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Popular Article

Self-Limiting Gene and Their Application in Insect Pest Management: Benefits and Drawback

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Introduction

The approach is inspired by the Sterile Insect Technique (SIT). Self-limiting gene approach doesn't rely on radiation to sterilize male insects. Self-limiting is the name given to Oxitec's innovative solution for the control of dangerous and damaging insects. This self-limiting approach uses modern biotechnology and advanced genetics to provide effective, safe, and sustainable control of insect pests. Self-limiting gene controls invasive moth and resistant pest like DBM, Pink bollworm etc sans pesticides. The self-limiting gene technique, developed by the British company Oxitec, has already been trialed against dengue fever carrying mosquitoes, successfully reducing their populations by over 90% in Brazil, Panama and the Cayman Islands. A self-limiting gene is carried by the insects. The engineered male pests are released to mate with the pest females, and because their female offspring do not survive to reproduce, the number of pest dwindles. Those pests also carry a color marker for monitoring. The female-specific self-limiting effect means that after a few generations the gene disappears from the population unless continued releases occur.

How the Self-Limiting Gene Works

The self-limiting gene comprises tetO (tetracycline Operator) sites, which bind tTAV (tetracycline repressible Trans-Activating factor Variant) protein, a promoter and coding sequence for tTAV. Without tetracycline (A), tTAV protein is produced, which simultaneously binds to transcriptional machinery and tetO sites, thereby enhancing the expression of the self-limiting gene.

This positive feedback system produces large amounts of tTAV, which binds to more and more transcriptional machinery, without the need to bind to the tetO, eventually making the transcriptional machinery unavailable for other essential gene expression (B). The inhibition of essential gene expression leads to cell death (C) and the death of the insect before it reaches adulthood (D). When tetracycline is added to the larval aquatic diet, it binds and inactivates tTAV (E), switching off the positive feedback system. The transcriptional machinery is not depleted as only a small amount of tTAV is produced (F), which does not affect normal cell function (G), and the insects survive (H).

The pest control gene (Self-Limiting Gene) produces a protein called tTAV (tetracycline repressible activator variant), which is able to act as a switch to control the activity of other genes. In the modified insects, when the tTAV gene is expressed, the nontoxic protein ties up the cell's machinery so it's other genes aren't expressed and the insect dies. But how do we produce insects if they die? There's an antidote given to the insects in the rearing facility that acts like a switch to turn off the tTAV gene preventing the tTAV protein from working. This antidote, tetracycline, an antibiotic, binds to the tTAV protein and disables it. So, in the presence of the antidote, the Oxitec insects are able to survive and reproduce in the rearing facility, but when the males are released into the wild, their offspring can't access the antibiotic in the quantities needed to survive, so they die before reaching adulthood.

How to identified GM pests or NOT

All of self-limiting gene strains contain a heritable, fluorescent marker to distinguish them from native pest insects and to help scientists with the management of pest control programmes. Oxitec's strains contain genetic fluorescent proteins that glow when viewed under certain filters. Because it is integrated into the insects' DNA, the marker can always be detected, it won't fade with age and it is inherited if offspring are produced. Importantly, identification of moths on traps by PCR screening was 100% accurate.

Why only female kill

Fist situation: Development of genetic sexing strains in Lepidoptera by transgenic approaches. In Lepidoptera, the female is the heterogametic sex, with most species having a WZ sex chromosome pair, whereas the males are ZZ. This means that if a conditional lethal gene can be inserted into the W chromosome, then all females should die after the application of the restrictive condition. Second situation: A major modification to the sterile insect technique is described, in which transgenic insects homozygous for a dominant, repressible, female-specific lethal gene system are used. In this the first system uses a sex-specific promoter or enhancer to drive the expression of a repressible transcription

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factor, which in turn controls the expression of a toxic gene product. The second system uses non-sex-specific expression of the repressible transcription factor to regulate a selectively lethal gene product.

Two key sex determination genes of insects, transformer (tra) and doublesex (dsx), are both regulated by sex-specific alternative splicing. The tra coding region is interrupted by additional exons in males; only females produce a splice variant encoding functional Tra protein. We have exploited this female-specific excision of a cassette exon to prevent expression of an engineered protein in males, thereby making its expression female-specific.

tTAV exposure is allergenic or toxic to vertebrates:

tTAV exposure is not allergenic or toxic to vertebrates using internationally recommended bioinformatics searches, as well as an analysis of peer-reviewed literature, sequence searches were commissioned from an external expert to identify possible allergenic (localised and/or systemic) or toxic effects in humans. No data were found that suggested any possible risk from the introduced proteins and no significant sequence matches of the required length, with any known or putative allergen or toxin were identified.

Who developed this technique?

Oxitec is a British biotechnology company which develops genetically modified insects to assist in insect control. Oxitec is working to develop a genetic modified insect: Mosquito (aedes aegypti), Pink bollworm (Pectinophora gossypiella), Diamondback moths (Plutella xylostella), olive fruit fly (Bactrocera oleae), Mexican fruit fly (Anastrepha ludens), and Mediterranean fruit fly (Ceratitis capitata). For more information we can visit site: http://www.oxitec.com/dbm.

Application in Pest Management (Transgenic insect):

- **Mosquito:** Tetracycline acts as the repressor of the positive feedback system within Oxitec's genetically engineered *Aedes aegypti* mosquito strain, OX513A. The self-limiting gene is able to tie up the normal processes in an into the mosquito's cell and as a result, the modified mosquitoes can't develop properly and die before they become adults. Mutation can occur in insects, and that made the self-limiting gene ineffective. The introduced genetics have been stable for over 140 generations; the introduced genes cannot be transferred to other species.
- **Pink bollworm:** Pink bollworm (*Pectinophora gossypiella)* is one of the most destructive pests of cotton in many areas of the world, including in India, China, Brazil and the western USA. Oxitec have now developed pink bollworm (Pectinophora gossypiella) strains, OX4319A and OX4319C, with self-limiting genes which affect only females. The self-limiting

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gene is repressible with tetracycline, so we can propagate the strain by providing this 'antidote' to insects.

- **Diamondback moths:** Diamondback moths (*Plutella xylostella*) is an invasive species. New pesticide-free and environmentally-friendly way to control diamondback moths (*Plutella xylostella*) with a "self-limiting gene". Oxitec research scientist develop Oxitec Diamondback Moth (OX4319L). Oxitec male moths are released to mate with female moths of their own species. They pass on a 'selflimiting' gene that prevents the female offspring from reaching adulthood. This reduces the number of reproductive females and the pest population in the release area shrinks. The Oxitec DBM also have a fluorescent marker (DsRed2) to identify the Oxitec moths and distinguish them from wild ones. This colour marker is used to monitor control of the pest population.
- **Olive fruit fly**: The olive fruit fly, *Bactrocera oleae*, (Rossi) (Diptera: Tephritidae) is the major arthropod pest of commercial olive production especially in the Mediterranean region. OX3097D is a female-specific Oxitec strain. It can be used for population suppression without the need to irradiate insects. OX3097DBol is able to provide 100% male only release generations when reared in the absence of a dietary transgene repressor. Introgression of susceptibility alleles through the female line provides a resistance management option for other control methods used with fsRIDL in an integrated pest management programmes.
- **Mexican fruit fly**: The Mexican fruit fly (*Anastrepha ludens*), or mexfly, is a prominent agricultural pest in Mexico, parts of Central America and the Rio Grande Valley of Texas. Oxitec Mexfly (OX3097B) is a female-specific Oxitec strain. The strain provides 100% female lethality. The Oxitec Mexfly is homozygous and is currently being tested at Oxitec for conditional lethality, marker performance and mating competitiveness.
- **Mediterranean fruit fly:** The Mediterranean fruit fly (*Ceratitis capitata),* or medfly, is one of the world's most destructive agricultural pests. OX3864A is a female-specific Oxitec strain. Our earlier prototype, OX3864A contained green fluorescent marker Transgenic insects are made by using transposable elements that seem to have a broad host range.

Future aspects of self-limiting gene in Entomology:

It may be effective control techniques for pest where mostly females damage the crop: Fruits sucking moth, etc. It may be effective control techniques for insecticide resistant insect-pests. It may be effective control techniques for pest. It may be effective production techniques for beneficial insects where male are most effective producers. Example: Silkworm (Transgenic silkworm)

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Benefits of self-limiting gene techniques:

Self-limiting gene technology can enhance their cost effectiveness by replacing the radiation that reduces the fitness of the sterile insect's male are healthy and compete to wild species. Female-specific lethal genes can also be used to separate the sexes to allow male only releases, which is especially cost-effective**.** Oxitec technology is species-specific because the insects only produce viable offspring with their own species so the genes don't spread. No risk of 'horizontal' gene transfer. The insects and their offspring die so the genes don't persist in the environment. The proteins of the introduced colour marker and pest control genes are nontoxic and non-allergenic.

Oxitec technology is a form of biological control which is completely species-specific because the insects only produce viable offspring with their own species, so other beneficial insects like bees are not targeted. It can be used to reduce a target population to below the threshold for damage or disease transmission, then low level 'preventative' releases can keep it there. It can be used as part of a broad IVC (integrated vector control) programme or IPM (integrated pest management). The self-limiting gene is also nontoxic, so the moths can be eaten by birds or other animals with no adverse effects.

Drawback of Self-Limiting Gene Techniques:

They are high technical. It is costly as compare to pesticide management. Not applicable to all insects due to lack knowledge of genetic sequence. There are little chances of biodiversity loss.

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