



## **Overview of Technological Advances Toward Greater Efficiency and Efficacy in Sterile Insect-Inherited Sterility Programs Against Moth Pests**

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### Overview of technological advances toward greater efficiency and efficacy in sterile insect-inherited sterility programs against moth pests

Marc J. B. Vreysen<sup>1,\*</sup>, Waldemar Klassen<sup>2</sup> and James E. Carpenter<sup>3</sup>

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#### Abstract

Lepidopteran species are amongst the most damaging pests of food and fiber crops world-wide. Pest lepidopterans are often managed injudiciously by spraying crops with large amounts of broad-spectrum—and often—persistent insecticides. In view of increased occurrence of resistance against these insecticides, and their negative impacts on the environment and ecosystems, the need for control tactics that are not only effective but also friendlier to the environment is becoming more and more pressing. Both the sterile insect technique (SIT), and the related inherited sterility (IS) technique offer great potential as additional control tactics for integration with other control methods in area-wide integrated pest management approaches against lepidopteran pests. However the SIT/IS can only be applied successfully when the released sterile insects can effectively compete with their wild counterparts for mating with wild females. Although there are a number of programs where the SIT has been used very effectively against key lepidopteran pests, there is great potential for further expansion of the SIT/IS technology to target other key lepidopteran pests, or to improve the SIT/IS for already targeted Lepidoptera. Such expansion could be facilitated through improvements of mass-rearing, measurement and control of quality, handling, irradiation, shipping, release and field assessment technologies that would increase program efficiency and efficacy. To foster such advances, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, implemented a Coordinated Research Project (CRP) from 2008 to 2014 entitled “Increasing the efficiency of Lepidoptera SIT by enhanced quality control”. This Project organized and sponsored research and development on the following key objectives: (1) identify and investigate factors and variables affecting the quality of the produced and released insects and their field performance, (2) identify and develop new tools and methods to assess and predict the field performance of sterile insects, and (3) improve the artificial rearing of several moth species through a better understanding and management of genetic resources. The key research outputs of the CRP are summarized in this paper.

Key Words: biological control, colonization, competitiveness, mass-rearing, irradiation, dispersal, field performance, mating status, modeling, mating compatibility, mating competitiveness, mating status, flight ability, cold adaptation, cage studies, open field studies, female attractant, host-derived kairomone, conspecific larvae-infested host, marking, natural isotope signature

#### Resumen

Algunas de las especies de lepidópteros son de las plagas más perjudiciales de los cultivos de alimentos y fibras en todo el mundo. Las plagas lepidópteras a menudo son manejadas imprudentemente al rociar los cultivos con grandes cantidades de insecticidas de amplio espectro — que a menudo son —persistentes. En vista del aumento de la aparición de resistencia contra estos insecticidas, y sus efectos negativos sobre el medio ambiente y los ecosistemas, la necesidad de tácticas de control que no sólo son eficaces, pero también más amigables con el medio ambiente es cada vez más apremiante. Tanto la técnica del insecto estéril (TIE), como la técnica de esterilidad heredaria relacionada (EH) ofrecen un gran potencial como tácticas adicionales de control para la integración con otros métodos del manejo integrado de plagas enfocados contra plagas de lepidópteros en todo lado. Sin embargo, la TIE/EH sólo se puede aplicar con éxito cuando los insectos estériles liberados pueden competir eficazmente con sus contrapartes salvajes para el apareamiento con hembras salvajes. Aunque hay una serie de programas en los que la TIE se ha utilizado de manera muy eficaz contra las plagas de lepidópteros clave, hay un gran potencial para una mayor expansión de la tecnología de TIE/EH para dirigirse a otras plagas de lepidópteros clave, o para mejorar la TIE/EH para los lepidópteros ya seleccionados. Tal expansión podría facilitarse mediante la mejora de la cría en masa, las tecnologías de medir y de control de calidad, manipulación, irradiación, envío, y su liberación y evaluación en el campo incrementaría la eficiencia y la eficacia del programa. Para fomentar

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estos avances, la División Mixta de Técnicas Nucleares en la Alimentación y la Agricultura de FAO/IAEA implementó un Proyecto Coordinado de Investigación (PCI) desde el 2008 hasta el 2014 titulado «El aumento de la eficiencia de la TIE de Lepidóptera por el mejoramiento de control de calidad». Este proyecto organizó y patrocinó la investigación y desarrollo de los siguientes objetivos clave: (1) identificar e investigar los factores y variables que afectan a la calidad de los insectos producidos y liberados y su rendimiento en el campo, (2) identificar y desarrollar nuevas herramientas y métodos para evaluar y predecir el comportamiento en el campo de los insectos estériles, y (3) mejorar la cría artificial de varias especies de polillas a través de una mejor comprensión y manejo de los recursos genéticos. Se resumen los resultados de la investigación clave de la PCI en el presente documento.

Palabras Clave: control biológico, colonización, competitividad, cría en masa, irradiación, dispersión, desempeño en el campo, modelado, compatibilidad del apareamiento, competitividad del apareamiento, estado del apareamiento, capacidad de vuelo, adaptación al frío, estudios de jaula, estudios de campo abierto, atrayente de hembras, kairomona derivada del hospedero, hospederos infestados con larvas conespecíficas, marcado, firma de isótopos naturales

Numerous species of Lepidoptera cause severe losses to agricultural crops in all tropical, sub-tropical and temperate regions of the world and are considered amongst the most damaging pests of food and fiber crops world-wide (Peters 1988; Bloem et al. 2005). To date these pests are mainly managed by injudiciously spraying large amounts of broad-spectrum, and often, persistent insecticides. This approach remains unsustainable in view of the enormous direct and indirect economic, social and environmental consequences of the wide use of these insecticides. Loss of yield, limitations on market access, and high control costs are only a few examples of the economic impact of the injudicious use of insecticides. In addition, sustainable management of these pests using insecticides is hampered by the rapid development of resistance to every newly developed insecticidal chemical (Suckling & Khoo 1993; Varela et al. 1993; Tabashnik 1994). Moreover, in view of increased scientific evidence and public recognition of the long-term effects of their excessive use on human health and the environment, the uses of many effective insecticides have already been curtailed and many have been removed from the market (UPI 2008). Linked to this is the increasing market pressure to reduce insecticide residues on or in food, especially in shipments to lucrative export markets. Finally, global increases in trade and travel—since 1986 when the 8th round of the General Agreement on Tariffs and Trade was agreed—have resulted in an alarmingly great increase in the rate of transcontinental invasion by lepidopteran species, which threaten agricultural systems, markets, communities, and biodiversity on a worldwide basis. As an example, an analysis indicated that 19 of the 37 or 51% of the worst insect pest threats facing US agriculture are Lepidoptera species (ESA 2001).

There is broad international consensus that the management of these key pests is ideally based on the concept of area-wide integrated pest management (AW-IPM) (Klassen 2005; Hendrichs et al. 2007; Koul et al. 2008), i.e., control tactics should be integrated based on their suitability for a given pest species and local ecological characteristics, and the control tactics should target an entire insect population (total population control) (Knippling 1972, 1979; Vreysen et al. 2007). In view of the above made considerations on the use of insecticides, control tactics that are effective but more friendly to the environment such as the sterile insect technique (SIT) offer great potential (Dyck et al. 2005) and should be increasingly adopted.

Unlike insecticide-based control tactics that aim at increasing the mortality rate of a given target pest population, the SIT/IS aims at compromising its hereditary machinery and thereby reduces the overall fertility and productivity of that population (Vreysen & Robinson 2010). This autocidal pest control tactic requires the colonization and mass-rearing of the target pest species, the induction of sexual sterility in a large fraction of the insects by an appropriate dose of gamma radiation followed by their release into the field on a sustained basis and in sufficient numbers to achieve appropriate sterile to wild insect over-flooding ratios (Knippling 1979). Wild virgin females that mate with released sterile males bear no offspring, and the target population's progressively reduced replacement rate inevitably leads to either a diminished wild population or local or regional eradication.

Because Lepidoptera possess a special chromosome structure with diffuse centromeres, they are more radiation-resistant than Diptera, which have monocentric chromosomes (LaChance 1967). Radiation doses above 250 Gy are usually required to induce complete sterility in male moths (IDIDAS Database; Bakri et al. 2005), but these high radiation doses negatively affect their competitiveness (Carpenter et al. 2005). In most moth species, the females are more radiation-sensitive than the males (LaChance 1985; Robinson 2005). For this reason and because of the absence of genetic sexing systems for lepidopteran species—although progress in developing such a system has been reported (Marec et al. 2007)—, the SIT requires that both genders must be irradiated with a dose sufficiently great to almost fully sterilize the released males. However in order to take advantage of the fact that irradiation of chromosomes with diffuse centromeres with somewhat reduced doses leads to dominant lethal chromosomal aberrations in subsequent generations, a derivative of the SIT—called  $F_1$  sterility or inherited sterility (IS)—was developed for lepidopteran pests (Proverbs 1962; North 1975; LaChance 1985; Carpenter et al. 2005). In this IS technique, the irradiation dose that moths receive is selected that would completely sterilize irradiated female moths and partially sterilize the males. Moths treated with this lower sterilizing dose live longer, are stronger fliers, and mate more frequently than moths treated with higher radiation doses. Moreover, the sterility effects are inherited by the offspring of the sub-sterile males mated with wild fertile virgin females to produce completely sterile  $F_1$  moths—predominantly males. These male descendants are produced in their natural environment—and not in an artificial rearing facility—and because they have not received any radiation dose, they are more competitive than males irradiated with a fully sterilizing dose (Carpenter et al. 2005). Implementing IS systems for the management of lepidopteran pests can greatly improve program efficiency as compared with classical SIT programs that release fully sterile male moths.

The use of SIT/IS has many advantages for pest population suppression, including species specificity and compatibility with other area-wide control tactics such as mating disruption, biological control, cultural control methods and the use of bio-rational insecticides (Knippling 1972, 1979; Carpenter et al. 2005). It is an environmentally-friendly technology that can be used to address many of the world's most difficult pest control problems.

The SIT and IS techniques have been used very successfully in AW-IPM programs with strategic objectives including containment, suppression and eradication against a number of major insect pests: New World screwworm, *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae) (Wyss 2000), tsetse flies, *Glossina* spp. (Diptera: Glossinidae) (Vreysen et al. 2000), various species of fruit flies (Diptera: Tephritidae) (Calkins et al. 1994; Enkerlin et al. 2015) and several moth species, i.e., the codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae) (Canada) (Bloem et al. 2005), the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) (USA) (Henneberry 2007), the cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) (USA) (Carpenter et al. 2008), the Australian painted apple

moth, *Teia anartoides* (Walker) (Lepidoptera: Lymantriidae) (New Zealand) (Suckling et al. 2007), and the false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) (South Africa) (Hofmeyr et al. 2015, 2016).

Moreover, there is great potential for further expansion of the SIT/IS to target other key lepidopteran pests, and to improve the SIT/IS in currently on-going programs against lepidopteran pest species. Such expansion could be facilitated by increasing the quality of the released moths through substantial improvements in mass-rearing, handling, irradiation, shipping, release and field assessment activities. To address this topic, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture implemented a new Coordinated Research Project (CRP) entitled “Increasing the efficiency of Lepidoptera SIT by enhanced quality control” from 2008 to 2014. The following key areas for research and development were addressed: (1) identify and research factors and variables affecting the quality of the produced and released insects and their field performance, (2) identify and develop new tools and methods to assess and predict the field performance of sterile insects, and (3) improve the artificial rearing of several moth species through a better understanding and management of genetic resources.

Seventeen researchers from Argentina, Australia, Chile, China, India, Israel, Malaysia, Mexico, New Zealand, South Africa, Syria, Tunisia, and the USA, participated in the CRP by doing their research on 13 Lepidoptera species that are potential targets for the SIT (Table 1). During the course of the CRP, the participants published 66 research papers in peer-reviewed journals (not including the papers in this special issue) and presented 50 papers at national, regional or international conferences. In addition, 2 MSc and 7 PhD theses were completed that contained some of the research implemented under the CRP. This special issue of the Florida Entomologist compiles some of the final findings of the CRP in a total of 24 original research papers.

In this introductory chapter, we present the overall framework of this CRP, highlight its main findings and summarize the papers presented in this special issue. The paper also identifies topics remaining for research that could be addressed in a follow-up CRP.

## Theoretical and Practical Frameworks

### MAJOR FINDINGS

#### Modeling

A conceptual model was developed that was based on 2 basic spatial elements of AW-IPM programs, i.e., the core area and the buffer area. The model was developed to determine the minimum size of the

protected area of an AW-IPM program needed to assure its technical feasibility and economic viability. The model consisted of a biological part (insect dispersal) and an economic part. The biological part used random walks and diffusion equations to describe insect dispersal and to determine the minimum width of the buffer area required to protect the core area from migrating insects. The economic part calculated the minimum size of the core area at which the revenues of the core area would equal the control program costs (Barclay et al. 2011). Kean et al. (2011) developed a population model for the light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) that indicated that releasing 300 Gy-irradiated moths will result in a 95% probability of extinction when the ratio of released to wild moths exceeds 6.4:1. Higher over-flooding ratios would achieve eradication more rapidly. The model indicated an optimal release interval of 1 wk, and predicted only little advantage to releasing only male moths as compared with both sexes. A dose of 200 Gy was considered optimal for an IS program because the resulting  $F_1$  sterility would reduce by  $\frac{1}{2}$  the needed number of factory moths compared with releasing moths irradiated with 300 Gy. Two models were developed to describe the population dynamics of an *Eldana saccharina* Walker (Lepidoptera: Pyralidae) population that was targeted by partially sterile insects. The first mathematical model described the population growth and the interaction between wild and sterile moths in a temporally variable but spatially homogeneous environment. The primary objective of this model was to determine suitable parameters that allowed quantification of population growth and interactions between released and wild moths (Potgieter et al. 2012). The second model, a reaction-diffusion model, described the growth of the wild population and the interaction between wild and sterile *E. saccharina* moths in a temporally variable and spatially heterogeneous environment. The primary objective was to use the model in an AW-IPM program context to investigate the efficiency of different sterile moth release strategies without having to conduct formal field experiments, and to present guidelines according to which release ratios, release frequencies and spatial distributions of releases may be estimated that are expected to lead to suppression of the pest (Potgieter et al. 2013). As a follow-up of the 2 previous models, Potgieter et al. (2016, this special issue) developed a user-friendly simulation model for determining the impact of an IS program on *E. saccharina* populations. The model can be adapted for a number of pest-crop combinations and pest control scenarios. The elaborated model contained 4 interacting subsystems (pest species population dynamics, sugarcane dynamics, environmental dynamics and economics) within a specified spatial domain that describes the layout of the agricultural crop (position, size, shape, sugarcane age and variety of the different fields contained within the sugarcane area).

**Table 1.** Lepidopteran species on which investigations were conducted in the Coordinated Research Project.

Species	Family	Common name	Country of study
<i>Cactoblastis cactorum</i> (Berg)	Pyralidae	cactus moth	USA
<i>Conopomorpha sinensis</i> Bradley	Gracillariidae	litchi stem end borer	China
<i>Cydia pomonella</i> L.	Tortricidae	codling moth	Canada, China, South Africa
<i>Eldana saccharina</i> Walker	Pyralidae	sugar cane borer	South Africa
<i>Epiphyas postvittana</i> (Walker)	Lymantriidae	light brown apple moth	Australia, New Zealand, USA
<i>Hypsipyla grandella</i> (Zeller)	Pyralidae	mahogany shoot borer	Mexico
<i>Lobesia botrana</i> (Denis & Schiffermüller)	Tortricidae	European grapevine moth	Israel, Syria
<i>Pectinophora gossypiella</i> (Saunders)	Gelechiidae	the pink bollworm	USA
<i>Plutella xylostella</i> L.	Plutellidae	diamondback moth	Malaysia
<i>Spodoptera litura</i> (F)	Noctuidae	Oriental leaf worm	India
<i>Teia anartoides</i> (Walker)	Lymantriidae	Australian painted apple moth	New Zealand
<i>Thaumatotibia leucotreta</i> (Meyrick)	Tortricidae	false codling moth	South Africa, USA
<i>Tuta absoluta</i> (Meyrick)	Gelechiidae	tomato leaf miner	Argentina, Tunisia

## Population Genetics, AW-IPM and the SIT

Studying the population genetics of a targeted and an adjacent population can provide important information on gene flow and the level of isolation of the target population. This knowledge can guide informed decisions for developing AW-IPM strategies that include the SIT/IS. Thus significant genetic differentiation was found between codling moth populations—a highly invasive species in China—of 2 adjacent regions in the Hexi Corridor that were geographically separated by stone deserts, whereas populations within each region showed a similar genetic structure. The limited gene flow of codling moths between the 2 regions suggests that SIT can be implemented to control the pest in the Hexi Corridor (Duan et al. 2016, this special issue).

## The SIT and Biological Control

One of the advantages of the SIT and IS techniques for suppressing lepidopterans is that these related technologies are compatible with other biological control tactics such as the use of parasitoids (Carpenter et al. 2005). Cagnotti et al. (2016, in this special issue) investigated the possibility of combining the IS technique for the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) with the release of the egg predator *Tupiocoris cucurbitaceus* (Spinola) (Hemiptera: Miridae). *Tupiocoris cucurbitaceus* females consumed similar numbers of eggs oviposited by untreated parents and those oviposited by females that had mated with irradiated males. The mirid females preyed on significantly more eggs from irradiated females than on eggs from untreated females, however released irradiated females do not play a significant population suppressive role when deployed in a program with an IS component. Indeed the authors concluded that it is technically feasible to combine the release of this predator with releases of irradiated moths in a program with an IS component.

Lebdi et al. (unpublished data) studied the life cycle of *T. absoluta* in Tunisia and found that it needed 29–38 d to reach completion, depending on the temperature and humidity. In Tunisia, *T. absoluta* was multivoltine and had 12–14 generations per yr.

## Factors and Variables that Affect Quality of Lepidopterans Used in SIT/IS Programs

The SIT requires the mass-rearing of insects that are released in sufficient numbers to outcompete their targeted conspecific native counterparts. The maintenance of large insect cultures for long periods in an artificial environment imposes numerous selection pressures on the insects that tend to result in undesirable traits in field-released males. Genetic phenomena such as the founder effect, laboratory adaptation and genetic drift can cause important changes, as can the stressful environment (Simmons et al. 2010). These pressures might select for insects that are perfectly adapted to mass-rearing conditions, i.e., short reproductive cycles, large pupae, increased fecundity and fertility etc., but these insects are not necessarily the best adapted to the ecological requirements of the target zone, or have optimal behaviors that make them strongly competitive sexually with their native counterparts. In addition, handling, irradiation, storage, transport and release procedures may degrade qualities of the released insects that are critically important to their performance in finding and competing for mates in the target area. None of the effects of all of these stress factors are well understood.

Often mass-rearing programs have emphasized numbers of insects produced over those biological qualities of the produced insects that are prerequisite to their mate finding and sexual competitiveness in the field. Releasing sterile insects that lack the capacity to adequately compete for mates with the wild males can drastically impair program progress and success, i.e., lower efficiency at the program level, more insects required to obtain the same level of progress, extended duration of the program, etc.; all resulting in substantially increased program costs (Simmons et al. 2010). Much of the focus on quality control of mass- or laboratory-reared insects necessarily concerns maintenance of facility output (i.e., numbers produced), but much too little attention and resources have been devoted to in-depth considerations of individual trait effects, e.g., mating competitiveness, and flight performance and dispersal in the field. Trait performance is becoming acknowledged as an essential factor in integrated pest management success (Sørensen et al. 2012). Indeed, keen awareness of the importance of quality traits in the success of area-wide IPM programs is growing.

Although insect quality management is routinely practiced in many mass-rearing facilities, there is a dearth of in-depth knowledge of the factors and variables that affect the quality and the field performance of released moths. Currently there is little operational use of quality measures that reliably predict field performance, and there is scant knowledge of how such measures could be used to improve the outcomes of mass-rearing to enhance insect quality and success in the field (Simmons et al. 2010).

For the purposes of this CRP, we adopted a cross-cutting view to examine biological and operational factors affecting quality at all stages of the operation. Various biological attributes—such as development rates, diapause, temperature adaptation, circadian rhythms, mating habits, flight capabilities, etc.—and operational components—such as cold storage, packaging and transport, release technology, and irradiation techniques—were identified that warranted further investigation.

## MAJOR FINDINGS

Before studying the impact of certain factors or parameters on the quality of the mass-produced insects, it was important to develop an in-depth knowledge of the general biology of the target insect species. In that respect, Walton & Conlong (2016a, in this special issue) assessed fecundity, fertility, and mating frequencies of the sugarcane borer, *E. saccharina* that had been cultured in the laboratory since the 1970s. These authors found that male and female *E. saccharina* were able to mate more than once under controlled laboratory conditions, and they discussed the potential consequences of this finding for the release rates that would be required to obtain adequate sterile to wild male over-flooding ratios.

## Thermal Tolerances and Insect Quality

Understanding tolerance to thermal extremes by pest insects is essential for developing integrated management strategies, as tolerance traits can provide insights into constraints on their activity and survival. Insect thermal limits may vary with microclimatic fluctuations, and may be influenced by biotic or abiotic factors. Chill-coma temperature (CT<sub>min</sub>) and critical thermal maximum (CT<sub>max</sub>) were significantly different for *E. saccharina* moths collected from 2 different host plant species, i.e., sugarcane, *Saccharum officinarum* L. (Poales: Poaceae), and papyrus, *Cyperus papyrus* L. (Poales: Cyperaceae). However lower lethal temperatures (LLT) of these moths and freezing temperatures of the pupae did not vary with the host plant species. The *E. saccharina*, which experienced the lowest minimum temperature (in *C. papyrus*), did not have the lowest CT<sub>min</sub>, although the highest estimate of CT-

max was found in *E. saccharina* collected from *C. papyrus*; and this was also the microsite from which were reported the highest maximum temperatures. These results suggest that host plants may strongly mediate the lower critical thermal limits, but not necessarily the LLT or freezing temperatures (Kleynhans et al. 2014). Male and female laboratory-reared *E. saccharina* were more heat tolerant than wild moths. Conversely, wild moths were more cold tolerant than their laboratory-reared counterparts. Irradiation had a deleterious effect on the critical thermal maximum and on the critical thermal minimum. Moths irradiated with a lower dose were more heat and cold tolerant than those irradiated with a higher dose, which highlighted again the importance of treating lepidopterans with a lower dose rather than with the full sterilizing dose (Mudavanhu et al. 2012).

Exposing the false codling moth to  $-4.5\text{ }^{\circ}\text{C}$  for 2 h or to  $-0.5\text{ }^{\circ}\text{C}$  for 10 h resulted in a 50% probability of survival. Gender and early adult age did not affect low-temperature tolerance of the false codling moth. Limited evidence for rapid cold hardening was found as false codling moth survival could not be increased through exposure to non-lethal, low- and high-temperature pre-treatments (Stotter & Terblanche 2009).

Significantly more low temperature-acclimated codling moths were recaptured under cooler conditions in the wild than either warm-acclimated or control moths. However, improvements in low temperature performance of cold-acclimated moths came at a cost to performance under warmer conditions. Conversely, warm acclimated moths had improved field performance under warmer ambient conditions as compared to either cold acclimated moths or control moths. Laboratory data matched field results indicating that assessment of thermal activity of codling moths in the laboratory also may apply to their field performance (Chidawanyika & Terblanche 2011a).

Temperature and duration of exposure significantly affected the survival of codling moths, with more extreme temperatures and longer periods proving to be more lethal. Rapid thermal exposures scarcely affected the moth's responses to prolonged low temperatures, but the effects on survival of rapid thermal exposures were greater at prolonged high temperatures. Low temperature pre-treatments of codling moths did not improve their high temperature survival, but high temperature pre-treatment did improve their low temperature survival (Chidawanyika & Terblanche 2011b).

### Irradiation and Insect Quality

Extensive attention was given to irradiation as one of the factors having a potentially negative impact on the quality of released male moths. Irradiating male *E. saccharina* with increasing doses of gamma radiation did not affect the fecundity of the untreated *E. saccharina* females that had mated with irradiated males, but egg hatch declined significantly with increasing doses. Female *E. saccharina* were completely sterile when treated with 150 Gy, whereas the males treated with 350 Gy mated still had a residual fertility of 0.19% (Walton & Conlong 2016b, in this special issue).

Similar studies on the litchi stem-end borer, *Conopomorpha sinensis* Bradley (Lepidoptera: Gracillariidae), indicated that a dose of 200 Gy was sufficient to induce complete sterility in females, whereas males irradiated with 300 Gy still had a residual fertility of 0.67%. A dose of 250 Gy applied to P generation males resulted in complete sterility in  $F_1$  males (Fu et al. 2016, in this special issue).

Effects of radiation on fecundity and fertility of codling moths originating from either South Africa or Canada were very similar. Female moths were considerably more radiation-sensitive than males and a dose of 100 Gy was sufficient to completely sterilize female moths (Blomefield et al. 2010).

Adult emergence of the tomato leaf miner decreased with increasing X ray doses that had been applied to pupae, but these treatments did not affect the longevities of irradiated P adult males and  $F_1$  adult males, nor was the sex ratio affected by them. A dose of 200 Gy completely sterilized females of *T. absoluta* (Cagnotti et al. 2012, 2016, in this special issue).

Fertility levels of female light brown apple moths that had been irradiated as pupae with doses increasing up to 300 Gy, declined at a greater rate than those of similarly irradiated males. Male moths irradiated with 300 Gy had still a residual fertility of 2.5 to 5%. Males irradiated with 150 Gy and mated to untreated virgin females produced few—but highly sterile— $F_1$  offspring (Soopaya et al. 2011). Irradiating adult light brown apple moth males with either 100 or 200 Gy and mating them to non-irradiated females resulted in 54.1% and 8.8% egg hatch, respectively, and 2.3% and 1.9% egg hatch when males were irradiated with 250 and 300 Gy, respectively. Irradiating females with either 100 or 200 Gy and mating them to non-irradiated males resulted in egg hatch of 16.1% and  $< 0.3\%$ , respectively, and 0.1% and 0.0% egg hatch when females were irradiated with either 250 or 300 Gy, respectively. No female progeny from any of these treatments survived into the  $F_1$  generation. Egg hatch when  $F_1$  males were mated to non-irradiated females averaged 1.0% (Jang et al. 2012).

Adult males of the European grapevine moth, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), irradiated with 400 Gy and mated with non-irradiated females retained a residual fertility of 2.7%. In contrast a dose of 150 Gy was enough to completely sterilize females (Saour 2014).

Rearing codling moths through diapause improved their competitiveness in orchards, but their radio-sensitivity was similar to that of moths reared under non-diapause conditions (Carpenter et al. 2010).

There was no significant difference in the mating frequencies of female cactus moths when caged with either non-irradiated or with 200 Gy-irradiated males. No significant difference was found in the mating abilities of either non-irradiated or irradiated male cactus moths (Marti & Carpenter 2009).

Manipulations of atmospheric oxygen content have been shown to be an effective way of lowering post-irradiation somatic damage while preserving sterility and improving sterile insect performance. Irradiation induces undesirable side effects that can reduce the quality and performance of released sterile male insects. An exposure of 1 h to anoxia of cactus moths before irradiation increased their anti-oxidative capacity, and irradiation in anoxia after 1 h of anoxia preconditioning decreased radiation induced oxidative damage to lipids and proteins. Anoxia treatment that reduced oxidative damage after irradiation also produced moths with greater flight performance, mating success, and longevity, while maintaining  $F_1$  male sterility at acceptable levels for SIT/IS (Lopez-Martinez et al. 2014).

The effects of anoxia treatment on the irradiation-dose-responses of adults of the cactus moth with respect to sterility, longevity, and  $F_1$  performance were further assessed by López-Martínez et al. (2016, in this special issue). Anoxic conditions prior to and during irradiation with intermediate doses substantially rescued the fertility of females from the induction of dominant lethal mutations, but had no such ameliorating effect on the reduced levels of fertility induced in irradiated males; and the fertility levels of males were always greater than those of females at a given dose. The anoxia treatment had a strong effect in lowering the mortality rate of irradiated cactus moth males, and it extended their lifespan at each dose.

The genetics of the tomato leaf miner and the impacts of x-irradiation on some cytological traits were investigated by Carabajal Paladino et al. (2016, in this special issue). Irradiation of pupae with 200 Gy generated translocations, chromosome fragments, chromo-

some fusions and, hence, altered chromosome numbers, whereas the apyrene:eupyrene sperm ratio was not altered by irradiation with 100–250 Gy. But males irradiated with 300 Gy produced significantly more apyrene sperm than non-irradiated males. It was suggested that the modified eupyrene sperm bundles of irradiated males could be used as a bio-indicator for monitoring a program with an IS component after the release of irradiated males.

Seth et al. (2016a, in this special issue) considered sperm behavior in irradiated Oriental leaf worm, *Spodoptera litura* (F) (Lepidoptera: Noctuidae) males to be an important attribute to assure effective suppression of target populations, and they supported this view by making very detailed quantitative and qualitative assessments of sperm behavior—production, descent, activation, movement and transfer to females—in parental male moths that had received sub-sterilizing irradiation doses (either 100 or 130 Gy) and in  $F_1$  generation males.

### Mating Compatibility and Competitiveness

AW-IPM programs that have a SIT/IS component can only be successful if the released males are of good biological quality (Vreysen & Robinson 2011). In addition, it is important to establish that there are no mating barriers between the strain used for release and wild population in the target area (Cayol et al. 1999; Vera et al. 2006; Taret et al. 2010), and that the released males are able to successfully compete with wild males for wild females (Vreysen 2005). Mating studies in field cage settings have shown the complete absence of mating barriers between codling moth populations from very diverse regions of the world, i.e., Argentina, Armenia, Canada, Chile, New Zealand, Syria, and Switzerland (Taret et al. 2010). These findings were confirmed by a study of interactions between codling moths from Canada and South Africa. Male moths of both origins were equally attracted to calling females from Canada and South Africa, both in laboratory cages and in open field studies, which served to indicate that codling moths from Canada were compatible with codling moths established in South Africa (Bloem et al. 2010). A further study showed that long distance shipments of codling moth pupae and adults had little effect on moth emergence, longevity and ability to mate, as assessed in the laboratory (Blomefield et al. 2011).

Mating competitiveness and compatibility of non-irradiated and irradiated *E. saccharina* under laboratory and semi-field conditions were investigated by Mudavanhu et al. (2016, in this special issue). Wild moths did not discriminate against irradiated or laboratory-reared moths, indicating no negative effects due to laboratory rearing or radiation treatment. However, irradiated males mated significantly more times than their wild counterparts regardless of the type of female mate indicating that males of the laboratory colony were still as competitive as their wild counterparts. The mating indices generated showed no evidence of incipient pre-mating isolation barriers or sexual incompatibility with the wild strain.

Competition of irradiated *C. sinensis* males for mates with wild males was studied in field cages by Fu et al. (2016, in this special issue). By processing these data with the formula of Fried (1971), the authors derived a mean competitiveness  $C$  value of 0.48, when 250 Gy-treated male *C. sinensis* were competing with similar numbers of untreated males for untreated females, indicating that a sub-sterilizing dose of 250 Gy would be adequate for programs that include an IS component.

Release ratios must be sufficiently great to cause a downward trend in the growth of the target population. When released sterile insects are fully competitive with the wild insects in the targeted zone, Knipling's models, which were rough approximations, assumed that the wild population was increasing at the rate of 5-fold per generation ( $\lambda = 5$ ) and that a satisfactory rate of decline of the targeted population

would be achieved at an over-flooding ratio of sterile to wild males of 9:1. Barclay (2005) expressed Knipling's relatively simple model as follows:

$$F_{t+1} = \lambda F_t (M_t / (S + M_t))$$

where  $F_t$  is the number of fertile females and  $M_t$  is the number of fertile males at time  $t$ ,  $\lambda$  is the growth rate per generation,  $S$  is the number of sterile males released each generation, and it is assumed that the normal sex ratio is 1:1. Further Barclay (2005) defined the critical release rate as  $S^* = F(\lambda - 1)$ , i.e., the rate of release of sterile males that hold the population at the steady state. If  $S > S^*$  then the population will decline. Whether the population will decline depends not only on the value of  $\lambda$ , but also on the value of  $C$  (competitiveness as defined by the formula of Fried (1971), because if  $C$  is less than 1, then the release ratio must be increased to fully offset the reduction in competitiveness.

Cagnotti et al. (2016, in this special issue) assessed mating competitiveness of irradiated *T. absoluta* males in field cages into which they released the sub-sterile males with non-irradiated females. A release ratio of 10:1 (treated: untreated) caused declines in the production of larvae compared with the untreated control cages, but these differences were not statistically significant. However a release ratio of 15:1 caused significant declines in production of larvae compared with that in the untreated control cages. Apparently the value of  $\lambda$  was greater than that of many other lepidopteran species because the authors concluded that "sub-sterile males were as competitive as untreated males in mating with untreated females under the experimental conditions, and were also successful in producing sterile  $F_1$  progeny that reduced the wild population growth over time." In addition the authors noted that in previous studies "a treated:untreated over-flooding ratio of 5:1 in field cages was effective to suppress *C. cactorum*, the potato tuber moth, *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae), and the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), but a 10:1 ratio was required to suppress *C. sinensis*, and a 20:1 ratio was required to suppress *P. gossypiella*."

The relationship of radiation dose and mating frequency of the light brown apple moth was found to be significantly negative (Stringer et al. 2013). In addition, the production of the sex pheromone by the females declined significantly with increasing doses of radiation. Male catch in traps baited with females irradiated with 300 Gy was reduced to 11% of that in traps baited with non-irradiated control females. Isotope analysis of spermatophores found in the bursae copulatrices of females indicated that mating success of irradiated males inside the live (entry-only) traps containing virgin females was less than suggested by the male catch in pheromone-baited traps. Impacts of irradiation on the quality of male and female moths should be taken into account to improve estimates of irradiated to wild male *E. postvittana* over-flooding ratios needed for population suppression (Stringer et al. 2013).

### Flight Ability of Sterile Insects

Released sterile insects need to be sufficiently mobile and have adequate dispersal capabilities to reach—in a timely fashion—all the ecological niches that are occupied by the wild insects. Knowledge about the mobility and dispersal characteristics of released insects is essential for developing and designing appropriate release strategies (Vreysen 2005). Anemotaxis theory describes moth behavior in the presence of a pheromone plume and includes 3 components: upwind, zigzagging, and sideways casting behaviors. In series of mark-release-recapture experiments active or passive downwind dispersal movements were found to be appetitive behaviors, occurring prior to the

elicitation of pheromone-oriented flight patterns (pheromone anemotaxis). Given the potential for significant displacement during downwind dispersal, this behavioral component is likely to have an impact on final trap captures, and should be considered when constructing moth dispersal models (Guichard et al. 2010).

In head-to-head comparisons of flights of irradiated light brown apple moth males and non-irradiated males toward a pheromone lure in a wind tunnel, irradiated males reached the lure first only 31% of the time (Stringer et al. 2013). Light brown apple moth males were irradiated as pupae at intervals up to a dose of 300 Gy, and their flight success was assessed in a wind tunnel equipped with flight track recording software. A radiation-dose response was evident with reduced successful search behaviors at the higher irradiation doses. Irradiation with 250 Gy reduced arrival success to 49% of untreated controls during 2-min assays. Mark-release-recapture of males irradiated with 250 Gy indicated reduced male moth recapture in hedgerows (75% of control value recaptured) and in vineyards (78% of control value recaptured). Males dispersed similar distances in both habitats, and over-flooding ratios dropped off rapidly from the release point in both landscapes. Relative field performance of male moths was greater than suggested by wind tunnel data, which could be due to time differences between the 2 assays, i.e. wind tunnel tests that lasted 2 min compared with d in the field treatments (Suckling et al. 2011).

A flight assessment cage proved to be a valuable tool for measuring differences in the quality of untreated and irradiated *L. botrana* moths (Saour 2016, in this special issue). A radiation dose of 350 Gy (but not 150 Gy) significantly lowered the flight responses of males to calling females in the laboratory flight assessment cage. Similarly, in a field release, the performance of 150 Gy-irradiated males and untreated males were similar, whereas the dispersal of 350-Gy irradiated males was reduced.

Using a laboratory flight mill Zhang et al. (2016, in this special issue) found that flight distance, flight duration and speed of irradiated (either 150 or 200 Gy) and untreated males of the litchi stem-end borer were not significantly different. This was confirmed in field release and recapture experiments, where recapture rates, dispersal distances, and dispersal directions of 150 and 200 Gy-irradiated males were not significantly different from those of non-irradiated males. Also in a study on the Oriental leafworm, Seth et al. (2016b, in this special issue) found that orientation of parental generation males irradiated with either 100 or 130 Gy and that of  $F_1$  males toward pheromone-baited traps were not different from that of non-irradiated males.

A study on sperm use patterns in Oriental leafworm males revealed the precedence of sperm of the last male to mate; also their mating success, remating propensity and fertility were significantly influenced by mating sequences that included irradiated males (Seth et al. 2016b, in this special issue). Thus the age of the female at her first mating with an  $F_1$  male influenced her fertility. Also mating success and remating propensity were reduced in several of the studied sequences of matings involving non-irradiated and irradiated males and  $F_1$  males, with reductions being more apparent with older females.

## **Tools and Methods to Assess Field Performance**

The paucity of currently available information on tools and methods that can be used to link the quality of sterile males to their field performance indicates an urgent need for innovations in this area that can provide the basis for feedback systems to improve those insect quality parameters most required for more effective population suppression. The assessment of field performance requires an adequate array of tools and methods for this purpose. Laboratory, field cage and open

field methods need to be integrated in order to improve our understanding of how to assess and improve insect quality from the factory to the field. Insects showing poor performance in the field are likely to exhibit relevant characteristics in laboratory or semi-field assays, which are much less expensive than assessments in the field. Understanding the various expressions of moth performance in a hierarchy of linked assays such as flight ability tests, wind tunnel flight performance tests, mating cage tests, field cage tests, and release-recapture tests might lead to the routine operational use of appropriate assays to manage the quality of released moths (Simmons et al. 2010).

## **MAJOR FINDINGS**

### **Laboratory Bioassays, Field Cages and Open Field Studies**

Simple bioassays that can be carried out in the laboratory and that prove to be good surrogates for expensive and laborious field studies would be very cost effective for assuring superior field performance of sterile insects. Brown et al. (2016, in this special issue) described a commercially available insect locomotion activity meter for quality assessment of mass-reared sterile male moths. This device housed sexually mature wild and sterile light brown apple moth males that were stimulated with repeatable pheromone pulses. The results indicated similar baseline activity in clean airflow, but a significantly greater response after pheromone stimulation of non-irradiated males than with irradiated males. A high-temperature shock did not change the response of the non-irradiated moths, but it slightly diminished the response by irradiated moths. The system showed potential for the routine quality assessment of mass-reared moths and might be suitable for factory scale quality assurance (Brown et al. 2016, in this special issue).

The suitability of simple flight cylinders to assess differences in various batches of the quality of mass-produced moths was investigated by Carpenter et al. (2012). Cylinder diam and ht and the duration (h) after the test had been initiated influenced the numbers of male and female moths leaving the cylinder. The data showed that this simple flight cylinder bioassay was adequate in detecting differences in coding moth quality induced by various treatments whose influence could also be detected by more complex laboratory essays and field trials (Carpenter et al. 2012). Laboratory bioassays (flight ability and mating) and field bioassays (field cage and open field releases) were carried out simultaneously to assess the abilities of these various bioassays to measure or predict quality and field performance of mass-reared coding moths. Moth quality was found to have been degraded by irradiation. The laboratory flight bioassay and the field cage bioassay clearly detected quality and performance differences that were relevant to moth performance in the field. However the field cage bioassay was the better predictor of the daily performance of male moths released in the orchard than the laboratory bioassays (Carpenter et al. 2013).

Recent developments in machine vision to record and analyze insect behavior would offer the opportunity to quantify important quality factors such as flight ability, mating propensity and competitiveness. The flight tracks of irradiated Australian painted apple moth males in a wind tunnel were different from those of untreated moths, although both arrived at the calling females. This approach is likely to be amenable to automation, and could potentially be used in routine quality assurance (Suckling et al. 2011)

Standard field cage tests routinely used to measure the sexual competitiveness of factory-reared tephritid fruit flies were validated with the light brown apple moth by Woods et al. (2016, in this special issue). Moths irradiated with either 200, 250 or 300 Gy were similarly competitive in the field cage when competitiveness was expressed as the Relative Sterility Index, but when a Fried competitiveness test was used to generate competitive C values then slightly greater competitiveness



appeared to occur with the lower doses of irradiation. Modified test procedures were developed in which the moths in field cages—after having had sufficient opportunity to mate—were egged individually and dissected to determine the presence of 1 or more spermatophores. Results indicated that sterile-male-only releases have the potential to increase mating competitiveness of the released irradiated moths, but this conclusion requires additional experiments for confirmation.

### Sperm Differences between $F_1$ and Wild Male Moths

During an AW-IPM program that has a SIT/IS component against a lepidopteran pest it is possible that traps used to monitor wild moth populations and to determine over-flooding ratios capture unmarked  $F_1$  sterile males that cannot be distinguished from wild fertile males. A cytological technique based on orcein and Giemsa stains was found to distinguish adult  $F_1$  progeny of irradiated males and fertile males of 6 pest species in 5 families of Lepidoptera. The nuclei cluster in each of the eupyrene sperm bundles in the fertile males showed a regular and organized arrangement of the sperm and was homogeneously stained, whereas the nuclei clusters of sperm bundles of  $F_1$  males were disorganized, irregular and unevenly stained (Carpenter et al. 2009). A similar study with the Australian painted apple moth showed the feasibility to distinguish between the homogeneous nuclei clusters of eupyrene bundles of the normal fertile males and the heterogeneously stained nuclei clusters of  $F_1$  progeny. However the percentage of positive staining nuclei clusters of sperm was strongly correlated with duration of male survival, which was < 5 d. Moths that had spent 24 h on a sticky base in a monitoring trap were equivalent to freshly killed specimens, but the efficacy of the technique decreased with moths glued to sticky trap bases for longer periods (Wee et al. 2011).

### Trapping Systems

Plant chemical signals are important olfactory cues for the survival and reproduction of phytophagous insects. Wee et al. (2016b, this special issue) conducted a scanning electron microscopy study on the morphology and distribution of antennal sensilla of males and females of the diamondback moth. Seven morphological types of sensilla were identified of which 1 particular type was present in males only. The presence of numerous pores or deep longitudinal grooves on the surface of 5 morphological types of sensilla indicated an olfactory function. Single sensillum recordings, carried out on the trichoid sensilla of females showed that each sensillum contained 3 co-compartmentalized olfactory receptor neurons (ORNs). Each ORN class showed a narrow response spectrum, with some ORNs specialized for green leaf volatiles and ( $\pm$ )-linalool that are present in brassicaceous hosts, while several other ORNs responded to 2 non-host volatile sesquiterpenes, (*E*)- $\beta$ -farnesene and germacrene D, as well as (*E*)- $\beta$ -caryophyllene, a host-related sesquiterpene volatile.

Female moth trapping systems would offer considerable benefits to operational AW-IPM programs that have a SIT/IS component by enabling the monitoring of female moth populations in the field, their mating status, quality, and release distribution (Vreysen 2005). Female attractants are now routinely used for monitoring populations of some species (e.g., codling moth) (Light et al. 2001) and have improved the prospects for this application in the SIT/IS. Pear ester, acetic acid, and N-butyl sulfide are chemicals that attract or enhance attractiveness of codling moths. In apple orchards, acetic acid was attractive in both of 2 tests and N-butyl sulfide was attractive in 1 of 2 tests. N-Butyl sulfide increased catches of codling moth when used together with acetic acid. Moreover N-butyl sulfide increased catches of both male and female codling moths when used in combination with acetic acid and pear es-

ter. These results provide a new 3-component lure comprising N-butyl sulfide, acetic acid, and pear ester that was stronger for luring codling moth females than other attractants tested (Landolt et al. 2014).

The mating status of diamondback moth females was shown to have little effect on the female responses to host odor and flight duration in a wind tunnel, but female moths were significantly more attracted to conspecific larvae-infested cabbage, *Brassica oleracea* L. (capitata group; Brassicales: Brassicaceae) plants and had significantly shorter flights than in fields with intact uninfested cabbage hosts (Wee 2016a, this special issue). Female moths oviposited significantly more eggs on larvae-infested cabbage than on intact uninfested cabbage. The data indicates the potential of developing a brassica host-derived kairomone attractant that may target both virgin and mated *P. xylo-stella* females and be used as a monitoring tool for them.

Smart traps—wireless enabled sampling devices that automatically record the insects sampled and provide feed-back using appropriate software systems—were investigated for bio-security surveillance in several countries, including Australia, New Zealand and USA. The development and application of these systems for sterile insect programs would lead to more cost effective and efficient field surveillance. Real time feedback on released insects could lead to rapid and adaptive changes to improve their quality (Simmons et al. 2010). A new technology for the remote detection of insect catch was developed and tested. A multi funnel trap was baited with specific or generic baits, and equipped with a modified security camera that was connected with an internal modem for General Packet Radio Service. Images taken were stored on a memory card. Images were adequate to identify large insects such as the long horn beetles, and might be adapted for moths (Chinellato et al. 2013). Simultaneous trapping for multiple species, by baiting traps with several lures can improve cost efficiency without affecting surveillance coverage. Traps deployed in central and southern Europe with single or multiple lures provided no evidence of interspecific repellency (Brockerhoff et al. 2012).

### Insect Marking Techniques

Marking released insects is an important auxiliary component of the SIT, as it enables the assessment of program progress (Vreysen 2005). Incorporation of dyes into insect diets is a valuable approach, but may be toxic or affect behavior, whereas external dyes, such as fluorescent powders, are useful and simple to apply but they can diminish the responses of males to pheromone sources (Calkins & Parker 2005). In addition, marking insects with dyes is not 100% reliable and any misdiagnosis of trapped insects can have very costly consequences.

A genetically engineered strain of pink bollworm was developed with a heritable fluorescent marker, to improve discrimination of sterile from wild moths. Field trials showed, that attributes critical to SIT in the field—ability to find a mate and to initiate copulation, as well as dispersal and persistence in the release area—were comparable between the genetically engineered strain and a standard strain. These represent the first open-field experiments with a genetically engineered insect (Simmons et al. 2011).

Measurement of natural stable isotope signatures of insects has proven to be a useful method in determining their natal origin, feeding strategies and mating behavior, and has spawned a new discipline of insect isotope forensics. The technique has proven its usefulness with several insect groups. Larvae of the common house mosquito, *Culex pipiens* L. (Diptera: Culicidae) were labeled by enriching the water in which the larvae developed with  $^{15}\text{N}$ -labeled potassium nitrate and  $^{13}\text{C}$ -labeled glucose, which allowed the differentiation of marked from unmarked adult mosquitoes throughout their entire lifespans, e.g., for 55 d. The marking did not affect immature mosquito survival or

adult body size, and the technique was also applied to mark naturally breeding mosquitoes in standing water bodies in the field (Hamer et al. 2012).

The difference in isotopic signatures between wild and mass-reared released Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), proved to be a reliable and intrinsic secondary marker to complement existing marking methods. Mass-reared Mediterranean fruit flies fed on a larval diet containing C4 sugar could be distinguished with > 95% confidence from wild flies that had developed mostly within the fruits of C3 plants. The C4 marker was detectable up to 12 d after release (Hood-Nowotny et al. 2009). The technique also worked with the tsetse fly, *Glossina pallidipes* (Austen) (Diptera: Glossinidae) and distinct differences in isotopic signature were detected between wild and laboratory-reared flies. In addition, these flies could be isotopically labeled by enriching their diet, and they remained distinguishable from wild flies for more than 80 d (Hood-Nowotny et al. 2011).

The stable-isotopic-labeling of lepidopterans was accomplished by Hood-Nowotny et al. (2016a, in this special issue). They showed that mass-reared moths of 7 species—*C. cactorum*, *E. saccharina*, *S. litura*, *E. postvittana*, *P. xylostella*, *L. botrana*, and *P. gossypiella*, in various Families—could be distinguished from wild conspecific moths based primarily on the difference in the ratio of the stable isotopes  $^{13}\text{C}:^{12}\text{C}$  in sugar derived from either sugar beet, *Beta vulgaris* L. (Caryophyllales: Amaranthaceae) or other  $\text{C}_3$  plant species and in sugar derived from either sugarcane or other  $\text{C}_4$  plant species. Depending on whether the wild host plants had  $\text{C}_3$  or  $\text{C}_4$  metabolism, the diets were prepared with sugar derived either from sugarcane or sugar beet, so that the moth reared on the meridic diet had a ratio different from wild moths. However, the standard method of measuring isotope signatures uses elemental analysis-isotope ratio mass-spectrometry (EA-IRMS), which is so costly that it has hindered the advancement and wide spread adoption of this approach. Hood-Nowotny et al. (2016b, in this special issue) took advantage of the development of a simpler technology based on laser spectroscopy that is much cheaper and more convenient than EA-IRMS. Data obtained with combustion module-cavity ring-down spectroscopy (CM-CRDS) were in good agreement with those obtained using EA-IRMS in terms of accuracy and precision.

## Quality Control in Relation to Rearing and Handling of Moths

The quality and cost of mass-reared insects are the 2 most important factors that limit expansion of existing programs and the initiation of new AW-IPM programs with a SIT/IS component. Much effort on rearing systems is invested in measuring quality control parameters but there is little relation between these laboratory measures and actual field performance. Insect colonies can exhibit the loss of desirable traits during the colony establishment phase and subsequently over time. The processes of selection under laboratory conditions are understood at a theoretical level, but have not been widely implemented in mass-rearing of moths. Furthermore, efforts have been made to understand the impact of factors such as diapause and fluctuating temperatures on moth quality. However, the quantification of such factors on the effectiveness of sterile insects to achieve population suppression has not been widely achieved. Improvements in this area would be of great benefit. This could involve developing new and improved methods for enhancing rearing systems, facilitating the selection for performance or fitness traits that improve colony establishment, refurbishment and production, as well as the field performance of released moths.

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A modified larval diet was developed for the mahogany shoot borer, *Hypsipyla grandella* (Zeller) (Lepidoptera: Pyralidae) that replaced expensive components with cheaper equivalents. The use of well plates containing the modified diet allowed the rearing of larvae from 1st to 3rd instars without the need for fresh diet material. Mating and oviposition were accomplished inside the insectary with air flow directed through the rearing cages. This is the first report of rearing *H. grandella* successfully under artificial conditions for 7 generations (Barradas-Juanz et al. 2016, in this special issue).

Some codling moth colonies have been reared for decades on the diet of Guennelon et al. (1981), which includes maize meal as a major ingredient, making up 59% of the diet. During the past decade maize hybrids in South Africa have been replaced with genetically modified maize hybrids that contain the insecticidal gene from the soil bacterium *Bacillus thuringiensis* (*Bt*). Stenekamp et al. (2016, in this special issue) showed that the use of maize meal made from *Bt* maize as a component of the larval diet of the codling moth was causing larval mortality, delayed larval development and larvae leaving the diet prematurely. Suitable replacements for *Bt* maize meal could include non-*Bt* maize meal, whole wheat flour or soybean flour provided that they can be procured at affordable prices.

Genetic sexing strains or sex separating systems are lacking for most Lepidoptera and as a result, both irradiated males and females are released in programs that have a SIT/IS component (Marec et al. 2007). This however, may encourage assortative mating amongst the released moths, thereby reducing the competitive capability of the irradiated males. Among 4 methods tested to separate male from female *L. botrana*, size of pupae was the most effective method that would enable the release of irradiated moths that are highly male-biased, with only a small fraction of females (Steinitz et al. 2016, in this special issue).

Meridic diets that did not contain any host plant materials were assessed with respect to the development and survival of 3 strains of cactus moth. Although the cactus moth is an oligophage within the genus *Opuntia*, it accepted and developed on several diets containing non-host plant ingredients, yeast, and fish meal. The best performance of the moths was on diets that contained white kidney bean, *Phaseolus vulgaris* L. (Fabales: Fabaceae), brewer's yeast, wheat germ and/or soybean (Carpenter & Hight 2012). Laboratory and field bioassays were carried out with sterile males of the cactus moth that originated from 2 insectaries, 1 in Georgia, USA and the other in Florida. Bioassays and assessments included moth mass, moth longevity, percentage of female moths mated at time of collection from the insectary, percentage of female moths mated 24 h after collection, flight ability, percentage recapture after release in the field, and mean distance dispersed from the release site. Data from the quality assessments of the 2 insectaries were compared and used to make protocol changes in both rearing and handling that improved sterile moth quality and performance (Hight & Carpenter 2016, in this special issue).

In several Lepidoptera programs that incorporate the SIT/IS, cold temperature is routinely used to immobilize moths to increase the density of moths to facilitate their collection, holding, irradiation, transport and release (Bloem et al. 2001). However cold temperatures or rapid chilling and prolonged storage may negatively impact field performance of some insectary-reared insects (Terblanche et al. 2008).

*Thaumatiobia leucotreta* moths collected in the insectary air stream and rapidly chilled were less likely to be captured in pheromone traps and less likely to disperse great distances following release in citrus orchards as compared with moths that were held at room temperature (Boersma & Carpenter 2016, in this special issue).

## Future Challenges

It is commonly accepted that the management of key insect pests is ideally based on the concept of AW-IPM (Klassen 2005), and the use of the SIT/IS has played and will continue to play a critical role in these campaigns against key lepidopteran pests. The availability of a wide range of efficient control tactics against lepidopteran pest species is becoming all the more important in view of their dramatic geographic range expansions especially in recent decades (Kean et al. 2016). Significant improvements were made during the CRP presented in this review paper and special issue with respect to identification of factors that affect field performance of sterile male moths, the development of methods and tools to assess field performance and quality control in relation to rearing and handling of moths. The CRP, however, also identified some gaps with respect to key factors and variables in the rearing and release processes that may affect the quality of the moths and their field performance that need further research and development.

Factors that need to be tied to the field performance of released insects, include: (1) improved rearing and maintenance of colonies based on selection and preservation of desirable traits supporting competitive field performance, (2) better collection and irradiation methods, (3) a better understanding of the efficacy of male-only releases compared with bi-sex releases (both sexes together vs. males in one area and females in another), (4) improved handling, transport and release methods, (5) practical and effective methods for field quality assessment, and (6) improved strategies to deploy sterile insects over the landscape of the target area to improve cost-effectiveness and outcomes. These issues will be addressed in a follow-up CRP that started in 2016.

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