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NEXT GENERATION INSECTICIDE RESISTANCE MANAGEMENT: TRANSGENIC CONTRIBUTIONS

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SYNTHESIS – In recent decades insecticides have played an invaluable role in contemporary integrated pest management (IPM) strategies. Although such cultural, physical, biological and chemical combinations are complementary to one another and can reduce over-reliance on single components, true synergism between them is rarely reported. Transgenic technologies for the control of insect pests in an autocidal manner are developing at an increasingly rapid rate, and in the case of Oxitec's genetic technology, offer potential direct synergism with insecticides and a significant insecticide resistance management (IRM) benefit.

It is now over 30 years since the first transgenic insect, *Drosophila melanogaster*, was developed and it has since become a model species for novel transgenic techniques. Indeed, work on *D. melanogaster was* also a precursor to Oxitec's insect control technology. Numerous pest insects have been the target for genetic transformations including fruit flies such as *Ceratitis capitata* (Medfly) and *Bactrocera oleae* (olive fruit fly). The first transgenic coleopteran was *Tribolium castaneum* (red flour beetle) and the first pest moth was *Pectinophora gossypiella* (pink bollworm); the latter being a devastating global pest of cotton prior to the advent of transgenic *Bt* cotton. The first transgenic mosquito, *Aedes aegypti* (yellow fever mosquito), was published in 1998; this species is the primary vector of several human viral diseases including yellow fever, dengue fever, and chikungunya. The first transgenic strain of a malaria-transmitting mosquito, *Anopheles stephensi* (Asian malaria mosquito), was published in 2000. As these dates illustrate, the ability to insert exogenous DNA into the genomes of pest insects has only relatively recently been developed, nonetheless applications are rapidly being developed.

Oxitec have now successfully applied transgenic insect control techniques in the field. The biological characteristics of transgenic *P. gossypiella* were examined in large-scale open release experiments in Arizona, USA; they were shown to have similar mating competitiveness to wild-type counterparts and stable expression of the genetically incorporated fluorescent marker. Most notably, 80-95% control of populations of the dengue vector, *Ae. aegypti*, was achieved after several months of releases at distinct sites in the Cayman Islands and north-east Brazil. National regulatory authorities in Europe, the Americas, Asia, and the Middle-east have granted permission to import and study specific Oxitec transgenic insect lines in contained surroundings. Following appropriate biosafety assessments, four of these have granted permission to conduct deliberate open releases into the environment (USA, Cayman Islands, Malaysia and Brazil).

Classic sterile insect technique (SIT) has been in use for many years for area-wide suppression of target pest populations and in some cases even elimination of target pest populations on various scales. To implement SIT, millions of insects are reared under strictly controlled conditions before being subjected to sterilising levels of radiation treatment. Insects are then released at high densities throughout the target area. When the sterilised males mate with wild females, the fertilised eggs are largely non-viable due to radiation-induced lethal genetic mutations in the sperm and few or no viable progeny are produced. Successive releases over multiple generations result in a decline in the wild population. SIT was used effectively to eradicate the New World screwworm (*Cochliomyia hominivorax*) from the USA and Mexico in recent decades, and the control of Medfly has been enhanced by SIT in many regions. Other successful SIT programmes have targeted Queensland, Mexican, and West Indian fruit flies, codling moth in Canada, and pink bollworm in south-western USA.

SIT has several positive attributes including an environmentally friendly profile and good efficacy

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in specific cases. The control agent (sterile males) will actively disperse themselves to seek out the wild pest females, and since these males will only mate with females of the same species, the method is exquisitely species-specific. However, the complexities of consistently mass-rearing and releasing high-quality insects at the correct time and place present a significant challenge.

Oxitec genetic control using lethal transgenes works in a similar manner to SIT, except that it has a number of key advantages. Primarily, instead of sterilisation by irradiation the insects cannot reproduce because they pass on a dominant lethal gene to their offspring. This death of progeny circumvents the irradiation process and any associated concerns, which can include a reduction in fitness as a consequence of radiation exposure, financial, and safety considerations. Oxitec insects carry a genetic fluorescent marker that enables quick and easy identification - so all released insects can be tracked and their performance assessed. The heritable dominant lethals ensure that the strains cannot persist in the environment.

One common constraint to the successful implementation of SIT type strategies is the need for sexing: separation of useful males from females, which are also potentially damaging to crops or as disease vectors. These procedures are frequently unavailable, and can be labour-intensive and costly. Genetic sexing strains, in which both sexes carry the lethal transgene but only females express the phenotype, can offer dramatic improvements in programme efficiency. Transgenic sexing strains can be mass-reared in the presence of a dietary antidote, and withholding the antidote from the pre-release generation conveniently removes the females. Post-release, transgenic males mate with wild females and their female progeny die. Persistent release of such males will result in a crash in the number of females in the field, and a consequent reduction in the reproductive potential of the wild pest population. This effect has been demonstrated in caged experiments with both Medfly and olive fly.

The introgression of insecticide-susceptible genes as a consequence of segregation during mating between male heterozygotes and wild females can 'dilute' resistance in the wild population. Both modelling and semi-field studies of insecticide-resistance allele frequencies have demonstrated that repeated releases of insecticide-susceptible individuals carrying a female-specific lethal transgene can reduce resistance levels in pest populations, offering a potentially powerful secondary benefit for pest control. Providing the released transgenic insects are susceptible, this approach can provide resistance management benefits for all insecticides and would also serve to protect novel modes of action from the initial onset of resistance in a pre-emptive manner.

Insecticides are most efficient when pest populations are high, whereas Oxitec's genetic control is most effective when pest populations are lower. Consequently, their synergistic integration can provide:

- control of high pest populations quickly and economically;
- long-term suppression of pest populations below economic thresholds;
- reduction in the level and/or development of insecticide resistance;
- options for the control of sporadic outbreaks /hotspots;
- control of pests for which biocontrol is not effective (e.g. olive fly).

In combination, insecticidal and genetic insect control methods could provide sustainable, economical and safe management of pests. The approach provides, in principle, a contemporary method for controlling pest populations while managing the spread of pest resistance to both conventionally applied and genetically engineered insecticidal toxins.