



Biotechnology in Sterile Insect Technique

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Management of insect pests especially the vectors of human diseases demand safer ways where the human health is not impeded. Sterile Insect Technique (SIT) is one such efficient method which is being used for management of codling moth, *Cydia pomonella*, false codling moth, *Thaumatotobia leucotreta*, light brown apple moth, *Epiphyas postvittana*, painted apple moth, *Teia anartoides* and pink bollworm, *Pectinophora gossypiella*, mosquitoes and others. Utilization of biotechnological tools maintain transgenic sterile insects can reduce the ill effects of radiation technique which was previously used to induce sterility

Introduction

A sterile insect is defined as "an insect that, as a result of a specific treatment, is unable to reproduce". Globalisation and climate change has led to an increase in outbreaks of mosquito borne diseases and major insect pests. The SIT is one among the important components of IPM of these pests (Franz and Robinson, 2011). Use of sterile males was first described by the Russian geneticist A. S. Serebrovsky in 1940. Raymond Bushland and Edward Knipling (1954) developed the SIT to eliminate a calliphorid dipteran named screw-worm fly, *Cochliomyia hominivorax* using irradiation method in Panama. But the radiation method of producing sterile insects comes with its own drawbacks. Thus, biotechnological tools can be effectively utilized to replace the technique.

Advantages of SIT

- The International Plant Protection Convention categorizes sterile insects as beneficial organisms under biological insect pest control method in Area Wide-Integrated Pest Management (AW-IPM)
- Environmental protection through reduced use of insecticides
- Sterile insects are not self-replicating thus cannot be established in the environment
- Breaking the pest's reproductive cycle, also called autocidal control and is species-specific
- The SIT does not introduce non-native species into an ecosystem
- Diseases of cattle and humans are controlled and can be taken as a prophylactic measure
- No risk of resistance development
- A significant reduction in crop and livestock production losses

Nevertheless, radiation techniques has its major drawbacks related to large-scale sex-separation, reduced male mating fitness/ compatibility thus demanding a greater number of sterile males to be released repeatedly, financial requirements, species-specificity demanding the standardisation of dosage for each species, problems of fertile male or sterile female release, migration of wild insects from outside the control area etc. The biotechnological tools might help mitigating these problems.

Transgenic approaches in SIT

The FAO (Food and Agriculture Organization) and IAEA (International Atomic Energy Agency) assist their member states in developing and adopting nuclear-based technologies for optimizing agricultural insect pest management practices. The lab-grown transgenic *Aedes aegypti* mosquitoes released into the wild to mate with the wild population where their offspring's inability to grow to adulthood lowers the population of mosquitoes.

Lethal genes and sex specific promoters

For disease transmitting insects like mosquitoes where released sterile females would still act as disease vectors, release of only males is essential for SIT development. Control of gene expression can be achieved using either the tetracycline-controlled transactivator (tTA) or by heat inducible promoters. To generate transgenic sexing systems, female lethality was first developed and tested in *Drosophila melanogaster*. Early embryonic female-specific lethality and thus avoidance of larval survival is caused under the regulation of the tTA which is under the control of an early embryonic promoter. Addition of tetracycline (TET) to the food keeps the system in the OFF state (shut down of expression of the transgene) during rearing. Removal of tetracycline results in switching ON of sexing system in release generation (death). The tTA system has been shown to be functional in the Mediterranean fruit fly where over-expression of tTA was shown to be lethal, and 100% of females died when raised on media that lack tetracycline; The heat inducibility of the *C. capitata hsp70* promoter was several times more heat inducible than the *Drosophila hsp70* promoter (Franz and Robinson, 2011).

The first genetic sexing strains in the Mediterranean fruit fly were developed using a *white pupae* mutation. In mosquitoes, genetic sexing strains were developed using insecticide resistant genes so that female larvae remain susceptible and could be killed by adding a fixed amount of insecticide to the larval medium (Franz and Robinson, 2011).

Monitoring the sterile insects in the field requires a marker to distinguish the sterile male insects from wild insects following trapping in the field, e.g. incorporation of a marker into Mediterranean fruit fly genetic sexing strains using a mitochondrial DNA (mtDNA). This unique piece of DNA can either be monitored using PCR or it can be used to express a fluorescent protein. *Sperm marker*: Sperm from the released sterile males could be identified in wild females following mating, i.e. an important indicator of competitiveness of the sterile males. *Preventative release programmes* where distinguishing female mated with sterile male from that of fertile male is important.

High redundancy of reproductive sterility system can be achieved by having diverse endonucleases or endonuclease target sites causing chromosome shredding which can be achieved through CRISPR. Use of the $\beta 2$ *tub* promoter, Cas9 will be highly expressed during spermatogenesis and the mRNA still be highly translated during spermiogenesis to expose the sperm chromosomes to high amounts of the endonuclease leading to large chromosomal aberrations causing reproductive sterility (Eckermann et al., 2014).

Conclusion

The SIT has the ability to eradicate invasive pest outbreaks and to prevent their establishment. Species composition of an area must be properly understood before going for SIT to ensure mating compatibility between wild females and laboratory males. Integrated with other control methods, the SIT has been successful in controlling a number of high-profile insect pests, including fruit flies (Mediterranean fruit fly, Mexican fruit fly, Oriental fruit fly, melon fly), tsetse fly, blow flies, screw worm flies, moths (codling moth, pink bollworm, false codling moth, cactus moth, and the Australian painted apple moth), and mosquitoes. Biotechnologically engineered designer insects have already been released in small scale trials: pink bollworm moths in Arizona, USA, as well as yellow fever mosquitoes

in the Grand Cayman Islands, Malaysia and currently in Brazil. Biotechnological approaches based on genetically modified organism (transgenic organisms) are still under development.

References

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