019), sex-sorting incompatible male system (SSIMS) (Upadhyay et al. 2022), female-specific release of insects with dominant lethality (fsRIDL) (Alphey et al. 2014), and homing endonuclease gene-drive (HEGD) (Hammond et al. 2016). These technologies have qualities that lead to bottlenecks at different TRL stages. pgSIT is a non-persistent (i.e., the allele frequency reduces in relative abundance after release into a wild population until it disappears) genetic pest control method that uses CRISPR/Cas9 to knock out genes required for female vitality and male fertility in the pest control agents that are to be released (Kandul et al. 2019, 2022; M. Li et al. 2021). This approach results in the release of engineered male insects that can compete with wild males to copulate with wild females, but that do not produce functional sperm to fertilize eggs (Witherbee & Gamez 2025). pgSIT has been demonstrated in the model laboratory organism, *D. melanogaster* and translated to several target species (Kandul et al. 2022; M. Li et al. 2021). It has progressed to semi-field trials in outdoor hoop-houses via a partnership between the US Department of Agriculture and the company leading commercialization efforts, Agragene (ARS project number 2072-22000-044-042-T; https://www.ars.usda.gov/research/project?accnNo=446300). This puts pgSIT at TRL5.

SSIMS is a non-persistent genetic pest control method that combines Engineered Genetic Incompatibility with conditional female lethality (Upadhyay et al. 2022). Genetic incompatibility between the released pest control agent and the wildtype mates is provided by a sequence-programmable transcriptional activator encoded in the homozygous control agent's genome. When the SSIMS agent mates with a wildtype individual, the hybrid experiences lethal over- or ectopic-expression of an endogenous tightly controlled gene. This leads to hybrid lethality in high frequency. The conditional female lethal allele provides a convenient mechanism to ensure that only a single sex (male) is released. SSIMS has reached TRL3, as it has been demonstrated in a model laboratory organism (*D. melanogaster*) but not a target pest organism.

fsRIDL is a non-persistent genetic pest control method that uses a female-specific gene expression element (e.g., female-specific promoter or female-specific alternative splicing intron) to control the production of a lethal effector protein (Fu et al. 2007). Note that we focus on single-component fsRIDL in this manuscript, not the two-component

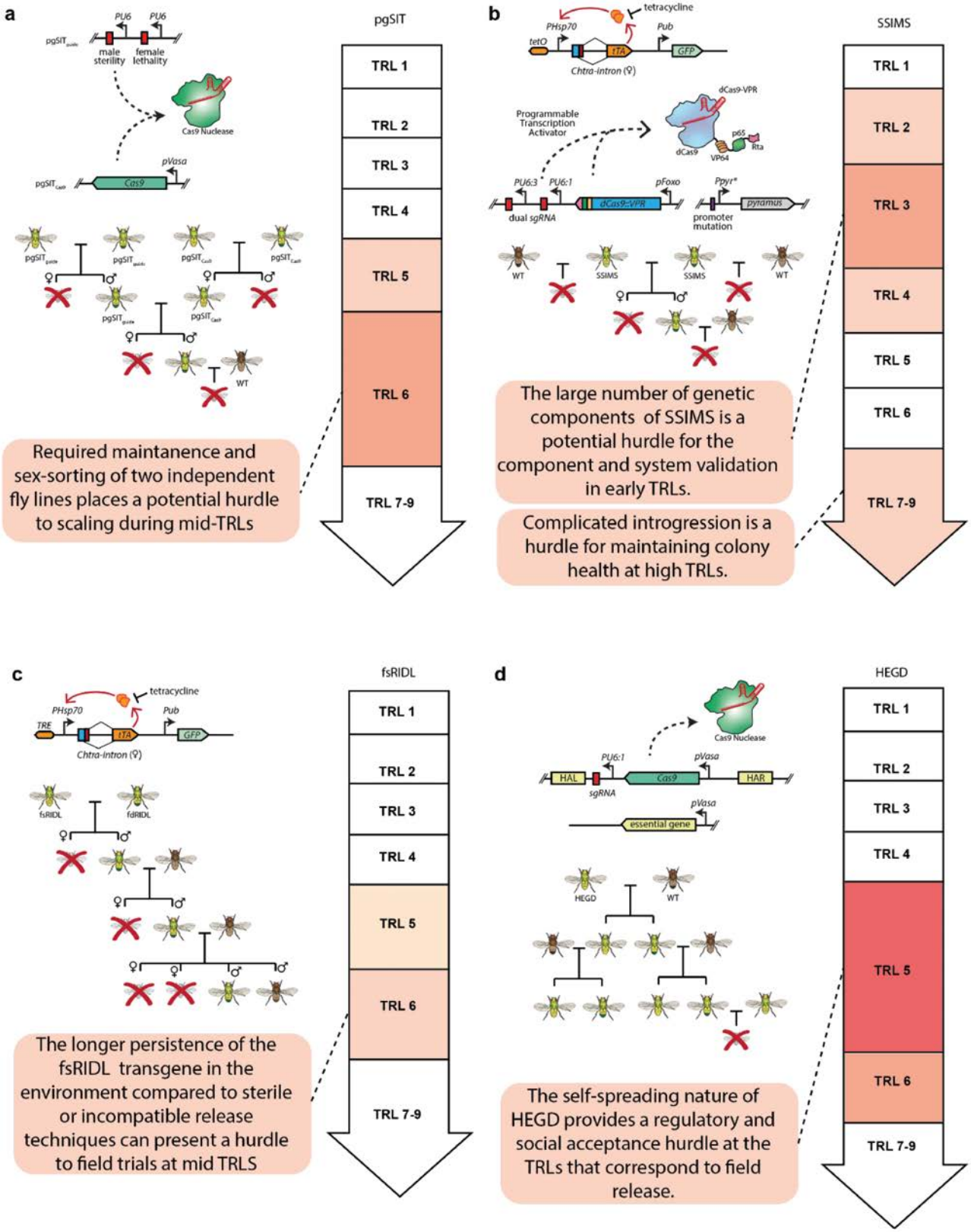

Fig. 2. Bottlenecks for technology development. Genetic design, behavior, and potential bottlenecks are illustrated for pgSIT (a), SSIMS (b), fsRIDL (c), and HEGD (d). Red shadings within the TRL progression highlight where each technique might expect to see longer residence times as they progress up the TRLs, with darker shades indicating more severe delays.

fsRIDL exemplified by TESS. When a male fsRIDL agent mates with a wildtype female, it can pass on the fsRIDL cassette to its offspring. Female offspring typically perish, and male offspring typically survive with the inherited cassette. These male offspring transmit the fsRIDL cassette to future generations, perpetuating the sex bias in survival. fsRIDL has reached the highest TRL (TRL8 in some countries for *Aedes aegypti*) of the case studies presented here, and it is commercially available in Brazil (https://www.oxitec.com/en/news/brazil-launch).

HEGD is a self-spreading (i.e., the allele frequency increases after release) genetic pest control method that uses a sequence-programmable endonuclease (e.g., Cas9) to cut a target wildtype gene (Burt & Crisanti 2018). The endonuclease gene is expressed from a specific locus on the chromosome that can be used to repair the cleaved target sequence via homology-directed repair. Such a repair event serves to copy the HEGD cassette to the corresponding locus on the chromosome inherited from a wildtype parent. In essence, organisms that are initially heterozygous for the HEGD cassette convert to homozygous in their germline and pass on the cassette to their offspring at super-Mendelian rates. HEGD has reached TRL4, as it has been successfully demonstrated in laboratory environments in the disease vectors *Anopheles gambiae* and *Ae. aegypti* but has not yet been tested in open-air field trials (Anderson et al. 2024; Hammond et al. 2016; M. Li et al. 2020; Yadav et al. 2023). Note that we focus this manuscript on single-locus HEGD, not split drives such as those demonstrated in *D. suzukii* by Yadav et al..

These four genetic pest control strategies have unique strengths and weaknesses, and each could be an appropriate choice to integrate into a pest management plan, depending on the location, target population, and management goals. The goal of this perspective is not to argue for one type of genetic pest control over another. In the sections that follow we highlight some of the key parameters by which they differ.

4 Unique characteristics of each genetic pest control strategy impacts the likely retention time at different TRLs

Nuanced assessment of these four potential genetic pest control strategies provides pathways towards commercialization that face major hurdles at different TRLs. Here we focus on three different sets of traits, which impact unique TRLs. Specifically, we discuss system complexity, environmental persistence, and the required scaling/economics of release as important distinguishing features. In assessing which genetic strategy might be appropriate for a given target pest population, there are additional considerations that could factor in decision-making (e.g., susceptibility to genetic resistance), but these are not considered here.

4.1 Complexity of genetic design

Demonstrating a proof-of-concept in laboratory environments, either in a model lab organism (TRL3) or in the target pest organism (TRL4) is in part a function of the system's genetic complexity. One measure of a system's complexity is the number of design variables that typically need to be optimized to make it work. On one end of the spectrum are mousetraps, admired for the simplicity of their design and function. On the other end are Rube-Goldberg machines, delightful when they work, but whose complexity is an obstacle to feasibility.

Genetic systems are composed of genetic parts. Most genes contain the following five parts. A **promoter** controls when, where, and to what degree a gene is turned on. A **5'-untranslated region (UTR**) can to a lesser degree impact these same things. A **coding DNA sequence (CDS**) encodes a protein and determines the function of the gene. A **3'-UTR** often impacts the stability and longevity of transcript. Lastly, a **terminator** is needed to mark the end of the gene. Because some genetic pest control approaches have more or less stringent requirements for when and where the component genes are active (e.g., turning on HEGD genes in the germline at the proper timing such that homing occurs just before gametogenesis), the impact of these genetic components on system performance differs from one technology to the next.

Of the four case-study approaches, HEGD is arguably the simplest (note that we use the word arguably here because there is not a great objective measure of genetic system complexity, and this section contains subjective assessments). The most common and most effective HEGDs developed so far use Cas9 nuclease, which is directed to its target location using a guide RNA. A minimal HEGD comprises two genes. Once a functional design is discovered to direct the production of a guide RNA in a target species, design changes to that component are straightforward. The guide RNA sequence (guide RNA genes do not require 5'UTR-CDS-3'UTR parts of typical protein-encoding genes) is a design variable that impacts where the nuclease will cut, but the promoter and terminator are rarely changed once a functional pair is discovered. The nuclease needs to be expressed only in limited cells that are involved in making gametes (sperm and eggs). There is some design flexibility in determining the correct combination of promoter, 5'-UTR, and 3'-UTR to control the spatiotemporal activity of a HEGD, but there is also a large amount of uniformity in published designs (Anderson et al. 2024; Hammond et al. 2016; M. Li et al. 2020; Yadav et al. 2023). Lastly, the genome-level design variable determining where in the genome to insert the HEGD genes is fairly constrained. To function appropriately, a conventional (i.e., not a split drive (Terradas et al. 2021)) HEGD is introduced precisely into the gene that it is targeting.

fsRIDL requires that a lethal effector gene is expressed only in transgenic females, but not in transgenic males. Sometimes this is achieved using promoters evolved to control biological processes related to female sexual devel-

opment (Heinrich & Scott 2000; Thomas et al. 2000). Alternatively, it can be achieved with specific CDS designs that leverage alternative splicing (alternative forms of RNA maturation) between males and females (Fu et al. 2007; F. Li et al. 2021). In either case, to allow females to survive in captivity to help propagate the genotype, a conditional expression system that can be controlled by a small molecule (typically tetracycline which disrupts the binding of the tetracycline transactivator (tTA) to the tetracycline response elements (TRE) in the promoter) added to the organism's food is used. Several different lethal effector genes have been demonstrated, including the pro-apoptotic gene *hid* (Heinrich & Scott 2000), the cytotoxic signaling gene *ras64B-V12* (Thomas et al. 2000), and *tTA* alone (Fu et al. 2007). Other design variables, including the copy number of the tet-operator within the TRE were optimized to give high levels of female-lethality. Unlike HEGD, the fsRIDL construct can, in theory, be expressed from anywhere in the genome. Because different chromosomal integration sites can vary in the level of gene expression, both the integration site and total number of copies are important design variables that can impact system performance (Das et al. 2020; Heinrich & Scott 2000; Upadhyay et al. 2022).

pgSIT is slightly more complicated in design than single-locus HEGD or single-component fsRIDL because it requires two unique, true-breeding lines that are sex-separated and crossed to produce the pest control agents (Kandul et al. 2019). One of the true-breeding lines is homozygous for the Cas9 nuclease gene, and the other line is homozygous for a pair of single guide RNAs (sgRNAs). Developing the system in model and applied target organisms requires empirically testing a small number of variables. These include the promoter that controls expression of Cas9 nuclease, the target genes for sgRNAs that direct both the male sterility and the female lethality (or flightless female) outcomes, and the chromosomal integration sites for sgRNAs (multiple independent strains generated with a randomly integrating transposase were assessed) (Kandul et al. 2019, 2022). While the performance of the full pgSIT system is robust to many of these design variables, it is noteworthy that species-specific changes in the genetic design – including the sequence and number of sgRNAs and the promoter used to control Cas9 expression – were present in the best-performing genotypes for closely related species (Kandul et al. 2019, 2022).

In terms of genetic design, SSIMS is the most complex of the case studies (Upadhyay et al. 2022). The proof-of-concept in the model organisms *D. melanogaster* required engineering four chromosomal loci. SSIMS combines a tetracycline-controllable fsRIDL design (design variables for this component match those described for fsRIDL above) (Fu et al. 2007), that was introduced in two copies on different chromosomes to achieve an expression level for effective sex-sorting. The EGI component comprised a tissue-specific promoter element controlling expression of a CDS encoding a catalytically inactive 'dead Cas9' (dCas9) fused to multiple transcriptional activation domains. Several promoter elements were tested (Maselko et al. 2020), and multiple CDS variants with different transcriptional activation domains were tested (unpublished). The 5'-UTR, 3'-UTR, and terminator were not important design variables for this gene. Linked to the dCas9 activator gene are two smaller genes controlling the expression of two sgRNAs, each from a different U6 promoter (Ewen-Campen et al. 2017). Several different dual-sgRNA designs were tested combinatorially against the different promoter variants, for a total of over thirty unique genetic designs assessed to optimize the dCas9 + sgRNA component. Finally, multiple target site mutations that protect the SSIMS agents from lethal overexpression were screened to find mutations that were benign, such that homozygotes were viable.

The differences in genetic design complexity across the selected case studies impact the overall technology maturation most during TRLs2–4. More complex designs (e.g., SSIMS) contain more component parts that need validating in TRL2, can be more finicky to optimize in model laboratory organisms in TRL3, and can be expected to require more retuning to translate from a model species to an applied target species (TRL4) compared to simpler genetic designs (e.g., HEGD). This leads to longer expected residence times in those TRLs. However, once the TRL4 stage is achieved, the impact of design complexity is less important for continued maturation to higher readiness levels.

4.2 Environmental persistence and social acceptance

In the United States, biotechnology is regulated by the Coordinated Framework for the Regulation of Biotechnology comprising the US Department of Agriculture (USDA), the Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA) (U.S. Coordinated Framework for Biotechnology, n.d.). Outlining the specific regulatory pathway that genetic pest control agents must follow is beyond the scope of this paper and may vary from organism to organism. For example, the complicated and circuitous path of the company Oxitec in seeking regulatory approval for their OX513A RIDL mosquitoes has been documented (Schairer et al. 2021). The detailed retrospective analysis highlights the differing timelines that comparable pest-control products experienced in seeking regulatory approval for field trials (Schairer et al. 2021).

However, we introduce some key steps below to provide context for the considerations that different genetic control technologies would face as they approach small-scale field trials to advance from TRL4 to TRL5.

Regulatory agencies consider genetic control agents to be pesticides. For a land-based pesticide trial covering greater than 10 acres, the EPA requires an Experimental Use Permit (EUP) as specified in section 40 CFR 172.3 in the Code of Federal Regulations and Chapter 12 of the Pesticide Registration Manual (https://www.epa.gov/pesticide-regis-

tration/pesticide-registration-manual-chapter-12-applying-experimental-use-permit). Importantly, the policy states:

"For testing operations for which acreage limits do not accurately reflect whether the testing is to be considered small- or large- scale, the Agency will determine on a case-by-case basis whether an exemption from requirement for an EUP is appropriate." This language and other stipulations in the policy give the EPA the right to make case-by-case exemptions or requirements for an EUP regardless of the size and scope of the intended field trial. Several genetic control technologies have already advanced to open-air field trials in the US and other countries. Oxitec has tested the RIDL mosquito internationally and an fsRIDL mosquito, OX5034, in the US (Schairer et al. 2021). The company MosquitoMate has tested a *Wolbachia*-based cytoplasmic incompatibility strain (they have products for *Ae. albopictus* and *Ae. aegypti*), including large urban trials in collaboration with Verily, Inc. (Crawford et al. 2020). More recently, Agragene has performed field trials of a pgSIT *D. suzukii* control agent in California and Oregon (Seydel 2024). However, no threshold-independent gene drive has been tested outside of contained laboratory environments. The self-propagating nature of gene drives means the consequences of a small-scale field trial and a full-scale release are similar. This contrasts with the other technologies highlighted by our case studies, which are inherently self-limiting, although they differ in precisely how persistent the transgenes would be following field release. For example, the fsRIDL system can be passed on by transgenic male offspring, while female carriers die. This approach provides a fitness disadvantage that will eventually lead the transgene to drop out of the population, but the transgenic components nevertheless persist for several generations.

Much has been written about the regulatory and social issues of gene drive field trials (Erickson et al. 2023; James et al. 2020; Jones et al. 2019; Kolopack & Lavery 2017; Long et al. 2021; Oye et al. 2014). HEGDs are seen as riskier than other forms of genetic pest control (Erickson et al. 2023), though there is more support for regulated use of HEGDs than an outright ban (Buchman et al. 2025). Implementing control measures to limit their spread increases HEGD public acceptability (Jones et al. 2019). The persistent nature of HEGDs in flying insects, like mosquitoes, further complicates regulatory pathways, because they could spread across country borders (James et al. 2023). The World Health Organization has recommended that widespread use of HEGDs should only proceed after field-trials confirm their efficacy and safety (Global Malaria Programme (GMP), n.d.). In theory, there exists a regulatory path for HEGD consideration (Eggers & Mackenzie 2000; FDA Center for Veterinary Medicine, n.d.). Nonetheless, no HEGD has been released in an open-air field trial, and to our knowledge, a field release application for a HEGD has never been formally submitted for regulatory evaluation. This makes it difficult to say with confidence how long the process will take or what the precise route to regulatory approval would be. Other examples of first-of-kind genetic pest control technologies have taken circuitous paths for regulatory approval (Schairer et al. 2021). Cumulatively, these examples suggest that the residence time at TRL4 (i.e., before TRL5 is attained) will be substantially longer for a gene drive technology compared to self-limiting technologies to account for the regulatory and social implications (e.g., buy-in from local communities at proposed testing sites) of even small-scale open-air field trials (Fig. 2).

4.3 Manufacturing/scale-up of genetic control agents

An important aspect of technology readiness that is often overlooked is scaling up the manufacturing. The US Department of Defense created a parallel Manufacturing Readiness Levels metric to de-risk and communicate the manufacturing capacity for a new technology (https://www.dodmrl.com/). We do not propose a separate MRL scale for genetic control agents but instead incorporate the requirement to scale production of pest control agents into the mid-level TRLs (e.g., TRL5–7).

Small-scale field trials could be executed with pest control agents that are reared in laboratory environments using unit operations and protocols which would be impractical for large-scale field trials or beta-testing. Large-scale manufacturing requires learning how to rear, feed, propagate, and process pest control agents for release in a way that is economical and retains agent efficacy. It also includes keeping the population healthy, from pathogens and parasites as well as from the deleterious effects of inbreeding.

Several unique challenges to scaling can be predicted for different genetic control approaches. First, the number of pest control agents needed for effective population control is unique to each approach. This includes both required release numbers and release frequencies. Because of this, the importance and burden of scaled-up propagation of the pest control agents is unique to each approach. Techniques with low environmental persistence (e.g., pgSIT) require larger-scale production of pest control agents. On the contrary, techniques with high persistence (e.g., HEGDs) will self-scale after release. Systems that rely on conditional expression of transgenes to control the sex-ratio commonly require addition of small molecules (e.g., tetracycline) to rearing media, which can impact the cost and fitness of the biocontrol agents (Arp et al. 2024; O'Shea & Singh 2015).

Many genetic control approaches either require (e.g., *Wolbachia*-mediated cytoplasmic incompatibility), or generally benefit from (e.g., RIDL) single-sex release (Papathanos et al. 2009). This can add extra labor and cost to at-scale manufacturing, particularly if the sex-sorting needs to be done mechanically. It also impacts the number of agents that need to be produced, since a sex-sorted release halves the number of pest control agents by eliminating one sex.

pgSIT requires separate maintenance of multiple lines that are sex-sorted and crossed to produce eggs/embryos for field release (Kandul et al. 2019). This necessity adds several

layers of complexity to the scaling process. The labor and cost associated with sex-sorting is required for both lines. Also, strain fidelity needs to be meticulously maintained and may dictate separate rearing facilities, as unintended mixing of the two distinct lines would require starting over from small colonies. There is precedent in agriculture for maintaining multiple independent lines that are crossed for seed production, for example in sorghum, where the final crop benefits from hybrid vigor of the distinct parental lines.

Lastly, maintaining the health of the pest control agent lines is important to ensure consistent field performance (Sørensen et al. 2012). Inbreeding in large-scale insect rearing facilities can decrease the genetic diversity of reared populations and negatively impact health and performance of the pest control agents. The challenge this presents to pest control programs will differ based on the species and specific approach employed. For example, SSIMS agents are genetically incompatible with wild-type individuals, which complicates the introgression of new genetic diversity. On the other hand, introgression would be straight forward in self-spreading approaches like HEGDs.

5 Conclusions

This is an exciting time for developers of insect genetic pest control technologies. The last decade has produced several diverse approaches that have been demonstrated in model and applied organisms. These applied organisms include species that are disease vectors, agricultural pests, and invasive species. The potential positive impacts that genetic pest control can have on society and the environment are substantial.

Combinations of existing methods (e.g., the SSIMS approach described above) and design permutations of existing methods can alter the behavior/performance of a control technology in important and nuanced ways. For example, splitting the Cas9 and guide RNA to produce a split-drive approach can limit the self-spreading nature of a HEGD (Terradas et al. 2021). Many of these approaches use similar molecular components that, because of the specifics of the system design, result in very different behaviors. For example, allele sails and HEGD both use Cas9 nucleases and sgRNAs to drive allelic changes in a population, but with unique dynamics (Johnson et al. 2024).

The four case studies described in this paper are not intended to be exhaustive. Instead, they are meant to highlight the main thesis that different approaches are expected to have bottlenecks at different stages in technology development. This is a simple idea with important implications. To illustrate this point, consider the following scenario. A funding organization seeks to de-risk the development of a genetic pest control strategy for a target organism by initially funding early-stage (TRL2–4) research and development efforts of several different strategies. It would be attractive to move forward with an approach that quickly matures to TRL4. However, it would not be prudent to terminate projects that are slower to advance to TRL4, if there is sufficient justification to expect it to progress faster through the middle or late TRLs.

The TRL classification system provides a framework and vocabulary to discuss the end-to-end process of technology maturation. It can also serve as a roadmap of what is needed for future technology development. While it is not the only stage-gate approach to project management (e.g., Front End Loading), the TRL scale has proven useful in many disciplines. The nuance that we hoped to add through this perspective article is that progression through the TRLs can look different for unique technologies. Anticipating the differing residence times at each stage along the TRL progression can help manage expectations of diverse stakeholders. We encourage technology developers and funders to consider a long-term view of the commercialization process when strategically investing time and resources in the development of genetic pest control approaches. We hope that this perspective is useful in focusing future discussions and decision making related to genetic control of pest insects. Time might seem to speed up and slow down based on the circumstances, but it always marches forward, just like a fruit fly towards a banana.

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References

Alphey, L. (2014). Genetic control of mosquitoes. *Annual Review of Entomology, 59*(1), 205–224. https://doi.org/10.1146/annurev-ento-011613-162002

Alphey, L., Koukidou, M., & Morrison, N. I. (2014). Conditional dominant lethals-RIDL. In *Transgenic Insects: techniques and applications* (pp. 101–116). CABI Wallingford UK. https://doi.org/10.1079/9781780644516.0101

Anderson, M. A. E., Gonzalez, E., Edgington, M. P., Ang, J. X. D., Purusothaman, D.-K., Shackleford, L., … Alphey, L. (2024). A multiplexed, confinable CRISPR/Cas9 gene drive can propagate in caged *Aedes aegypti* populations. *Nature Communications, 15*(1), 729. https://doi.org/10.1038/s41467-024-44956-2

Arp, A. P., Williamson, M. E., Vasquez, M., Quintero, G., Vargas Lowman, A., Sagel, A., & Scott, M. J. (2024). Doxycycline is a viable alternative to tetracycline for use in insect Tet-Off transgenic sexing systems, as assessed in the blowflies *Cochliomyia hominivorax* and *Lucilia cuprina* (Diptera: Calliphoridae). *Journal of Economic Entomology, 117*(2), 650–659. https://doi.org/10.1093/jee/toae023

Badger, J., Alirigia, R., Ameli, I., Bajer, P., Bartodziej, B., Dalgleish, V., … Smanski, M. (2025). Coproducing a Technology Readiness Level framework for non-persistent genetic biocontrol of aquatic invasive species. *Journal of Environmental Management, 391*, 126189. https://doi.org/10.1016/j.jenvman.2025.126189

Beach, S. J., & Maselko, M. (2025). Recombinant venom proteins in insect seminal fluid reduce female lifespan. *Nature Communications, 16*(1), 219. https://doi.org/10.1038/s41467-024-54863-1

Buchman, L. W., Goldsmith, C. L., Heitman, E., Kang, K. E., & Liu, X. (2025). Public trust in regulatory agencies and support for policies on agricultural gene drive. *The Review of Policy Research, 42*(1), 29–49. https://doi.org/10.1111/ropr.12610

Burt, A., & Crisanti, A. (2018). Gene drive: Evolved and synthetic. *ACS Chemical Biology, 13*(2), 343–346. https://doi.org/10.1021/acschembio.7b01031

Concha, C., Yan, Y., Arp, A., Quilarque, E., Sagel, A., de León, A. P., … Scott, M. J. (2020). An early female lethal system of the New World screwworm, *Cochliomyia hominivorax*, for biotechnology-enhanced SIT. *BMC Genetics, 21*(S2, Suppl 2), 143. https://doi.org/10.1186/s12863-020-00948-x

Crawford, J. E., Clarke, D. W., Criswell, V., Desnoyer, M., Cornel, D., Deegan, B., … White, B. J. (2020). Efficient production of male Wolbachia-infected *Aedes aegypti* mosquitoes enables large-scale suppression of wild populations. *Nature Biotechnology, 38*(4), 482–492. https://doi.org/10.1038/s41587-020-0471-x

Das, S. R., Maselko, M., Upadhyay, A., & Smanski, M. J. (2020). Genetic engineering of sex chromosomes for batch cultivation of non-transgenic, sex-sorted males. *PLOS Genetics, 16*(11), 1–11. https://doi.org/10.1371/journal.pgen.1009180

Eggers, B., & Mackenzie, R. (2000). The Cartagena protocol on biosafety. *Journal of International Economic Law, 3*(3), 525–543. https://doi.org/10.1093/jiel/3.3.525

Erickson, S. E., Lhewa, P., Rundquist, K., Schultz, S., Levers, L., & Smanski, M. (2023). Perceptions on the genetic biocontrol of invasive carp. *BioRxiv,* 2007–2023.

Ewen-Campen, B., Yang-Zhou, D., Fernandes, V. R., González, D. P., Liu, L.-P., Tao, R., … Perrimon, N. (2017). Optimized strategy for in vivo Cas9-activation in *Drosophila. Proceedings of the National Academy of Sciences,* 201707635. https://doi.org/10.1073/pnas.1707635114

FDA Center for Veterinary Medicine. (n.d.). *Clarification of FDA and EPA Jurisdiction Over Mosquito-Related Products*. Retrieved July 14, 2025, from https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cvm-gfi-236-clarification-fda-and-epa-jurisdiction-over-mosquito-related-products

Fu, G., Condon, K. C., Epton, M. J., Gong, P., Jin, L., Condon, G. C., … Alphey, L. (2007). Female-specific insect lethality engineered using alternative splicing. *Nature Biotechnology, 25*(3), 353–357. https://doi.org/10.1038/nbt1283

Global Malaria Programme (GMP). (n.d.). Guidance framework for testing of genetically modified mosquitoes, second edition. ISBN: 978 92 4 002523 3. Retrieved July 14, 2025, from https://www.who.int/publications/i/item/9789240025233

Hammond, A., Galizi, R., Kyrou, K., Simoni, A., Siniscalchi, C., Katsanos, D., … Nolan, T. (2016). A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. *Nature Biotechnology, 34*(1), 78–83. https://doi.org/10.1038/nbt.3439

Héder, M. (2017). From NASA to EU: The evolution of the TRL scale in public sector innovation. *The Innovation Journal, 22*(2), 1–23.

Heinrich, J. C., & Scott, M. J. (2000). A repressible female-specific lethal genetic system for making transgenic insect strains suitable for a sterile-release program. *Proceedings of the National Academy of Sciences of the United States of America, 97*(15), 8229–8232. https://doi.org/10.1073/pnas.140142697

James, S. L., Dass, B., & Quemada, H. (2023). Regulatory and policy considerations for the implementation of gene drive-modified mosquitoes to prevent malaria transmission. *Transgenic Research, 32*(1), 17–32. https://doi.org/10.1007/s11248-023-00335-z

James, S. L., Marshall, J. M., Christophides, G. K., Okumu, F. O., & Nolan, T. (2020). Toward the definition of efficacy and safety criteria for advancing gene drive-modified mosquitoes to field testing. *Vector Borne and Zoonotic Diseases, 20*(4), 237–251. https://doi.org/10.1089/vbz.2019.2606

Johnson, M. L., Hay, B. A., & Maselko, M. (2024). Altering traits and fates of wild populations with Mendelian DNA sequence modifying Allele Sails. *Nature Communications, 15*(1), 6665. https://doi.org/10.1038/s41467-024-50992-9

Jones, M. S., Delborne, J. A., Elsensohn, J., Mitchell, P. D., & Brown, Z. S. (2019). Does the U.S. public support using gene drives in agriculture? And what do they want to know? *Science Advances, 5*(9), eaau8462. https://doi.org/10.1126/sciadv.aau8462

Kandul, N. P., Liu, J., Buchman, A., Shriner, I. C., Corder, R. M., Warsinger-Pepe, N., … Marshall, J. M. (2022). Precision guided sterile males suppress populations of an invasive crop pest. *GEN Biotechnology, 1*(4), 372–385. https://doi.org/10.1089/genbio.2022.0019

Kandul, N. P., Liu, J., Sanchez, C. H. M., Wu, S. L., Marshall, J. M., & Akbari, O. S. (2019). Transforming insect population control with precision guided sterile males with demonstration in flies. *Nature Communications, 10*(1), 1–12. https://doi.org/10.1038/s41467-018-07964-7

Kimmel, W. M., Beauchamp, P. M., Frerking, M. A., Kline, T. R., Vassigh, K. K., Willard, D. E., Johnson, M. A., & Trenkle, T. G. (2020). Technology readiness assessment best practices guide. National Aeronautic and Space Administration. https://ntrs.nasa.gov/citations/20205003605

Knipling, E. F. (1959). Sterile-male method of population control: Successful with some insects, the method may also be effective when applied to other noxious animals. *Science, 130*(3380), 902–904. https://doi.org/10.1126/science.130.3380.902

Knipling, E. F. (1960). The eradication of the screw-worm fly. *Scientific American, 203*(4), 54–61. https://doi.org/10.1038/scientificamerican1060-54

Kolopack, P. A., & Lavery, J. V. (2017). Informed consent in field trials of gene-drive mosquitoes. *Gates Open Research, 1*, 14. https://doi.org/10.12688/gatesopenres.12771.1

Li, F., Yamamoto, A., Belikoff, E. J., Berger, A., Griffith, E. H., & Scott, M. J. (2021). A conditional female lethal system for genetic suppression of the global fruit crop pest *Drosophila suzukii*. *Pest Management Science, 77*(11), 4915–4922. https://doi.org/10.1002/ps.6530

Li, M., Yang, T., Bui, M., Gamez, S., Wise, T., Kandul, N. P., … Akbari, O. S. (2021). Eliminating mosquitoes with precision guided sterile males. *Nature Communications, 12*(5374). https://doi.org/10.1101/2021.03.05.434167

Li, M., Yang, T., Kandul, N. P., Bui, M., Gamez, S., Raban, R., … Akbari, O. S. (2020). Development of a confinable gene drive system in the human disease vector *Aedes aegypti*. *eLife, 9*, e51701. https://doi.org/10.7554/eLife.51701

Long, K., Alphey, L., Annas, G., Bloss, C., Campbell, K., Champer, J., … Akbari, O. (2021). Core commitments for field trials of gene drive organisms. *Regulation & Governance, 15*(4), 1144–1165. https://doi.org/10.1111/rego.12245

Maselko, M., Feltman, N., Upadhyay, A., Hayward, A., Das, S., Myslicki, N., … Smanski, M. J. (2020). Engineering multiple species-like genetic incompatibilities in insects. *Nature Communications, 11*(1), 1–7. https://doi.org/10.1038/s41467-020-18348-1

O'Shea, K. L., & Singh, N. D. (2015). Tetracycline-exposed *Drosophila melanogaster* males produce fewer offspring but a relative excess of sons. *Ecology and Evolution, 5*(15), 3130–3139. https://doi.org/10.1002/ece3.1535

Oye, K., Esvelt, K., Appleton, E., Catteruccia, F., Church, G., Kuiken, T., … Collins, J. P. (2014). Regulating gene drives. *Science, 345*, 6–9. https://doi.org/10.1126/science.1254287

Papathanos, P., Bossin, H. C., Benedict, M. Q., Catteruccia, F., Malcolm, C. A., Alphey, L., & Crisanti, A. (2009). Sex separation strategies: Past experience and new approaches. *Malaria Journal, 8*(S2, Suppl 2), S5. https://doi.org/10.1186/1475-2875-8-S2-S5

Schairer, C. E., Najera, J., James, A. A., Akbari, O. S., & Bloss, C. S. (2021). Oxitec and MosquitoMate in the United States: Lessons for the future of gene drive mosquito control. *Pathogens and Global Health, 115*(6), 365–376. https://doi.org/10.1080/20477724.2021.1919378

Schetelig, M. F., Schwirz, J., & Yan, Y. (2021). A transgenic female killing system for the genetic control of *Drosophila suzukii*. *Scientific Reports, 11*(1), 1–10. https://doi.org/10.1038/s41598-021-91938-1

Seydel, C. (2024). Pest control gets the CRISPR treatment. *Nature Biotechnology, 42*(11), 1633–1636. https://doi.org/10.1038/s41587-024-02449-z

Sørensen, J. G., Addison, M. F., & Terblanche, J. S. (2012). Mass-rearing of insects for pest management: Challenges, synergies and advances from evolutionary physiology. *Crop Protection (Guildford, Surrey), 38*, 87–94. https://doi.org/10.1016/j.cropro.2012.03.023

Teem, J. L., Gutierrez, J. B., & Parshad, R. D. (2014). A comparison of the Trojan Y chromosome and daughterless carp eradication strategies. *Biological Invasions, 16*(6), 1217–1230. https://doi.org/10.1007/s10530-013-0475-2

Terradas, G., Buchman, A. B., Bennett, J. B., Shriner, I., Marshall, J. M., Akbari, O. S., & Bier, E. (2021). Inherently confinable split-drive systems in *Drosophila*. *Nature Communications, 12*(1), 1480. https://doi.org/10.1038/s41467-021-21771-7

Thomas, D. D., Donnelly, C. A., Wood, R. J., Alphey, L. S., Knipling, E., Krafsur, E., … Randolph, S. E. (2000). Insect population control using a dominant, repressible, lethal genetic system. *Science, 287*(5462), 2474–2476. https://doi.org/10.1126/science.287.5462.2474

Upadhyay, A., Feltman, N., Sychla, A., Janzen, A., Das, S., Maselko, M., & Smanski, M. (2022). Genetically engineered insects with sex-selection and genetic incompatibility enable population suppression. *eLife, 11*, 1–25. https://doi.org/10.7554/eLife.71230

U.S. Coordinated Framework for Biotechnology. (n.d.). Retrieved July 14, 2025, from https://usbiotechnologyregulation.mrp.usda.gov/biotechnologygov/home

Wang, G.-H., Gamez, S., Raban, R. R., Marshall, J. M., Alphey, L., Li, M., … Akbari, O. S. (2021). Combating mosquito-borne diseases using genetic control technologies. *Nature Communications, 12*(1), 4388. https://doi.org/10.1038/s41467-021-24654-z

Witherbee, A. D., & Gamez, S. (2025). Dissecting the sterility phenotype in gene edited *Drosophila suzukii* pgSIT males. *Scientific Reports, 15*(1), 3903. https://doi.org/10.1038/s41598-025-88598-w

Yadav, A. K., Butler, C., Yamamoto, A., Patil, A. A., Lloyd, A. L., & Scott, M. J. (2023). CRISPR/Cas9-based split homing gene drive targeting doublesex for population suppression of the global fruit pest *Drosophila suzukii*. *Proceedings of the National Academy of Sciences of the United States of America, 120*(25), e2301525120. https://doi.org/10.1073/pnas.2301525120

Yan, Y., Aumann, R. A., Haecker, I., & Schetelig, M. F. (2023). CRISPR-based genetic control strategies for insect pests. *Journal of Integrative Agriculture, 22*(3), 651–668. https://doi.org/10.1016/j.jia.2022.11.003

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