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Transgenic Insects: Advancements, Applications and Implications

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Abstract:

Transgenic insects, genetically modified through the introduction of foreign genes, have emerged as a cutting-edge technology with significant implications in various fields. This review paper explores the advancements made in transgenic insect research, their potential applications, and the ethical, environmental, and regulatory considerations surrounding their deployment. The paper highlights the potential benefits and risks of using transgenic insects, emphasizing the importance of responsible and well-regulated research to harness their full potential while safeguarding the ecosystem.

Keywords:

SIT, rDNA technology, Transgenic insects, Ecological impact, Risk

1. Introduction

Transgenic insects are genetically modified organisms that have their DNA altered to benefit humans, such as increasing agricultural production, producing oil, and managing pests and vectors. These insects are created by inserting DNA sequences from another organism into the insect's genome, creating a mobile element that moves around the insect's genome. The primary goal is to manage pests causing damage to crops, the environment, and human health, and reduce the use of synthetic chemical pesticides.

Pest insects cause economic damage and harm to humans, transmitting diseases and affecting plant species and animals. Advanced research on genetically modified (GM) insects aims to control vectors of diseases like malaria and dengue, control crop pest populations like diamondback moth, olive fruit fly, and Mediterranean fruit fly, and control insect-borne diseases in livestock like bluetongue and *Schmallenberg* virus. This research also impacts the trade and the spread of diseases like malaria and dengue (GM Insects and Disease Control, 2014), and in wildlife conservation.

Chemical insecticides have been the primary method for controlling pests, but their reliability is limited due to pest resistance, bioaccumulation, contamination, and harmful effects. Genetics-based insect control strategies, based on the classical Sterile Insect Technique (SIT), are becoming more viable.

Genetic engineering techniques can be used to create transgenic insects, which can be used to address global challenges in agriculture, public health, and biodiversity conservation (WHO, 1998). This paper provides an overview of the current state of transgenic insect research, their mechanisms of gene manipulation, and their diverse application.

2. Genetic Engineering Techniques for Producing Transgenic Insects

2.1. Sterile Insect Technique (Sit)

The oldest and most effective technique involving direct genome manipulation is Sterile Insect Technique (SIT). Knipling was the first to utilize this method, which was based on the idea of using insect species against itself. The ultimate objective of SIT is to produce non-viable or infertile progeny; the sterile men inseminate the females, resulting in non-viable eggs, which lowers the population. (Davidson, 1974). The method involves the use of male insects that have been mass-reared and treated to high doses of a chemical mutagen or radiation. The gametes of the males are undergoing damage by this radiation or other mutagen exposure. Damage to the gametes results in non-viable offspring but it does not stop inseminating wild females. Next phase is the release. A population study must be conducted before these sterile individuals are released. Approximately ten to one hundred times as many of these males must be released as the normal population of males. These extra sterile insects are released to help compete with the wild type males (Nyaupane, 2015).

Application of SIT within an area-wide integrated pest management programme (AW-IPM) was implemented to eradicate New World screwworm (*Cochliomyia hominivorax*) from the USA and Mexico (Van der Vloedt & Klassen, 1991; Hendrichs & Robinson, 2009) and was successfully employed to eradicate the parasite in Libya just four years after it became established in the late 1980s (Lindquist *et al*., 1992). Additionally, SIT is used effectively in Florida and California to manage Mediterranean fruit fly (*Ceratitis capitata*) populations. It has also been used to manage codling moth (*Cydia pomonella*) and pink bollworm moth (*Pectinophora gossypiella*).

2.2. rDNA Technology

After selection of a particular trait, scientist have to for insert the desired gene into the insect's genome, this can be done in two was The first one is transposons. Transposons have the ability to cut and paste themselves into and out of genome or DNA. Researchers can put their gene of interest within a transposon using its molecular machinery and express it to insect cells. One of the major limitations of this technique is that the transposon integrates into a random region of the gene. There are certain sequences in DNA into which a transposon will preferentially insert itself, but other than that, insertion is random. Using viral vectors is the other method. A desired gene can be inserted into a viral vector. Viral vectors can then be utilized, to integrate into the genome. It is necessary to confirm if the gene has been correctly expressed and integrated. Adding a selectable marker to the gene's vector or determining whether the gene is expressed are two ways to accomplish this. (Nyaupane, 2015).

2.2.1. Gene Drive

Using specific endonuclease enzymes to cut a specific region in the organism's DNA is one possible technique for generating a gene drive. When one organism inherits a chromosome with the gene for this enzyme and another without the gene, the endonuclease will cut the latter, causing the cell to copy the endonuclease and its surrounding genes onto the chromosome that didn't have them. It has the potential prevent diseases carried by insect vectors by means of endonuclease drives. This method has the potential to rapidly modify an entire population, depending on generation times (Oye *et al.* ,2014).

Gene drive has become a practical tool with great potential to prevent disease-carrying insects thanks to the recently established "CRISPR" gene-editing method, which enables researchers to make precise modifications to DNA. Bier and Gantz (2015) demonstrated how a CRISPR-based mutagenesis chain reaction may be used to transfer a mutant gene onto a pair of chromosomes, passing on the genetic alteration to almost all of their progeny (Bier & Gantz, 2015).

2.2.2. Homing Endonuclease Genes

Homing Endonuclease Genes (HEG) are used in a gene drive system designed to suppress the population. HEGs, often known as "selfish" or "parasitic" genes, are naturally occurring genes that take advantage of host cell machinery to replicate into a specific DNA sequence, at a rate that is higher than that of mendelian segregation process. A gene's function can be disrupted by HEGs by cutting a DNA sequence in the middle of the gene. An example of this could be a gene that is necessary for the transmitting the disease but not necessary for the host. (Burt, 2003; Deredec *et al*., 2008).

HEG technique has been utilized to cut the paternal X chromosome in the malaria vector (*Anopheles gambiae*), inhibiting transmission of the diseases to the next generation. Transmission dynamics of a HEG have also been reported to be effective in caged mosquito populations. With this method, fully viable mosquito strains were created, and over 95% of the progeny were male. (Galizi *et al*., 2014).

2.2.3. Release Of Insects Carrying A Dominant Lethal (Ridl)

The RIDL technique, developed by Oxitec, British biotech company is a genetic enhancement of the Sterile Insect Technique, where transgenic technology is used to insert a lethal gene into the insects, thus produceing a non-toxic, lethal protein (tTAV) that allows larval development, but prevents the offspring of RIDL insects to metamorphose into viable adult (Oxitec Ltd).

Compared to SIT, RIDL offers a number of benifits, including a heritable visible genetic marker that allows the differentiation of sterile and wild insects. Fertile, mass-reared pests cannot accidentally escape, unlike with SIT. Moreover, a female-specific variation of RIDL (fsRIDL) generates large-scale cohorts of the insects that are exclusively male. (Slade & Morrison, 2014). Field trials using RIDL male mosquitoes (*Aedes aegypti*) have been undertaken in the Cayman Islands, with the aim of controlling dengue infections. This resulted in a suppression of the wild population by 80% relative to nearby untreated areas (Harris *et al*., 2012).

2.3. Paratransgenesis

Another way to alter insect gene is paratransgenesis. This technique involves altering the natural flora (or adding completely new flora) in the gut of an insect, without altering the insect's own genome. Vectors have an a hematophagous lifestyle. Scientist can use the interaction between the pathogen and the natural flora of the insect's gut to destroy the disease-causing agent. One example of paratransgenesis in altering the natural gut flora of kissing bug. The kissing bug (*Rhodnius prolixus*) transmits Chagas' disease (*Trypanosoma cruzi*). Researchers have been able to alter the bacteria commonly found in the gut of the kissing bug to produce an anti- trypanosome peptide, cecropin, when expressed, this peptide does not harm the insect, but is harmful to the pathogen (Beard *et al*., 2000).

3. Advantages

3.1. Human Health

One very critical aspect that fueled the development of genetically modified insects is human health. An estimated 80% of people on the planet are susceptible to one or more vector-borne illnesses. Infecting millions of people worldwide, viruses, parasites, and bacteria are spread by mosquitoes, flies, bugs, and other vectors. Leishmaniases, Chagas disease, dengue, chikungunya, yellow fever, malaria, and Zika virus disease the major vector borne disease of concern (WHO, 2024). Excessive usage of pesticides has had many adverse effects on both humans and the environment. The insects have grown resistant to those pesticides over time. Some poor nations lack cold storage conditions, which affects the efficacy of pharmaceutical medications used to treat a variety of tropical diseases. Drug resistance is another issue that comes up when developing chemotherapeutics. The targeted organisms are rapidly evolving even as pharmaceutical companies strive to create chemotherapy medicines. *Plasmodium falciparum*, the etiological agent of malaria, one of the deadliest vector-borne illnesses, is an organism that exemplifies this troubling phenomenon. Quinine, a medication originally made from the bark of the chinchona tree many centuries ago, has historically been used to treat this parasite. (Hayton & Su, 2008).

Additionally, in some countries, attempts to control and eradicate insect vectors by spraying insecticides on their habitats have a significant negative impact on the environment and human health. Studies unequivocally demonstrated that exposure to certain chemicals, such as Dichloro Diphenyl Tricholorethane (DDT) which is converted to Dichloro Diphenyl Dicholorethane (DDE) during metabolism, impairs children's cognitive and neuromuscular development. (Sagiv *et al*., 2008). Another study has also documented that insecticide or pesicide can cause precocious puberty on children (Ozen *et al*., 2012).

According to one study more over 500 insect species have developed some type of insecticide resistance. (Hoy, 1998). These resistances often develop to more than one class of insecticide which makes the control effort more difficult (WHO, 1998). In order to treat infectious diseases, spread by insects, chemotherapeutics must be developed and implemented with great care. Insecticide resistance can also result from direct attacks on insects, which can be expensive for the environment and human health. The quest for genetically engineered insects is therefore motivated by serious concerns about human health.

3.2. Agriculture

The research and use of genetically modified insect aims of increasing agricultural productivity and yield by efficient management of major agricultural pests which are causing agricultural yield and quality loss. Agricultural concerns were the major motivation for modifying insects to alter the native population. The original use of GM insects was implemented to deal with the burden of the New World screwworm (WHO, 1998). To get rid of the economic problem associated with screwworm infestations, ‘altered’ insects were used in the U.S.A. Obviously those are not the genetically modified insects but this has led to the development of GM insects.

Furthermore, a number of well-known insect pests of agricultural crops have accelerated the need for the development of genetically modified insects. In terms of their economic impact, Mediterranean fruit flies rank among the most significant plant and crop pests. It typically targets over 250 different kinds of fruits, nuts, and vegetables and is polyphagous in nature. Reluctance to import different fruits and vegetables is also a result of the fear of importing this pest. The fruit fly is an invasive insect that will destroy native plants once it is released. (Gong *et al*., 2005). Like this pest, other pests have also similar effect on trade and agriculture. This huge economic concern is a strong motivator for development of GM insects that can control such pest.

3.3. Other Associated Benefits

The development of genetically modified insects has several advantages beyond agriculture and human health. The silkworm, *Bombyx mori*, is a good example of this. GM silkworms can produce huge quantities of silk, within a short period of time (Goldsmith *et al*., 2005). Recently created a GM strain of silkworms that produce human antibodies (Park *et al*., 2009). Other advancements could be developed from this use. Bioreactors could be made from silkworms. Additionally, like the methods employed in the Pharming movement, insects can be genetically altered to produce advantageous products. In general, there is a great need for these insects to develop.

4. Possible Risks

Transgenic insects are controversial due to concerns about their potential impact on the environment and human health. Organisations like GeneWatch UK and EcoNexus argue that relying on genetic modification may detract from more effective measures. Environmental NGOs like Greenpeace also worry about the potential for new insects or diseases to fill ecological niches, and the potential for horizontal transfer of genes. Researchers acknowledge the need for cautious approach but argue that traditional control methods cause more harm (POSTNOTE, 2010).

Horizontal gene transfer is a concern in insects, but no study has identified a mechanism through which it can occur in insect. Transgenes can be inactivated to prevent their transfer to other species. Self-limiting strategies remove GM individuals from the environment, while self-propagating strategies maximize their spread. Recall mechanisms are being developed to reverse the spread of GM if needed (POSTNOTE, 2010).

5. Ecological Impacts Of Gm Insects

Environmental risks are also associated with the use of genetically modified insects over the open environment. Once they released, they can't be taken back so lots of investigation and regulation should be done for this research. The environmental concerns surrounding the use of the SIT and other technologies to eliminate or control a native population stem from a permanent alteration of the ecosystem. If the operation is a success, and a population is either eliminated or vastly reduced, the ecosystem is permanently altered. It is difficult to predict all of the broader ecological impacts of these changes. It is possible that the elimination or vast reduction in numbers of this organism could have dire consequences for other non-targeted organisms. A likely food source and predator will have been eliminated and this could have a serious impact. Scientists have emphasized that pre- release investigation of the targeted organism's ecology must be performed to plan for these environmental impacts.

5.1. Number Of Insects Released

Suppression technologies like SIT and RIDL involve mixing of local populations with transformants, with RIDL requiring 1.5-2.2 times more insects for local elimination. This mass release of insects into target locations may have ecological implications (Bonsall *et al*., 2010).

The radiation methods used to sterilize males in SIT can result in a small proportion of fertile males, which does not efficiently reduce the population. In mosquitos, the release of males does not increase bite rate, but a larger release of transformant insects is needed (Bonsall *et al*., 2010).

The incomplete penetration of the lethal gene in RIDL is feasible, but it would still result in a proportion of transgenic offspring retaining the transgene, enhancing the overall suppressive effect of RIDL (Yakob *et al*., 2008).

5.2. Migration

Yakob et al. (2008) analyzed the risk of inadvertent population increase in *Aedes aegypti*, the vector for dengue, through the release of SIT and RIDL. They found that survival from larval to adult stages is limited by resources, so a reduced density of pre-adult stages may increase the adult population. This effect may be seen in SIT control by lowering the number of offspring in the next generation.

Yakob et al.'s study revealed an increase in wild vectors in non-target areas where sterile males migrated, but this decrease declined with distance from the release site. Simulations using RIDL, which acts after density dependent processes, showed that neighboring wild vectors re-stabilize at lower populations and stabilize more quickly than with SIT (Yakob *et al*., 2008).

5.3. Interspecific Competition

Interspecific and intraspecific competition is crucial for species coexistence and ecological community structure. Bonsall et al.'s (2010) study analyzed the effects of SIT and RIDL control strategies on coexistence and exclusion in two vectors, such as mosquito species. The study found that conventional and transgenic control techniques can affect the local existence or exclusion of vector species and allow coexistence of species that would not otherwise occur. This could have significant consequences for disease persistence, depending on the competitor's competence or less competence than the target species. This should be investigated on a case-by-case basis. For example, malaria is transmitted by multiple mosquito species, while *Aedes aegypti* is the principal dengue vector globally (*Aedes albopictus* is a vector in some regions). GM transformants may need to be developed and released for several species in the locality.

5.4. The Food Chain

The local eradication of insects may affect organisms at higher trophic levels that rely on them as food sources. However, there is limited evidence to determine the impacts on transformant insects. A study in Germany showed that despite a 90% reduction in mosquitoes using non-GM technologies, the ecosystem has not been damaged. Other insects continue to develop without large mosquito populations, providing food for birds, amphibians, and bats. (Becker, 1997).

The eradication of mosquitoes in the Arctic could affect diets of migratory birds and caribou migratory routes. The absence of mosquito larvae in water pools could affect fish and other animals' diets. Mosquitoes also act as pollinators for thousands of plant species, although few of which humans rely on as food sources. Experts agree that these services would be filled by other organisms inhabiting the empty ecological niche. But some effect on the food chain should be evident if the mosquito population wiped out, for example, the mosquitofish (*Gambusia affinis*), a specialized predator used for mosquito control, could go extinct, impacting the food chain and affecting many insect, spider, salamander, lizard, and frog species (Fang, 2010).

It is relevant to highlight the well-established ecological impact of insecticides, the most common method of controlling insects today, and ensuing harmful impact of buildup of toxins throughout the food chain by the chemical insecticide (INTECH, 2012).

5.5. Disease Free Wildlife

Dyck *et al*. (2021) discuss the effects of screwworm eradication on white-tailed deer in the US, highlighting the benefits for both domestic and wild animals, including endangered species. However, the increase in deer numbers led to an increase in the Gulf Coast tick, which in turn infected cattle. These interactions vary depending on ecosystems (Dyck *et al*., 2021)

5.6. Resistance

Genetically modified (GM) technology can lead to resistance to modified genes, which can be effectively monitored and seen in other control methods like insecticides. However, the risk of developing more virulent pathogen strains is more hazardous (Alphey, 2014). Research on the evolutionary impact of genetically modified (GM) insect is limited, but study of Medlock et al. (2009) on evolutionary impact of different GM mosquito strategies on dengue virulence in both humans and mosquitoes found that control strategies that increase mosquito mortality have less risk of increasing virulence to humans than those that block disease transmission or reduce mosquito biting. Further research is needed to test these models (Medlock *et al*., 2009)

Conclusion

The field of insect pest management and disease management and vector control has made significant progress, but there is still much work to be done towards a sustainable and eco-friendly approach. Expanding this approach could help alleviate hunger and diseases, and with appropriate social policies, it could be a panacea. While genetically modified insects have advantages, sound research on public safety and sustainable ecological balance is necessary to ensure the preservation of ecology and self-sustained nature for future generations.

Abbreviations

GM: Genetically Modified.

SIT: Sterile Insect Technique.

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats.

HEG: Homing Endonuclease Genes.

RIDL: Release of Insects Carrying a Dominant Lethal.

WHO: World Health Organization.

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