



Genetic, Biological and Sterile Insect Techniques: Insect Pest Management Strategies: A Review

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ABSTRACT

Insect pests indicate serious hazards to agriculture, human health and global food security, which are compounded by climate change and changing pest behaviors. Traditional pest management systems, such as chemical controls and integrated pest management, are under threat due to resistance and environmental concerns. This review explores innovative pest control methods, focusing on Genetic Pest Management (GPM) and the Sterile Insect Technique (SIT). GPM technologies, such as CRISPR-based gene editing and gene drives, offer promising solutions by introducing targeted genetic modifications to pest populations. SIT, which involves releasing sterilized insects to reduce pest populations, is enhanced by advances in sexing and genetic techniques. Biological control agents (BCAs), including entomopathogenic bacteria and fungi, provide sustainable alternatives for pest management. The effectiveness of these methods depends on understanding the underlying mechanisms and optimizing application conditions. Regulatory frameworks play a crucial role in the adoption of these technologies, with varying requirements across countries. We believe that ongoing advancements in biological and genetic approaches, when effectively regulated and thoughtfully integrated, will be crucial in shaping the next generation of pest management techniques. This review highlights the importance of sustained research and comprehensive regulation to maximize the benefits and applications of these innovative pest management strategies.

Key words: Biological agent, Genetic control, Regulation, Sterile insect technique.

Insect pests are insects that inflict considerable harm to crops, livestock, or human health by diminishing agricultural yields or spreading diseases, with recent research underscoring their effects on global food security and ecosystems (Saito *et al.*, 2022). A warmer climate is projected to change insect pests' behavior, potentially increasing yield losses by 10%-25% (Deutsch *et al.*, 2018). The estimated yield loss experienced by farmers due to insect pests showed a significant correlation with the estimated cost of pest control (Sadat and Chakraborty, 2017). Insect pests cause enormous harm to both human health (*via* disease transmission such as dengue fever and malaria) and agriculture (*via* crop or livestock damage). Pesticides, biological control and integrated pest and habitat management are all existing control methods (Hawkins *et al.*, 2018). Climate change, global food security, environmental sustainability and vector-borne diseases have all emerged as major issues for the twenty-first century. Insect pests have played significant roles in agricultural losses over the last two decades and infectious diseases transmitted by vector insects have spread globally (WHO, 2014). Genetic control, also known as genetic pest management (GPM), is a biological pest control method that introduces desirable and heritable genetic changes into a wild population through intraspecific mating (Devos *et al.* 2022). Synthetic DNA sequences, or "transgenes," inserted into the pest genome are the heritable changes that have been introduced into wild populations. Compared to existing techniques like the SIT, these "next generation" GPM technologies offer several advantages, including the potential for increased efficiency and flexibility in the control traits they induce. Additionally, they permit engineering and

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re-engineering by design and are applicable over a wider taxonomic range (Black *et al.* 2011).

By mass-producing, sexually sterilizing and then releasing conspecific individuals, the sterile insect technique (SIT) seeks to lower populations of a target pest insect (Dyck *et al.*, 2021). Combining SIT with complementing control methods can increase the likelihood of eradication (Klassen *et al.*, 2021). Specifically, combining SIT with classical biological control is a workable approach for an AW-IPM program (Hendrichs *et al.*, 2009). However, if the following requirements related to the biology of the target pest are met, an appropriate application of an SIT program may be effective for suppression and eradication in specific geographic and/or infrastructural conditions (*e.g.*, greenhouses, silos and isolated crop areas) if multivoltine, gregarious behavior and the unaffected mating behavior by irradiation are met. For the SIT to be used successfully, the raised sterilized males must be able to mate and compete with their wild

counterparts and must be capable of communicating with females by sending and receiving signals (Lance *et al.*, 2021). The scale at which SIT can be effectively utilized is determined by the biology and ecology of the target species, the ease of production and release and landscape characteristics, as well as the influx of wild, fertile males from outside the target area (Lance and McInnis, 2021). Genetic transformation will make some impact on the SIT, especially regarding the introduction of markers for released flies and the construction of genetic sexing strains. It is now present in almost all cotton-growing countries and is a key pest in many of them. The Mediterranean fruit fly ('Medfly') *Ceratitis capitata* (Wiedemann) is a highly invasive generalist attacking more than 250 host plants and is one of the world's most economically important pests (CABI, 2016).

Biological control agents (BCAs) are a promising alternative control measure, with a rapidly expanding market for BCA products (van Lenteren *et al.*, 2018). BCAs can reduce pest or pathogen damage to levels below an economic threshold (Adivitiya *et al.*, 2017). The entomopathogenic bacterium (EPB) *Bacillus thuringiensis* (Bt) is the most widely used biological control agent (BCA) (Sanchis and Bourguet, 2008). So far, only a few studies have looked into this option, with mixed results. These included some lab, greenhouse and field experiments using nematode combinations with either fungi or bacteria against various insect pests (Jaffuel *et al.*, 2019). Pesticide concerns have shifted agricultural focus towards biological pest management. Among these methods, biological control stands out as a sustainable and long-term solution for vegetable crop protection (Ambethgar *et al.*, 2024). Our review centers on theoretical analysis, taking into account both biology and genetics to aid in the creation, comprehension, testing and application of these innovative approaches together with regulatory techniques for agricultural insect pest management.

All of the data and information gathered came from a secondary source. This review did not use the primary source of data gathering. During the creation of this review paper, many national news portals, government websites, thesis papers, publications and research papers were consulted.

Genetic pest management

Genetic control technologies for insect population suppression that are currently being developed aim to improve older systems by disseminating female-targeting genetic loads throughout a population or converting female progeny into functional males. These newer technologies also extensively use modern molecular biology tools, particularly those used in gene editing, such as CRISPR/Cas9. They all have in common that they use knowledge of the target species' sex determination pathways, demonstrating the importance of incorporating fundamental biological principles to underpin applied science in Genetic Pest Management (Leftwich *et al.*, 2021). CRISPR

(clustered regularly interspaced short palindromic repeats)-based gene-editing technologies have seen rapid development and widespread application in the life sciences over the last decade (Cannon and Kiem, 2021). The relevant derivative is made up of a Cas nuclease that cleaves DNA by forming site-specific double-stranded breaks (DSB) and a single chimeric guide RNA (sgRNA) that recognizes its target sequence (Wiedenheft *et al.*, 2012). The CRISPR system-induced DSB can be repaired by one of two cellular DNA repair pathways: Non-homologous end-joining (NHEJ) or homology-directed repair (HDR) (Sander and Joung, 2014). CRISPR-based gene drives have been tested in two species of Anopheles mosquitoes, which are vectors for malaria. In *A. gambiae*, these drives have been shown to suppress reproduction (Hammond *et al.*, 2016). By providing the corresponding DNA template for the repair, the HDR pathway can generate specific mutations or introduce genes of interest into the genome, a process known as sequence knock-in. Both repair strategies have been widely used in various insect species for functional genomics (Sun *et al.*, 2017). Furthermore, both approaches have been investigated in various genetic control strategies against insect pests, such as CRISPR-based homing gene drives that work *via* HDR (Gantz and Bier, 2015). Genetic control strategies that generate more effective and robust release agents would improve the SIT program's overall efficacy and broaden the technology's range of targeted species and control goals.

The engineering of female-specific tetracycline-repressible lethality in Mediterranean fruit flies, *Ceratitis capitata* (Fu *et al.*, 2007) and diamondback moths, *Plutella xylostella* (Jin *et al.*, 2013), was based on a conserved sex determination mechanism based on alternative splicing (Fig 1 a and b). Gene drives can introduce desirable traits into wild populations by promoting higher-than-Mendelian inheritance rates. This technique can be used for population suppression or modifying specific genes, such as reversing insecticide resistance (Champer *et al.*, 2016), (Fig 1a and d). Fig 1 c) illustrates the Medea gene drive mechanism, which involves expressing a maternal toxin during oogenesis. This toxin affects all progeny, but embryos carrying the Medea gene are rescued by an antidote expressed during embryogenesis (Fernández *et al.*, 2021). The development of self-limiting gene drive systems, such as daisy-chain drives (Noble *et al.*, 2019) (Fig 1e and f), offers potential for broader deployment of these technologies, especially for targeting invasive and non-native pests. Genetic pest management (GPM) technologies, including gene drives and CRISPR, offer promising, targeted solutions for pest control. Recent developments highlight the need for ongoing research and careful regulation to ensure effectiveness and mitigate risks (Lefebvre *et al.*, 2023).

The temporary introduction of transgenes into a population that would revert to its original state were releases to stop is the fundamental component of self-limiting GPM strategies. It is therefore necessary to

periodically release new transgenic individuals to preserve the control effect (suppression or replacement). It is generally believed that to maintain disease-refractory transgenes in wild populations at frequencies high enough to significantly lower transmission rates, population replacement strategies will necessitate the deployment of gene-drive systems (Sinkins and Gould 2006). Insects with a transgene exhibiting a dominant lethal phenotype are known as RIDL (release of insects carrying a dominant lethal genetic system) insects. Before being released into the field, RIDL lethality can be reduced by giving insects an antidote (tetracycline or other suitable analogs), typically included in their larval diet. When tetracycline is not available in sufficient amounts in the field, offspring of released insects carrying RIDL transgenes perish before they can reproduce. To continue suppressing the target population, transgenic insects must be kept in the field, much like with the SIT, through periodic releases of RIDL insects (Curtis, 2015). Compared to bi-sex-lethal designs, transgenic

systems that exclusively kill females can be more effective at suppressing the population, especially if they are designed with multiple independently segregating loci. The survival of male transgene heterozygotes, who spread the transgene in succeeding generations, is the source of this increased efficiency (Bax and Thresher, 2009).

Insect sterilization techniques

SIT programs typically release both males and females, lacking a practical method to sort the sexes easily in large numbers. This is inefficient because the released sterile females and males tend to court and mate with each other rather than seek out wild mates. Male-only releases are generally more efficient than mixed-sex releases, a large-scale study of irradiated Medfly quantified this as being three-to five-fold more efficient per male (Rendón *et al.*, 2004). Sexing or sex-separation systems are required for operational-level implementation because they eliminate females, which in the case of mosquitoes can cause

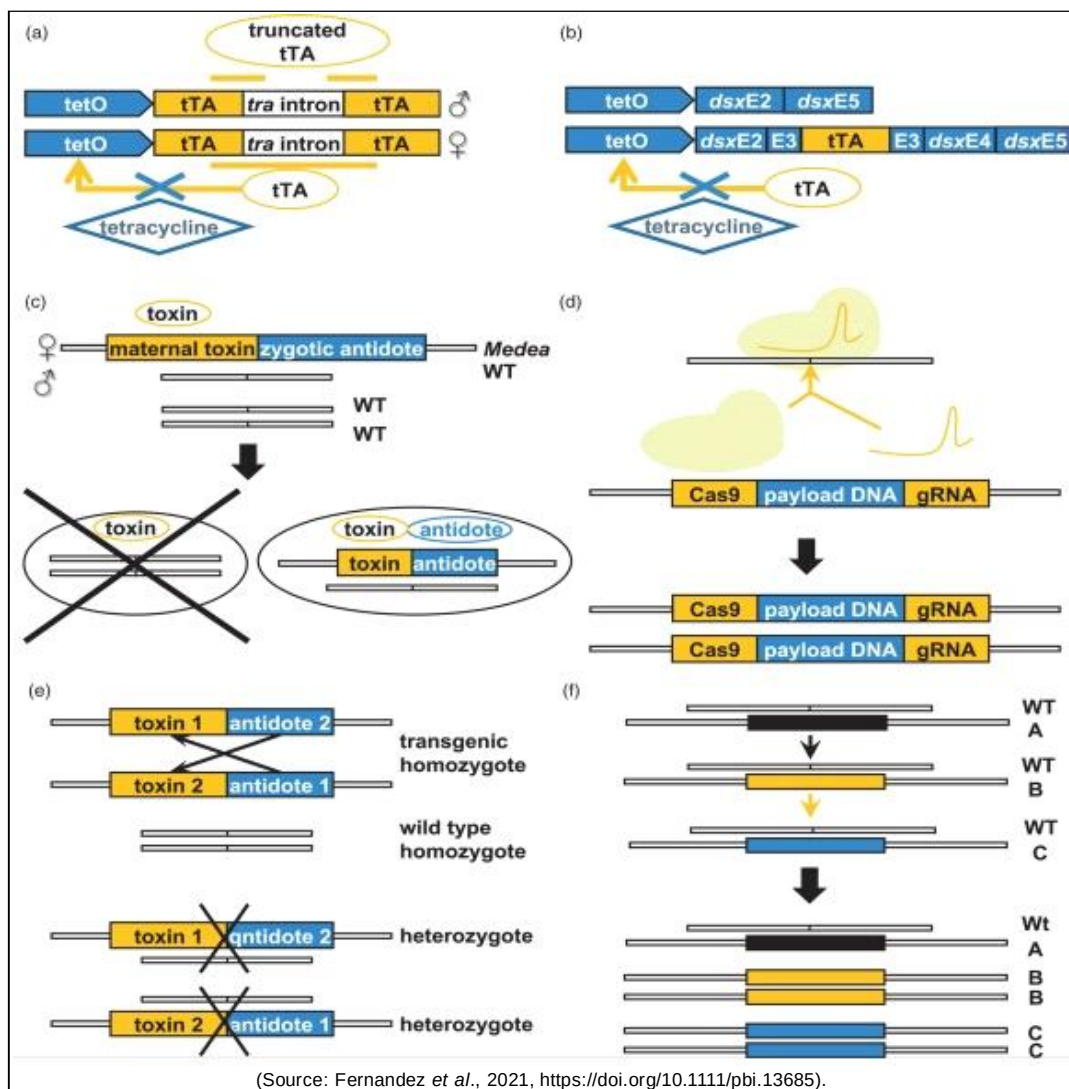


Fig 1: Strategies for genetic control of insect populations.

nuisance and transmit pathogens (Mashatola *et al.*, 2018). The use of insects sterilized by irradiation (gamma rays and X-rays) is referred to as sterile insect technique *sensu stricto* (IAEA, 2021). Ionizing radiation exposure causes chromosomal damage due to random dominant lethal mutations. The use of the appropriate radiation dose results in sterile insects that are still sexually competitive (Bakri *et al.*, 2021). Transgenic techniques, such as the release of insects containing dominant lethal (RIDL), cause no radiation damage to the organisms and typically allow for sex separation. Females can only develop in the presence of an antidote in insects that carry the genetic construct; once it is removed from the diet, only males can develop and be released (Alphey, 2014).

An alternative, non-genetic variant of the SIT, known as the incompatible insect technique (IIT), is being developed in mosquitoes. Although IIT may apply to the integrated management of vectors, as far as we are aware, there are

(as yet) no proposed applications of this technology to plant pest insects; one key reason is the need for a highly effective sexing strain or sex-separation method to enable only males to be released because any released females could establish the *Wolbachia* infection in the wild population, thereby removing the incompatibility (Bourtzis, 2008). This involves the sustained release of males infected with *Wolbachia*, an intracellular bacterium that interferes with reproduction, rendering matings between released males and wild *Wolbachia*-free females infertile (Bourtzis *et al.*, 2014). The ability of the SIT to suppress, contain, prevent (re)introduction, or even locally eradicate populations of specific key insect plant and livestock pests has been continuously demonstrated. However, there's also a constant need to improve effectiveness and cost-effectiveness (Bourtzis and Vreysen, 2021).

It has been proposed to develop genetic sexing strains through a transgenic method in which females carry a

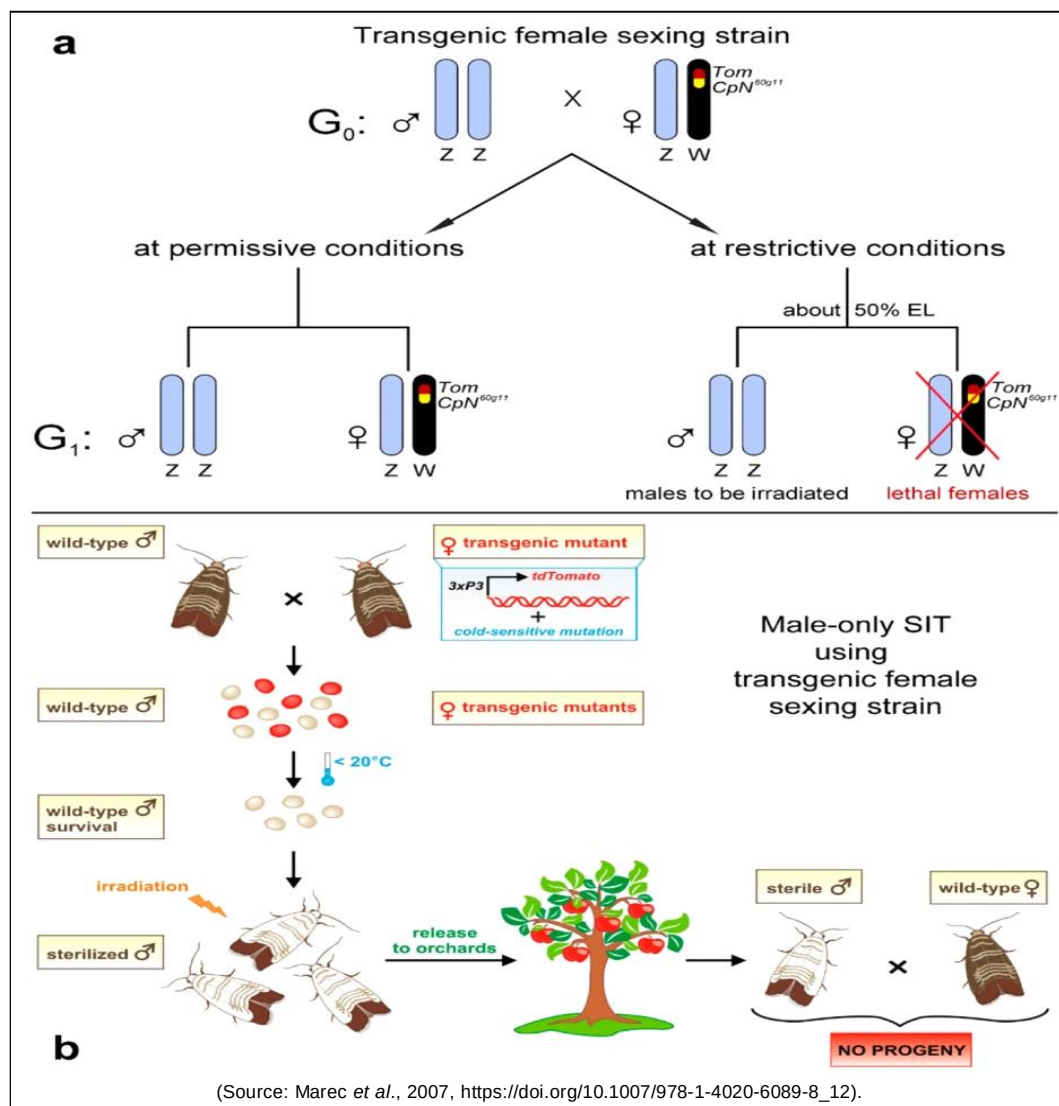


Fig 2: Proposed transgenic female sexing strains aim to control codling moth populations using the Sterile Insect Technique (SIT).

transgene with a dominant, conditional lethal mutation located on their W chromosome. This mutation would be activated during embryogenesis under specific conditions. If the eggs from these transgenic females are maintained at restrictive conditions, they will produce progeny that are male-only and non-transgenic. These males can then be irradiated and released for pest control purposes (Fig 2 a, b), (Marec *et al.*, 2005). Genetic sexing strains were initially developed for codling moths but abandoned due to low transgenesis efficiency (Marec *et al.*, 2007). However, recent success in silkworms, demonstrated by inserting an EGFP reporter transgene into the W chromosome, shows the approach's feasibility (Ma *et al.*, 2013). The Sterile Insect Technique (SIT) and Incompatible Insect Technique (IIT) have been crucial in Area-Wide Integrated Pest Management (AW-IPM) strategies for controlling major lepidopteran pests. Their importance is expected to grow, particularly as these pests have significantly expanded their geographic range in recent decades. By effectively managing and suppressing pest populations, SIT and IIT contribute to mitigating the impacts of these pests on agriculture and ecosystems. Their role in AW-IPM campaigns is increasingly vital as the spatial distribution of these pest species continues to change and spread (Kean *et al.*, 2016). The effective use of SIT/IS in controlling various Lepidoptera pests highlights the efficiency and broad applicability of these eco-friendly strategies for managing lepidopteran pests (Marec and Vreysen, 2019).

Biological controls

Biological pest control methods have become an important part of integrated pest management strategies. To control insect infestations, natural predators, parasitoids, competitors and pathogens are all used. However, artificial selection has been used to improve biocontrol agents (Lirakis and Magalhaes, 2019). Natural enemies can effectively reduce the populations of harmful insects that act as vectors for plant pathogens. By controlling these pest populations, they help in managing the spread of plant diseases (Chandi, 2020). Transgenic Bt crops are engineered to express insecticidal toxins derived from *Bacillus thuringiensis*, causing mortality to susceptible insects eating the plant. Even where its main assumptions appear to hold, the high-dose/refuge strategy is predicted only to delay resistance and, after two decades of commercially grown Bt cotton and Bt corn (maize), some field-evolved resistance has now been observed and reported in a variety of insect species. In some cases, this has already led to reduced efficacy of crops or even crop

failures. A comprehensive review is provided by (Tabashnik *et al.*, 2013). Bt generates a diverse range of insecticidal proteins. During sporulation and the stationary growth phase, it produces δ -endotoxins as parasporal crystalline inclusion bodies. It also generates vegetative insecticidal proteins, which are first released during the bacterial vegetative growth stage. Insecticidal proteins are widely used as biopesticides, either through spraying or incorporation into transgenic crops (Chakrabarty *et al.*, 2022).

The majority of biocontrol agents on the market are entomopathogenic agents such as fungi, bacteria, viruses and nematodes (Lacey *et al.*, 2015). Biotechnology allows for the improvement of these species' efficacy through genetic engineering. Transgenic approaches, for example, have been used to increase the efficacy of entomopathogenic fungi by overexpressing virulence factors, toxins and proteins that disrupt physiological homeostasis (Fan *et al.*, 2012). Potential insecticides include toxins from bacterial symbionts of entomopathogenic nematodes such as *Photorhabdus luminescens* and *Xenorhabdus nematophilus* (French-Constant and Bowen, 2000), soil-dwelling bacteria *Pseudomonas chlororaphis* (Schellenberger *et al.*, 2016) and numerous other naturally occurring peptides (Paul and Das, 2021). The biocontrol potential of *P. chlororaphis* against a root-feeding pest insect was investigated using a variety of experiments from the greenhouse to the field. In a first field trial, strain PCLRT03 significantly reduced the survival of the cabbage maggot *Delia radicum* and increased the marketable produce. *P. chlororaphis* has traditionally been marketed for its plant-growth-promoting and disease-suppressive properties, but our findings suggest that new *P. chlororaphis* products, as well as existing ones, could be developed for insect control (Spescha *et al.*, 2023).

Bacterial strains were stored at 80°C in 44% glycerol and cultured on King's B medium with antibiotics. KB+++ contained cycloheximide (100 mg/l), chloramphenicol (13 mg/l) and ampicillin (40 mg/l), while KB++G used gentamycin (10 mg/l) instead of ampicillin for GFP-tagged strains, as shown in (Table 1), (Vesga *et al.*, 2021). To investigate basal compatibility and potential synergistic effects of the three BCAs, a simplified sand-radish bulb test system was used for pseudomonads and adapted for nematodes and fungi, (Table 1), (Flury *et al.*, 2016). The commercially available nematode product was dissolved in tap water to create suspensions, which were then used to infect *Galleria mellonella* larvae, (Table 1), (Jaffuel *et al.*, 2018).

Table 1: Biocontrol agents.

Species	Strain/population	Origin	Reference
<i>P. chlororaphis</i>	PCLRT03 (P)	Potato root, CH	Vesga <i>et al.</i> (2021)
<i>P. chlororaphis</i>	PCL1391	Tomato root, ESP	Flury <i>et al.</i> (2016)
<i>S. feltiae</i>	RS -5 (RS5) (N)	Soil wheat field, CH	Jaffuel <i>et al.</i> (2018)

(Source: Spescha *et al.*, 2023, <https://doi.org/10.1016/j.agee.2023.108414>).

Biocontrol agents use a variety of mechanisms to protect plants from pathogenic invasion. They may engage with pathogens either directly or indirectly, employing one or a combination of processes to reduce plant disease (Ayaz *et al.*, 2021). Grasping the biocontrol mechanisms of plant pathogens is crucial for creating an optimal environment to manage various plant diseases effectively. Over the past two decades, extensive research has been dedicated to examining the role of biocontrol agents in crop disease prevention, rhizosphere colonization and plant growth promotion (Rabbee *et al.*, 2023). Understanding the mechanism of action behind a biocontrol agent's protective effect facilitates the optimization of biological control. It helps in establishing ideal conditions for interactions between the biocontrol agent, the pathogen and the host, as well as in developing effective formulations and application techniques to enhance plant health and promote sustainable agriculture (Ayaz *et al.*, 2023).

The disease triangle model in Fig 3 illustrates the interaction between three critical factors that influence the success of biological control: the biocontrol agent, the pest and the environment. Sustainable biocontrol is achieved when a potent biocontrol agent, such as a virulent entomopathogenic fungus, effectively targets a susceptible pest under favorable environmental conditions. These conditions include positive socio-economic returns, efficient delivery technology and an environment conducive to the pathogen's activity (Nchu, 2024).

Policy and regulations in genetic strategies

Most jurisdictions require public authorities to grant permission for the release of sterile insects, such as the

Hazardous Substances and New Organisms Act (HSNO) in New Zealand and the US National Environmental Policy Act (NEPA) in the United States. However, how these regulations are implemented varies greatly across countries. In the United States, for example, irradiated, sterile insects are evaluated by the Department of Agriculture's Animal and Plant Health Inspection Service (USDA-APHIS), as are biocontrol agents (WHO and IAEA, 2020). SIT is increasingly in demand for pest control, including commercial agricultural commodities. Due to their experience in mass-rearing and marketing insects and other beneficial organisms, commercial producers of beneficial insects are expected to become important players in privately driven demand for SIT. Thus, further adoption of SIT may depend on a well-defined regulatory framework that provides commercial insectaries with the legal approval to produce, trade and coordinate the release of sterile insects (Hendrichs and Robinson, 2021).

Article 2001/18 of the European Union mandates that Member States assess the risks associated with the release of genetically modified organisms (GMOs), including plants, animals and vaccines. This method of purposefully releasing genetically modified organisms (GMOs) is risk (cost) based and relies on recombinant DNA technology, or genetic modification, as the regulatory trigger. Different regulatory procedures are in place. For instance, the "plants with novel traits" legislation in Canada states that an environmental risk assessment is triggered more "product-based" by considering the phenotypic effects of the plant (novel traits) (Canadian Food Inspection Agency, 2016). For GM insects, several guidance frameworks have

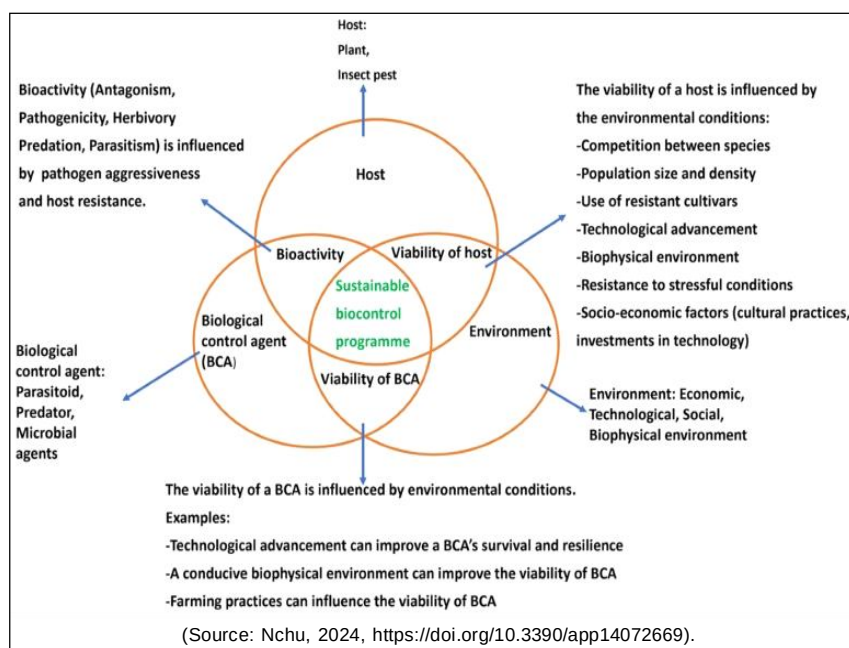


Fig 3: A diagram illustrates the host, biological control agent (BCA) and environment interactions.

been produced in recent years. The European Food Safety Authority (2012) published a regulatory framework for GM animals and the World Health Organization (WHO/TDR and FNIH, 2014) issued guidance for the testing and regulation of GM mosquitoes. Both of these asserted that a tiered approach from laboratory studies focused on molecular biology and simple ecological processes) through contained or confined field trials to commercial implementation should underpin environmental risk assessment in support of the development of GM insect technologies. Public acceptance of GM technologies has varied across products (crops, insects, vaccines and insulin), as well as between countries and communities and public engagement concerning GM insects will continue to be an important issue for developers and regulators (House of Lords Science and Technology Committee, 2015). Determining harm and choosing suitable comparators for genetically modified insect pests necessitates more sophisticated methods than in the case of plants, where genetic alterations are compared with an unmodified (traditional) plant. Appropriate techniques for evaluating the environmental risk could center on changes in crop yields or other indirect measures of assessment according to the technology and logically consistent with other pest intervention approaches (Alphey *et al.*, 2018).

CONCLUSION

The integration of Genetic Pest Management (GPM) and the Sterile Insect Technique (SIT) marks a significant advancement in pest control. GPM technologies, such as CRISPR-based gene editing and gene drives, offer targeted solutions by introducing genetic changes to control pest populations. SIT, which involves releasing sterilized insects, remains valuable, especially when combined with other methods. Effective use of these technologies demands a thorough understanding of their mechanisms, optimal conditions and proper formulations. Biological control agents (BCAs) are also crucial for regulating insect populations and plant diseases, with additional research needed to improve their efficacy and incorporation into pest management methods. Regulatory frameworks are crucial for developing and deploying these technologies. Harmonizing regulations, enhancing public acceptance and managing potential risks are key to progress. Future research should focus on refining genetic and sterilization techniques, optimizing biocontrol applications and ensuring that regulatory practices not only foster innovation but also safeguard environmental and public health. Effective and adaptable regulatory frameworks, alongside robust interdisciplinary collaboration and active stakeholder engagement, will be essential for advancing these pest management strategies and achieving long-term, sustainable agricultural practices.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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