CHAPTER 8.1.

PROSPECTS FOR THE FUTURE DEVELOPMENT AND APPLICATION OF THE STERILE INSECT TECHNIQUE

J. HENDRICHS¹ AND A. S. ROBINSON²

¹*Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture,
International Atomic Energy Agency, A-1400 Vienna, Austria
Email: jorgehendrichs@gmail.com*

²*Liverpool L21 7LA, United Kingdom*

TABLE OF CONTENTS

1. INTRODUCTION.....	1120
2. UTILIZATION OF THE SIT TECHNOLOGY	1123
2.1. Commercialization	1123
2.2. Non-Native Pest Incursions.....	1124
2.3. SIT Application to Environmental Problems.....	1125
2.4. New Target Species.....	1126
2.5. Integration with Other Measures	1129
2.6. Regulatory Issues.....	1130
2.7. Resistance to the SIT.....	1131
3. TECHNICAL IMPROVEMENTS	1132
3.1. Improving Insect Quality.....	1132
3.1.1. Colony Initiation and Maintenance.....	1133
3.1.2. Strain Replacement Procedures and the Filter Rearing System (FRS).....	1135
3.1.3. Larval Diets	1136
3.1.4. Post-Factory Sterile Male Performance.....	1137
3.1.5. Sterile Males as Carriers of Bioactive Materials	1138
3.1.6. Hybrid Vigour.....	1140
3.1.7. Radiation Dose	1140
3.2. Improving Technology	1141
3.2.1. Shelf-Life and Shipment	1141
3.2.2. Rearing Systems	1142
3.2.3. Sterilization.....	1142
3.2.4. Release Technology.....	1143
3.2.5. Field Monitoring.....	1144
3.2.6. Suppression.....	1145

Pages 1119–1170

V. A. Dyck, J. Hendrichs and A. S. Robinson (eds.), Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management. Second Edition.

© 2021 IAEA. CRC Press, Boca Raton, Florida, USA.

4. TRANSGENESIS AND GENE EDITING	1147
4.1. Genetic Sexing	1147
4.2. Marking	1148
4.3. Sterilization.....	1149
4.4. Paratransgenesis	1150
4.5 Technical and Regulatory Constraints.....	1151
5. CONCLUSIONS.....	1152
6. REFERENCES	1154

SUMMARY

Science-based modern agriculture and international trade in agricultural commodities have achieved that, even though the world population has doubled in the last 40 years, the absolute number of people in poverty and hunger has been falling steadily. The major challenge in the immediate future is to consolidate these positive gains, while simultaneously expanding sustainability through environment-friendly agricultural and public-health practices. Within this context, the sterile insect technique (SIT), as part of area-wide integrated pest management (AW-IPM) programmes, will continue to gain momentum for application against certain key insect pests. This is in response to the demands for cleaner food and a better environment, the need to facilitate increasing international trade by overcoming pest related trade barriers to the movement of agricultural commodities, and the imperative of dealing with the increasing invasion of invasive pests and disease vectors. As the use of the technology increases, changes will continue to be made to improve the overall efficiency of the technique for those species where the SIT is already being used, and to expand the use of the technique to new key species. Modern biotechnology and paratransgenesis will increasingly also contribute to improving SIT efficiency in terms of strain marking, genetic sexing, molecular sterilization and disease refractoriness. However, whereas the shipment and use of *Wolbachia*-infected male insects as part of combined SIT/IIT (Incompatible Insect Technique) strategies for disease vectors is making considerable progress, public opposition and regulatory hurdles have largely limited to a few countries the release of transgenic insects. There appears to be much promise in improving sterile male performance by exposing male insects to hormonal, nutritional, microbial, and semiochemical supplements. Furthermore, the management of mother colonies will be significantly improved to reduce the effects of colonization and to slow down mass-rearing effects on key behavioural parameters that often result in rapid colony deterioration. Progress will also need to be made in the cost-effectiveness of all components of SIT implementation, from cage design to facility design, and from programme planning to evaluation. The trend of increasingly using sterile insects for routine pest suppression rather than eradication, particularly in commercially important commodities, will favour the involvement of the private sector and hence accelerate these improvements. Commercial producers of beneficial insects will probably be the natural investors, in view of the complementarities with sterile insects, experience in managing living organisms, and understanding the biological control market. As programme implementation is logistically complex, management will remain the key issue determining the success or failure of any area-wide approach to insect control. Thus, in spite of the many successes achieved and to be expected, in many least-developed countries the SIT may be a technology that is “ahead of its time” and beyond the animal and public health, as well plant protection infrastructures. Failures in SIT application, mostly confined to such countries, have not been due to science but to the poor management of large-scale operations. Increased involvement of the private sector in such countries probably would assure effective implementation.

1. INTRODUCTION

It is likely that the use of sterile insects in area-wide integrated pest management (AW-IPM) programmes will continue growing as increasing constraints are placed on the use of chemical pesticides responding to the public’s growing demands for a cleaner environment and residue-free food. Global food chains increasingly link agricultural production with powerful supermarket chains that compete to offer their customers higher quality food in terms of maximum residue levels of insecticides. Even without these constraints, resistance problems and the cost of development and

regulatory approval of new insecticides will continue to increase. Added to this, more species-specific methods to control key pests, such as mating disruption or sterile insects, will play a more important role to complement the increasing use of biological control agents, which are often disrupted by insecticide applications. Moreover, as a result of the global spread of mosquitoes and other vectors, vector-borne and zoonotic diseases are on the rise (Higgs 2018). In the absence of vaccines and efficient, safe and inexpensive drugs to combat the emerging diseases they transmit, interest in the genetic control of public health pests is undergoing a revival (Lees et al., this volume). Furthermore, the international standards that regulate global agricultural trade, including measures for pest management where these affect trade (under the World Trade Organization's (WTO) Agreement on Sanitary and Phytosanitary Standards (SPS), are having a major impact on agricultural production and trade, and this is creating an environment in which area-wide approaches to the management of insect pests have a comparative advantage (Devorshak 2007; Klassen and Vreysen, this volume).

With this expansion in the use of the technology, the basic components of the sterile insect technique (SIT) — mass-rearing and release of sterile insects — are hardly likely to change. However, changes will continue to be made to improve the overall effectiveness and efficiency of the technique for those species where the SIT is already being used, and research needs will be addressed to expand the use of the technique for new key pest species. For some potential candidate species, technical bottlenecks have already been identified that prevent the development of the technology, e.g. artificial rearing systems or obligate diapause, which often can be addressed by a concerted research effort, whereas other pest species, because of their biology, are not amenable to SIT application (Lance and McInnis, this volume).

Expanding globalization will inevitably lead to a further rise in the invasion of non-native or invasive insect pests into new areas, where they increasingly survive in previously inhospitable areas due to a changing climate. The concept of using the SIT against such pest incursions to preclude the establishment of such invasive populations has proven effective (Hendrichs, Enkerlin et al., this volume) and will probably gain increasing support. This can take the preventive approach of the California model in which sterile Mediterranean fruit flies *Ceratitis capitata* (Wiedemann) are continuously released in areas of high risk of establishment (due to the regular incursions into the region) (Dowell et al. 2000; Enkerlin, this volume). Alternatively, it can take the form of the Australian approach where the technology, expertise, and contingency plans are developed to be able to respond should an introduction of the Old World screwworm *Chrysomya bezziana* (Villeneuve) occur (Tweddle and Mahon 2000). The trend to counter the impact of increasing globalization, by moving plant protection “offshore” for risk mitigation of non-native pest incursions, will open up new opportunities to integrate the SIT into the process of creating pest free or low prevalence areas under a systems approach (FAO 2016a, b, 2017a), from which agricultural products can be safely exported.

Currently there is no interest from pesticide companies to become involved with the SIT technology, probably because it is not impacting on their market to any significant extent. Even with continued expansion, the SIT will remain a niche market. Commercial producers of pollinators and other beneficial insects will probably be the

natural investors, in view of their experience in managing living organisms and their understanding of the biological control market. Such companies aim to offer a complete “biological package”, i.e. the SIT can be used to manage some of the major key pests, while the reduced insecticide applications allow a significant expansion of the sales of parasitoids and predators to control secondary pests. The use of sterile insects for routine pest suppression rather than eradication, thus creating a sustainable demand analogous to the current use of augmentative biological control, will favour the involvement of the private sector (Hendrichs et al. 1995). However, for commercial companies to become more involved in the SIT, some issues including intellectual property rights (IPR) and regulation may have to be addressed (Barnes 2007; Bassi et al. 2007) (although see section 2.6.).

Programmes with the strategic goal of eradication can suffer from the price of success, e.g. maintaining staff and infrastructure in the face of a successful but declining programme. In such cases it is important to have a long-term vision, developing alternative uses of the rearing facility and programmes for other relevant pests. Converting the pink bollworm *Pectinophora gossypiella* (Saunders) mass-rearing facility to navel orangeworm *Amyelois transitella* (Walker) production, and from melon fly *Zeugodacus* (formerly *Bactrocera*) *cucurbitae* (Coquillett) to sweet potato weevils, are examples in the USA and Japan, respectively.

For future expansion of the SIT, a major constraint that will always need to be addressed is the significant upfront cost of constructing a facility. Of course, industrial plants producing and formulating insecticides represent larger investments, and compared with the costs of insecticide development, the costs of SIT development are minimal. Nevertheless, raising funds is not easy, and so far most rearing facilities have been constructed with public funds, and the beneficiaries often pay, partially or fully, for the sterile insects and/or the field operations. (The costs to beneficiaries usually do not include costs for constructing the facility; this is in contrast to the costs of insecticide where the development costs are recovered in the pricing policy of the company). In some situations, true commercialization may be a way out of the dilemma (section 2.1.).

Another constraint that must be overcome at the start of an area-wide programme is to involve, and get the commitment of, all or at least a majority of stakeholders in the programme (Loosjes 2000). Again, this free-rider problem is not faced in a local pesticide situation; an individual farmer can make his/her own decision, and be approached on an individual basis by chemical companies.

Quality control protocols, both for the product, i.e. the sterile insect, and the process, i.e. the procedures used to produce, sterilize, and distribute the sterile insect, will need to be further improved, and in some way standardized (Parker, Vreysen et al., this volume). This has already been done for sterile fruit flies, where an internationally agreed set of protocols is used to monitor the quality of the sterile insect (FAO/IAEA/USDA 2019). It is reasonably straightforward to develop the protocols and to update them regularly. This is a pre-requisite for commercialization. Producers and users require harmonized protocols to measure sterile insect quality, and the increasing transboundary shipment and trade in sterile insects will gradually ensure that all programmes implement them (Enkerlin and Quinlan 2004; Dowell et al., this volume).

Management remains the key issue determining the success or failure of any area-wide approach to insect control (Dyck, Reyes Flores et al., this volume). Since the SIT is logistically complex and management intensive, its implementation requires flexible procedures and non-bureaucratic management structures. In general, the scientists who have been closely involved with developing the technology should not be responsible for programme implementation, as other skills are needed. Also, research activities should not be part of an operational programme; instead, a separate unit (but associated with the programme) is needed for problem-solving and continuous fine-tuning of procedures; technology can always be improved to increase cost-effectiveness. The “secret” is to find a balance between sterile insect quality and operational stability, predictability, and innovation (Rull et al. 2012).

2. UTILIZATION OF THE SIT TECHNOLOGY

2.1. *Commercialization*

Almost all of the field programmes integrating the sit described in this book have been carried out by public-funded organizations, with or without some financial support from the direct beneficiaries. Although area-wide programmes often address a public good, in the long run full reliance on governments may not be sustainable. Furthermore, continued expansion of the technique will be facilitated when commercial enterprises become involved. Even if public funds are used initially to establish a programme, governments, to increase implementation efficiency, subcontract private companies to provide services or operate the whole programme or parts thereof. However, thus far commercialization of the sit has been a difficult concept to promote, for the following reasons: (1) there are no equivalent models through which commercial confidence can be generated, with the possible exception of the growing biological control industry (Steinberg and Cayol 2009), (2) currently there are only some international agreements or regulations for the production and transboundary shipment of sterile insects (but see section 2.6.), (3) only a very few detailed business plans for the production and use of sterile insects have been developed (FAO/IAEA 2008), (4) the initial capital cost of constructing a mass-rearing facility is rather high (although this can be mitigated by following a gradual modular approach, where new rearing modules are added only with expanding sterile insect demand (Tween 1987)), (5) some public-funded programmes have been providing sterile insects at subsidized prices, thus undercutting competition from private sector companies that have to sell at real prices that include the cost of capital (Barnes 2007; Bassi et al. 2007), and (6) the area-wide nature of sit programmes requires at least some degree of organization and coordinated participation of stakeholders. In spite of this list of constraints to the commercialization of sterile insect production, rather paradoxically there is currently no shortage of customers who would purchase sterile insects if they were available. While the use of sterile insects only for eradication of pest populations is normally not an attractive proposition for commercialization because these are time-limited programmes, the permanent use of sterile insects for suppression, containment and prevention programmes (Hendrichs, Vreysen et al., this volume) provides some continuity in the

need for sterile insects, and has already resulted in some private investment (Steinberg and Cayol 2009; Boersma 2021; Venter et al. 2021).

Commercialization of the SIT can involve the delivery of a complete package, or more likely be partitioned into several different components, depending on the type of programme. A commercial company could be responsible for rearing, shipping, emerging, and releasing sterile insects, and charge customers (who would only manage the field monitoring) according to the number of insects released or the area treated. This would be suitable where there are large agricultural areas growing mainly one crop, or where governments contract such turnkey operations to deal with pests of animal or public health importance. In an alternative strategy, a grower cooperative could buy sterile insects from a supplier, manage the monitoring and fly emergence, and subcontract the release activities; the Mediterranean fruit fly programme in Patagonia, Argentina, already follows such an approach. On the other hand, in The Netherlands, onion growers individually contract with a commercial producer of sterile onion maggots for the combined service of monitoring and suppression to guarantee maintaining the pest in their onion fields below a pre-agreed threshold level (Loosjes 2000; Fournier 2014; Everaarts 2016).

Another strategy that lends itself to commercialization is the production of eggs in a large facility for satellite facilities (maybe even in another country) which then only rear, sterilize, and release the insects. This removes the necessity for every programme to manage mother colonies and to maintain a large egg production colony, an expensive and highly skilled operation, especially if genetic sexing strains are being used (Caceres et al. 2004; sections 3.1.1. and 3.1.2.).

2.2. *Non-Native Pest Incursions*

Globalization, increased movement of agricultural products, and changes in climatic conditions will all lead to increases in the spread of pests and in their ability to establish in new locations. This increasing problem of non-native or invasive species incursions and outbreaks is causing much concern; often no remedial actions can be taken when such a pest enters a new area, or no environment-friendly eradication tools are available that are acceptable to the public (Hendrichs, Enkerlin et al., this volume). A good example of how such problems can be addressed is the accidental introduction into Libya in the 1990s of the New World screwworm *Cochliomyia hominivorax* (Coquerel). In the case of this incursion, fortunately a solution was at hand; the technology was already available for this pest, and sterile pupae could be brought from Mexico. For the eradication in 1990–1991, 40 million irradiated pupae pre-packed in boxes for release were air-freighted from Mexico each week in aircraft (especially outfitted for long-distance transport of chicks). The insects were released in Libya, and the large outbreak was successfully eradicated (FAO 1992; Lindquist et al. 1992). Of course, the major drawback to this approach is that a monitoring and rearing/release system must be in place, and the procedures must be implemented rather quickly.

The Australian government has taken this proactive approach and developed offshore a mass-rearing system for the Old World screwworm; should there be an incursion of this devastating pest into the country, the needed expertise and experience

are available (Mahon and Ahmad 2000; Vargas-Terán et al., this volume). The recurrent incursions (through cargo ships) of the Asian gypsy moth *Lymantria dispar* (L.) into British Columbia, Canada, and north-western USA, but also as far as New Zealand, are also potential targets for integrating the SIT with repeated aerial *Bt* sprays, increasing the effectiveness of ongoing efforts to eradicate such outbreaks (Suckling 2003). New Zealand also has contractual arrangements in place with mass-rearing facilities in Australia in the case of early detections of incursions of Mediterranean and Queensland fruit flies *Bactrocera tryoni* (Froggatt) (Horner et al. 2016).

Countries or regions may decide to identify their most important non-native insect threats, and then in advance develop the required pest-specific technology and expertise, as well as the general legal and physical infrastructure, that provide some degree of remedial action (Suckling 2003; Horner et al. 2016). This could include the SIT; it has a unique ability to eradicate incipient outbreaks of non-native insects in an environment-friendly way and with minimum public resistance (Hendrichs, Enkerlin et al., this volume). For example, one of the most feared non-native pests is the false codling moth *Thaumatotibia leucotreta* (Meyrick), still largely confined to the southern part of the African continent. To prepare for any future outbreak, the US government has supported in South Africa the development of the SIT against this major polyphagous pest (Carpenter et al. 2007; Boersma 2021).

2.3. SIT Application to Environmental Problems

Non-native insects, as well as causing problems for humans, their livestock and crops, can have a devastating impact on the environment. A textbook example of successful classical biological control is the deliberate introduction of the cactus moth *Cactoblastis cactorum* (Berg) to Australia to control invasive *Opuntia* cacti. However, after its accidental arrival in North America (where *Opuntia* cacti are endemic), *C. cactorum* has become an invasive pest. It has spread from the Caribbean to the south-eastern USA, attacking endangered cacti. If this pest reaches the American southwest and Mexico, it is expected that it will have a devastating effect on whole ecosystems based on *Opuntia* cacti. In response, radiation biology, mass-rearing, and pheromone studies have been carried out on *C. cactorum*. The aim is to apply SIT/inherited sterility to contain this environmental pest, in its westward spread along the Gulf of Mexico, before it reaches the south-western USA (Zimmermann et al. 2004; Bloem et al. 2007).

Since the SIT has virtually no environmental impact, it is an ideal tool for use in protected areas such as natural parks and biological reserves where other kinds of control are prohibited. One example is the eradication of an outbreak of the cactus moth on the island of Contoy, Mexico, which is a natural park where the mechanical removal of host cacti was not possible (Bello-Rivera et al. 2021). Other examples are some national parks in Africa where conventional tsetse control is no longer acceptable, but sterile insects can be released without any impact on biodiversity (Nagel and Peveling, this volume). Similarly, the SIT plays a special role in the large organic coffee production areas of Central America where suppressing the Mediterranean fruit fly using conventional bait sprays is prohibited.

SIT/inherited sterility also has considerable potential to assess under natural conditions the environmental impact of introducing non-native herbivores as biological control agents of invasive weeds (Hendrichs et al. 2009). There is increased awareness of possible risks of non-target effects (Follett and Duan 2000). To obtain importation and release permits for such species, increasingly stringent host specificity testing is required. Tests carried out under unnatural quarantine conditions often overestimate the host range, leading to rejection of effective candidates. Releasing sterile herbivores can be another risk-management tool for host-range assessment under natural conditions. There can be no leakage of genetic material into the gene pool because there is no native population present, and in the case of inherited sterility, released females are fully sterile. The survival of F₁ larvae under various abiotic and biotic conditions can be assessed, and if non-target species are attacked, releases can be suspended with no risk of permanent establishment.

This concept is being developed in Florida for an herbivore of the Brazilian peppertree *Schinus terebinthifolius* Raddi (Anacardiaceae), a major invasive non-native that is altering native plant communities in many subtropical regions. The South American leaf-rolling moth *Episimus utilis* Zimmerman (Tortricidae) has been imported into Florida for quarantine evaluation, and radiation biology studies have already been conducted to determine the dose that results in full female sterility (Moeri et al. 2009).

2.4. New Target Species

The biological criteria, to be considered before embarking on an AW-IPM programme integrating the SIT for a particular species, are described by Lance and McInnis (this volume), but the external conditions under which a pest population can become a candidate for the SIT are changing continually. For example, problems in the citrus trade between Spain and the USA led to the incorporation of the SIT into the area-wide control of the Mediterranean fruit fly in Valencia's large citrus-growing areas (Juan-Blasco et al. 2014), which until then had been based largely on insecticide bait sprays. The same has been occurring in Morocco's main citrus exporting region in Agadir (Qadda et al. 2017). Therefore, increasing concerns about biosecurity, food safety, and residues will also impact positively on the economics and desirability of using sterile insects.

The rapid adoption and expansion in the area planted to crops expressing *Bt* toxins had been expected to reduce the demand in some candidate species for SIT integration. For example, the use of *Bt*-cotton would remove insect pests of this crop from consideration as potential SIT targets. Nevertheless, even though transgenic cotton is now planted annually on many million ha, there is increasing resistance developing in some of the targeted moth pests because the area-wide compliance of farmers in following the refuge strategy has not always been adequate (Tabashnik et al. 2010). The systematic maintenance of refuges, by planting non-*Bt* plants on parts of their fields, is supposed to delay development of pest resistance by producing susceptible insects to mate with resistant insects. Another disadvantage of this strategy, besides the potential for resistance development, is that populations of the pest need to be maintained so that refuges can fulfil their role (Fig. 1) (Wu 2010).

Figure 1. Use of the SIT together with transgenic cotton expressing the Bt transgene suppresses the growth of the pink bollworm population and facilitates management of resistance to Bt toxin. The pink bollworm feeds only on cotton bolls and does not damage other tissues. (a) Sustainable use of Bt-cotton to control pink bollworm populations is threatened by the emergence of resistance. (b) Although costly, repeated release of sterile pink bollworm moths (red) in vast excess to the number of wild moths (brown) can suppress the growth of pink bollworm populations. (c) Combined use of Bt-cotton and the SIT ensures that the release of fewer sterile moths can suppress the growth of pink bollworm populations while preventing the emergence of resistance to Bt toxin. (Figure from Wu 2010, reproduced with permission.)

An alternative strategy in such situations where refuges may be insufficient to mitigate permanently the development of resistance, and taking advantage of the *Bt*-suppressed pest populations, is to combine the use of the *Bt* crop with the simultaneous release of sterile insects so that they mate with, and suppress the reproduction of, the resistant insects (Fig. 1) (Tabashnik et al. 2010; Wu 2010).

This synergistic approach was successfully applied against the low pink bollworm populations (resulting from the extensive growing of *Bt*-cotton) and motivated the upgrading of the existing containment programme to an eradication programme (Henneberry 2007). The application of the complementary SIT, mating disruption, and transgenic technologies, requiring a less extensive release of sterile moths, succeeded in eradicating all populations of this pest from the south-western USA and northern Mexico, while also eliminating insecticide sprays against this key invasive pest (Staten and Walters 2021; Simmons et al., this volume). Therefore, while area-wide application of the SIT can help delay the establishment of resistance in a pest population exposed to *Bt* crops, and allows growers to avoid planting non-*Bt* refuges and the related damage, the area-wide expression of the *Bt* toxin without refuges suppresses drastically the pest population, thereby increasing efficacy and economic feasibility of SIT application (Wu 2010).

Pest lepidopterans constitute a major threat to many agricultural crops, resulting in the use of large amounts of insecticide. Currently there are already several operational programmes being implemented against this group of pests (Simmons et al., this volume), supported by much research that has been conducted in this area (IAEA 2002; Marec et al. 2005; Kean et al. 2007; Vreysen et al. 2010, 2016; Marec et al., this volume). Thus, the number of lepidopteran species, for which SIT technology will be developed, will probably increase, but this will be only gradual since its application is usually more expensive compared with dipteran species, transgenic crops primarily target moth pests, and mating disruption is most effective in lepidopteran pests (Cardé 2021).

Insect vectors of malaria and other human diseases continue to exact a huge toll in human lives and livelihoods, and in the 1970s many SIT pilot projects against vectors were implemented with varying degrees of success, including a very successful field trial in El Salvador (Weidhaas et al. 1974; Klassen et al., this volume). Since that time, many technologies related to SIT development and implementation have changed dramatically, and the use of this technology against important vectors is being re-evaluated. This trend will be accelerating because of the increasing burden of mosquito-borne diseases and the global spread of emerging diseases such as chikungunya, dengue, yellow fever and Zika, growing public awareness and concerns about the impact of chemical control on human health and the environment, the increasing incidences of resistance to insecticides, and recognition by the WHO (World Health Organization) and other major stakeholders of the need for more sustainable, effective and biologically based methods that are complementary to current control (WHO 2017).

The technical problems in suppressing mosquito populations using the release of sterile males are quite formidable, but significant progress is being made in developing the SIT and the combined SIT/IIT (Incompatible Insect Technique) (Zabalou et al. 2004) strategies for important vectors such as *Aedes aegypti* (L.), *Ae.*

albopictus (Skuse), and *Anopheles arabiensis* Patton (Bourtzis and Robinson 2006; Beier et al. 2014; Lees et al. 2015; Bourtzis et al. 2016; Zheng et al. 2019). The problems in developing and implementing the SIT are significantly smaller for *Aedes* than *Anopheles* populations, and a number of mosquito pilot programmes that are ongoing or in preparation are focusing mainly on suppressing *Aedes* populations in urban areas (Bellini et al. 2010; Lees et al., this volume).

2.5. Integration with Other Measures

The SIT is always applied in combination with other methods of pest management; in eradication programmes it is often the final component of an integrated sequential approach (Mangan and Bouyer, this volume). Knipling (1992) suggested that the combinatorial approach involving the simultaneous release of natural enemies together with sterile insects may be extremely efficient in population suppression and eradication. Theoretical modelling (Barclay, this volume) has confirmed the effectiveness of this integration. While sterile insects have their impact on receptive adult females, parasitoids target the immature stages, and pathogens and predators all life stages of the pest. Although there has been limited experimental work to assess the veracity of this prediction, an expansion of this approach can be foreseen.

In Lepidoptera, where the release of partially sterile insects offers greater suppressive potential than the release of fully sterile insects, the synergism of combined augmentation of natural enemies and inherited sterility has been repeatedly demonstrated, with released biological control agents reproducing/feeding/infecting on sterile or substerile offspring (Mannion et al. 1995; Carpenter et al. 1996; Hamm and Carpenter 1997; Bloem et al. 1998; Seth and Barik 2009).

Seeding substerile or sterile supplemental hosts or prey in the field to increase the initial survival of inoculatively released biological control agents, or to increase the early build-up of native biological control agents in advance of pest population build-up, also has potential (Fatima et al. 2009). This approach is currently providing effective management of several species of sugar cane borers in a 40 000-ha area of sugar cane in Pakistan.

Integration of augmentative releases of natural enemies and sterile insects can also increasingly be expected in protected or greenhouse crops, where insecticide use must be avoided so as not to interfere with the release of pollinators and biocontrol agents controlling other pests (Kaspi and Parella 2006, 2008; Steinberg and Cayol 2009; Robinson 2018). Already a number of SIT studies are ongoing to address *Drosophila suzukii* (Matsumura) (Lanouette et al. 2017; Nikolouli et al. 2018) and other greenhouse pest problems, such as whiteflies (Calvitti et al. 2000), leafminers (Cagnotti et al. 2012; Walker 2012; Sultan et al. 2017), and the pepper weevil *Anthonomus eugenii* Cano (Robinson 2018), for which other biological control solutions are not available or sufficient as part of a complete biologically based control package. Also, in terms of integration of mass-production processes, biological control and the SIT can be complementary, reinforcing the applicability and cost-effectiveness of these augmentative technologies (Steinberg and Cayol 2009).

Today the control of mosquitoes relies heavily on the use of *Bti* for the suppression of immatures, and insecticide-treated bednets that target female mosquitoes during

their host-seeking behaviour. These technologies are very compatible with the release of sterile male mosquitoes; they do not seek the host for feeding but rather target the mate-seeking behaviour of female mosquitoes. Similarly, success in New World screwworm eradication programmes requires the simultaneous treatment of wounds with insecticides, which are directed at the immature stages, and the systematic release of sterile insects, which are directed at the adult stage.

There will be increased efforts to achieve field sterilization or inoculation through deploying pathogen- or chemosterilant-baited traps or autodissemination devices (Charmillot et al. 2002; Navarro-Llopis et al. 2011, 2015; Maniania and Ekesi 2013; Bouyer and Lefrançois 2014; Toledo et al. 2017). Such approaches attempt to achieve autosterilization and dissemination through slow-acting compounds (such as insect growth regulators, chitin synthesis inhibitors and pathogens) that are transferred by contact or the ingestion of a bait and spread in the target population through intraspecific interactions. In the case of chemosterilants, this effect can be especially complementary to the release of sterile insects because, unlike wild insects, they are not affected. Nevertheless, the potential for large-scale application is limited to special areas and situations; high-density deployment of such devices is not practical for area-wide implementation, and the environmental impacts on non-target species must be thoroughly assessed.

2.6. Regulatory Issues

Future expansion of the SIT will be facilitated by a regulatory framework to support the commercial production, trade, shipment, and release of sterile insects. Historically, since they are not self-replicating, sterile insects were not considered to be biological control agents, and therefore not included in the regulatory framework provided by the International Plant Protection Convention (IPPC) in the form of International Standard for Phytosanitary Measures (ISPM) Number 3 “Code of Conduct for the Import and Release of Exotic Biological Control Agents” (FAO 1996), which specifically dealt with the import and release of biological control agents. Notwithstanding this lack of a regulatory framework, over the last 60 years there have been significant transboundary shipments of sterile insects, totalling over a trillion (million million) insects (Enkerlin and Quinlan 2004; FAO/IAEA 2018a). This significant international transport of live sterile insect pests testifies to the inherent safety of radiation-induced sterility and its general acceptance by the international plant protection community.

The increasing interest shown by some commercial companies in producing sterile insects emphasized the importance of having a regulatory framework. In response, in 2005, ISPM Number 3 was revised by representatives of national and regional plant protection organizations, resulting in the “Guidelines for the Export, Shipment, Import and Release of Biological Control Agents and Other Beneficial Organisms” (FAO 2017b). In this revised ISPM 3, the definition of biological control agents has been broadened, sterile insects have been specifically included as “beneficials”, and the SIT is now officially accepted internationally as a type of biological control. In addition, the terms “sterile insect” and “sterile insect technique” have been defined for inclusion in the ISPM Glossary of Phytosanitary Terms (FAO 2017c; Klassen and Vreysen, this volume). Thus, the application of sterile insects as part of the integrated

management of plant pests is now recognized by the IPPC, and this should facilitate their transboundary shipment and use, especially in terms of their commercial use. Since this framework only covers those cases where plant pests are involved, the use of the SIT against insects of veterinary or public health importance, that are vectors of disease, will require other regulatory action, possibly through the Office Internationale des Épizooties (OIE) and the WHO. While the shipment and use of sterile and/or *Wolbachia*-infected male mosquitoes as part of the SIT, IIT (EPA 2017), or combined SIT/IIT strategies (Bourtzis et al. 2016; Zheng et al. 2019; Lees et al., this volume) for such vectors are recently making considerable progress, the lack of regulation has limited (largely to a few countries) the transport and release of transgenic male mosquitoes and other insects (Carvalho et al. 2015; Miller and Cochrane 2018).

2.7. *Resistance to the SIT*

The increased use of sterile insects to suppress pest populations may bring with it concerns about the development of resistance (Itô et al. 2003; Lance and McInnis, this volume; Whitten and Mahon, this volume). As suppression programmes by definition are long-term, this will provide opportunity for natural selection to select individuals that can, in some way, differentiate between a released sterile male and a fertile wild male. This may be especially relevant where an island population is targeted on an area-wide basis because it represents an isolated genetic environment (Itô et al., this volume). The danger for resistance to occur is probably more remote when a population is targeted for eradication, although the development of resistance in suppression programmes will be modulated by the occurrence of immigration and refugia from where “susceptible” individuals re-enter the target population.

For resistance to develop and be selected, three conditions must be fulfilled: (1) a recognizable difference or differences between a sterile male and a wild male that a wild female can recognize, (2) a fitness cost to the wild female if she mates with a sterile male, and (3) a genetic basis for the recognition of the sterile male by the wild female. It is not inconceivable that, for certain species, all these conditions could be met. For condition (1), routine application of quality management systems should identify any significant anomalies (Cáceres et al. 2007), allowing correction through the introduction of a new strain and better mother-colony management; for (2), as the fitness of a wild female mating with a sterile male is zero, this condition will always be met in a programme integrating the SIT, and for (3), for most species this is largely unknown. The development of resistance in a target population can be dealt with by re-colonizing a population from the target field site and replacing the original colony. This becomes slightly more problematic when specialized strains, such as genetic sexing strains, are used for release (Robinson et al. 1999). Field cage tests under semi-natural conditions, that continuously monitor the effectiveness of sterile males when competing with wild males to mate with wild females, become a very critical component of all programmes (Hendrichs et al. 2002).

In those programmes where sterile females are also released, tests need to evaluate the ability of wild males to discriminate between sterile females and wild females. If wild males mate only with wild females, but sterile males cannot distinguish and mate with both types of females, modelling indicates that a doubling of the sterile insect

release rate is required to overcome this wild male discrimination against sterile females (Vreysen et al. 2006).

3. TECHNICAL IMPROVEMENTS

3.1. Improving Insect Quality

A critical factor determining the success or failure of a programme that includes an SIT component is the ability of the released sterile males to effectively inseminate wild females with sperm that is competitive with normal sperm (although in some species aspermic sterile males can also inhibit female remating at the same rate as normal matings involving sperm transfer (Itô et al. 1993)). This ability is generally termed “competitiveness”, and is determined by many factors related both to the treatment of a particular cohort of released insects and the developmental history of the mass-reared strain used to produce the cohort for release. Both sets of factors can impact negatively on the competitive ability of released insects, and efforts will continue to be made to minimize these negative effects. As the “production philosophy” still tends to dominate decision-making, even though theoretical models continue to stress the importance of insect quality over the number of insects released (Itô et al., this volume), more efforts to educate decision-makers and managers are needed. A major component of any quality improvement strategy is to have in place a series of quality control protocols that can be used as a baseline to evaluate the effect of any experimental changes on insect quality (Cáceres et al. 2007; FAO/IAEA/USDA 2019; Parker, Vreysen et al., this volume).

Since quality refers only to a field parameter, then it would seem appropriate to develop field-based protocols for its assessment, unless laboratory-based protocols can be identified that correlate directly with field competitiveness (Boller and Chambers 1977). In the past, probably too much attention was focussed on laboratory-based protocols that had very little predictive value in terms of field performance. Quality is relative; it is dependent on the space and complexity of the testing arena. In a restricted space, for example in a small laboratory cage with a reduced distance between individuals, sterile males are often more effective than wild ones, but in larger cages the effectiveness of the same sterile males decreases significantly, and in field cages with vegetation it decreases even further (Soemori et al. 1980; Miyatake and Haraguchi 1996). It is also of little value to develop quality control parameters using only mass-reared insects, especially for species with a mating system in which the females choose among mating partners (Lance and McInnis, this volume). Therefore, the quality of mass-reared insects must increasingly be measured directly under semi-natural or natural conditions and in competition with wild insects (FAO/IAEA/USDA 2019; Parker, Vreysen et al., this volume).

In fruit flies, a standard field-cage system, including host plants, has been developed, where mass-reared sterile insects have to compete with wild insects (Cáceres et al. 2007; FAO/IAEA/USDA 2019). A similar system has also been developed for tsetse flies (Mutika et al. 2001). This type of approach needs to be expanded and refined to include semi-field cage systems that contain a cycling wild population, and in which the competitiveness of sterile insects can be maintained and

assessed. This has already been done for the Mediterranean fruit fly (McInnis et al. 2002) and for mosquitoes (Knols et al. 2002).

The key field parameter to assess the quality of mass-reared sterile males is the amount of sterility induced in wild females; improved protocols need to be developed that provide information on this parameter. In screwworms (collection of egg masses), tsetse flies (aberrations in the reproductive system of dissected wild females), and moths (mating tables), the opportunity to monitor the behaviour and fertility of a field population under challenge from sterile insect releases is a tremendous advantage to routine programme evaluation (Vreysen, this volume). In fruit flies, on the other hand, this is not practical, although molecular tools now allow identifying the origin of sperm in captured wild females as an indicator of the degree of induced sterility in the target population (San Andrés et al. 2007; Juan-Blasco et al. 2013, 2014). In mosquitoes, sugar-feeding of males before release with a rhodamine dye can be used to stain their sperm and seminal fluid that can then be detected in the wild females of the target population (Johnson et al. 2017).

To a limited extent, compensation for low insect quality is possible by releasing a larger number of sterile insects. However, there is a limit to this in terms of the overall economy of a programme, and there is a biological limit in that, below a certain quality value, increasing the number of released insects will simply not be effective (Barclay, this volume; Itô et al., this volume). In future, much more attention should be paid to sterile insect quality as opposed to quantity, irrespective of the cost of producing a specific insect.

The introduction of a colony mass-rearing protocol based on the filter rearing system, currently used to maintain stability in genetic sexing strains (Parker, Mamai et al., this volume), can also make a major contribution to the quality of mass-reared insects (section 3.1.2.).

3.1.1. Colony Initiation and Maintenance

There are virtually no procedures or protocols on how to establish an initial colony of a species for future mass-rearing (McDonald 1976). Questions about the number of insects that should be sampled, where they should be sampled, and when the sampling should be done, remain largely unanswered. Even if guidance could be given on the above points, there is still the well-known phenomenon of a “bottleneck” which occurs during the first one or two generations of laboratory rearing, and which can negate the effects of any science-based approach to field sampling (Cayol 2000a; Rull and Barreda-Landa 2007; Hoffmann and Ross 2018; Parker, Mamai et al., this volume).

The answer to the question, “How many insects should be sampled?” is generally, “As many as possible”. Of course this is not very satisfactory, and the number of insects used is usually influenced by the logistics involved in collecting insects in the field, coupled with the known size of the bottleneck that occurs during initial colonization. If the aim of the initial sampling of the field population is to sample the available genetic variability, then, based on population genetic studies of natural populations, samples of 20–100 insects generally reveal most of the genetic variability present in a field population, indicating that a sample in the hundreds is probably sufficient for most situations, provided that most individuals reproduce in the

laboratory. Then the major task becomes the preservation of that variability during initial colonization and subsequent mass-rearing in the laboratory (Hoffmann and Ross 2018).

From where in its distribution should the field population be sampled? Preferably this should be from the target population, although again logistics tends to determine the specific location of sampling sites and hence the parts of the insect distribution that are sampled. A related question to this concerns the strengths and weaknesses of using a single mass-reared strain for all regions where a particular species is a pest. This is especially important for species that are distributed worldwide, e.g. the codling moth *Cydia pomonella* (L.) and the Mediterranean fruit fly, and even for other species showing very discontinuous distributions. To a point, this can be tested by conducting mating compatibility studies, using the target populations from the different regions and the mass-reared strain (Cayol 2000b; Taret et al. 2010). Even if tests reveal no mating problems between the two populations, still programme managers often request that a particular genetic background be backcrossed into the strain to be used, despite the absence of any evidence that this is beneficial. In biological control circles, there has been much discussion on whether to sample marginal or central populations, but some of the hypotheses proposed to answer this question are, in practice, very difficult to test (Mackauer 1976).

The underlying assumption made during this discussion is that the level of genetic variability, per se, in the mass-reared colony is correlated with the overall quality of an insect once sterilized and released in the field, and hence is important to maintain. Krawfsur and Ouma (this volume) challenge this assumption, and suggest that inbreeding, i.e. reduction of overall variability during colonization and mass-rearing, may not always affect competitiveness in the field. At the moment, probably there are insufficient data to make a general conclusion regarding the correlation of genetic variability in the mass-reared colony with competitiveness of the insects in the field. As suggested by Krawfsur and Ouma, in the future, quality control procedures should be expanded to include routine measurements of genetic diversity in the colony over time, coupled with effective tests to monitor the competitiveness of the insect in the field. In the colonization strategy for the New World screwworm, procedures have been developed that involve the establishment of isofemale lines, followed by their evaluation and subsequent hybridization to establish a strain for mass-rearing (Mangan 1992).

The decision-making procedure, as to when a strain needs to be replaced, has to be improved. Since strain domestication or deterioration is normally correlated with the number of generations under selective mass-rearing conditions, at present programme managers usually establish a time-based replacement schedule. Better tools need to be developed to monitor the quality of released sterile insects in the field. A science-based and data-driven approach to strain initiation and replacement is needed (but see next section). Also, mother-colony maintenance procedures that slow strain deterioration should be developed, thus extending the life of a strain under mass-rearing. These will probably include a combination of the following actions:

- Maintaining founder/back-up strains under cryopreservation to guard against genetic drift and laboratory adaptation (Leopold et al. 2001; Leopold 2007),
- Holding mother colonies under relaxed, preferably semi-natural, rearing conditions that introduce some variation and complexity, and from which the rearing colonies are regularly derived (see filter rearing system below),
- Making simple modifications to the rearing processes and holding conditions of the mass-reared colony that reduce the selective pressures that result in directional changes in some of the key behavioural processes (Miyatake and Haraguchi 1996; Liedo et al. 2007), and
- Implementing an “active” quality control programme that establishes heritabilities and genetic correlations between desirable traits such as mating behaviour and traits that can be counter-selected during routine mass-rearing (Miyatake and Yamagishi 1993; Miyatake et al. 1997; McInnis et al. 2002; Sánchez-Rosario et al. 2017).

3.1.2. *Strain Replacement Procedures and the Filter Rearing System (FRS)*

For all insect species that are mass-reared, the artificial environment of a facility presents the insect with a tremendous challenge, i.e. to adapt to the artificial conditions, both biotic and abiotic. Such conditions impose new selective pressures that are not present in nature (for example, high densities), while selective pressures that are strong in nature are weakened or absent (for example, predation) (Hoffmann and Ross 2018). While holding large colony sizes and refreshing them with new wild insects can help minimize genetic drift, they will generally not ameliorate adaptation (Schneider 2009). Therefore, successful colonization will inevitably result in the often rapid selection for adapted genotypes, and this can impact negatively on the competitiveness of a sterile insect once it is released in the field (Miyatake and Haraguchi 1996; Briceño and Eberhard 2002; Hoffmann and Ross 2018).

Most mass-rearing protocols follow the principle of large cycling colonies, whereby a proportion of the production is used for sterilization and release, while the remainder is returned to maintain the production colony. In this system, over time there is an inevitable accumulation of highly selected genotypes, such as early reproduction, faster development, and simplified courtship behaviour (Briceño and Eberhard 2002), some of which can significantly compromise the quality of the insects released in the field. This fact has prompted most operational programmes to replace their strains regularly, and to establish improved quality control procedures that monitor the overall competitiveness of the mass-reared strain. Strain replacement is a major logistical exercise, and invariably the new strain requires considerable time before it is as productive as the old strain. It is not clear how much of the overall quality of a strain is lost during this adaptation process, and how often a strain needs to be changed. Rull and Barreda-Landa (2007) found that new colonies established by exclusively crossing wild males and laboratory females enabled the restoration of male mating competitiveness without compromising mass-rearing production. Nevertheless, improved methodologies to monitor strain quality are needed.

As discussed by Franz et al. (this volume) and Parker, Mamai et al. (this volume), the use of genetic sexing strains in Mediterranean fruit fly programmes incorporating the SIT required the development of a filter rearing system (FRS) to maintain the

integrity of the sexing strain (Fisher and Caceres 2000; Caceres et al. 2004). The FRS relies on the maintenance of a mother colony that each generation is checked for unwanted individuals (which are then removed). Eggs from this colony are harvested as required, and following 3–4 generations of mass-rearing amplification the males are sterilized and released. In the FRS, no insects that have been through mass-rearing are returned to the mother colony, and therefore there is no accumulation of highly selected genotypes in the colony.

The FRS can, of course, be used for purposes other than maintenance of the integrity of genetic sexing strains, and can make a major contribution to the overall competitiveness of mass-reared strains. The mother colony can be kept under more natural environmental conditions, at reduced adult and larval densities, and with reduced selection pressure for genotypes adapted to mass-rearing conditions. In addition, a more natural environment, preferably under greenhouse conditions with hosts and natural light, could also help address the major problem of loss of irritability and predator-evasion behaviour in mass-reared insects, requiring significantly higher overflooding ratios as a result of the high mortality suffered by released insects due to predation (Hendrichs et al. 2007).

A major advantage of developing a mass-rearing system based on the FRS is that strain replacement becomes a much simpler procedure, and can be done without major disruption of the production process. The size of the population in the initial small mother colony will, of course, depend on the production level required in the facility, but it will probably not exceed several thousand individuals. Therefore, a new strain can be introduced into the FRS in a sequential manner during one generation, and there will be little interruption in production. This procedure has been used to introduce new Mediterranean fruit fly genetic sexing strains into production facilities (Caceres et al. 2004).

3.1.3. *Larval Diets*

All insects that are mass-reared are maintained on an artificial diet. For most species, the larval and adult stages need to be fed. One exception is tsetse flies where larval development takes place in the female, and only adults need to feed. The larval diet of many species is an expensive component of mass-rearing costs, and presents logistical problems if the diet includes components that are poorly defined, requiring bioassays before undertaking large bulk purchases to verify their quality. From the perspective of quality control, defined diets would be preferable, but very few are available, and anyway these would be too costly for mass-rearing purposes. Nevertheless, one can foresee the commercial availability of pre-mixed diets (Thomas et al. 2018).

Disposal of large quantities of spent larval diet can also be a concern, although some facilities sell it as livestock feed, after sterilizing with a steam extruder or other method to kill any live insects remaining in the diet. Since the spent larval diet can still be rich in organic matter, recycling of some components has been considered (the larvae do not actually exploit part of the nutritional value of the diet), but the problem of metabolite accumulation in the diet has yet to be overcome, and thus far no effective practical procedure for any larval diet has been identified.

A challenge for the future of larval diets is to include micro-organisms that naturally contribute nutrients (Kyritsis et al. 2017; Stathopoulou et al. 2021; Augustinos et al., this volume). Inoculation of diets with such probiotic symbionts would not only reduce the need for diet preservatives, but also for the protein supplements, normally the costliest ingredient that larvae often only partially use (Chan Jr. et al. 2000). Whether such probiotic bacteria can be mass-produced cost-effectively for the larval diet, compared with protein supplements, is being investigated (Stathopoulou et al. 2021).

Books by Schneider (2009) and Cohen (2015) review and highlight many critical issues related to insect diet development and rearing. More research on ways to artificially rear several important pest species otherwise amenable to SIT application is required. The lack of cost-effective mass-rearing procedures for these species has been the major obstacle to the implementation of area-wide control programmes.

3.1.4. *Post-Factory Sterile Male Performance*

By applying nutritional, probiotic, hormonal or semiochemical supplements or other bioactive materials to post-teneral sterile males before their release, there is a great potential to improve their subsequent performance or impact in the field (Pereira et al. 2013). Until recently, research on the critical period between the arrival of sterile pupae at emergence facilities and the release of adults in the field has been neglected. Current and future research could result in significant breakthroughs, and it may be possible to recover some of the quality deterioration that has occurred due to colonization, mass-rearing, and irradiation (FAO/IAEA 2017a; Pereira et al., this volume). Thus far, operational programmes have only gradually been adopting the findings already made on this subject.

Juvenile hormones (JH) are known to regulate the development of reproductive capacity and sexual signalling in Diptera and other insect orders. Hormonal treatments in the form of JH mimics such as methoprene, applied to emerging sterile males, have been shown to advance significantly sexual maturation. For example, in *Anastrepha* spp. fruit flies, treated males mate 5–7 days earlier than untreated males (Teal et al. 2000). Also, in *Bactrocera* spp., early sexual maturation is promoted by methoprene- or raspberry ketone-treatment of immature sterile males (Akter et al. 2017; Akter and Taylor 2018; Adnan et al. 2019). This acceleration of reproductive development is crucial for improved SIT application in insects with a long imaginal pre-maturation period, where normally a majority of released sterile males are lost to predation and other causes before they reach sexual maturity in the field (Hendrichs et al. 1993).

Nutritional supplements are equally critical for sexual development and signalling in anautogenous species, in which the imaginal stage has to forage for protein sources to be able to reach sexual maturity. Therefore, adding protein to the diet fed to sterile males prior to release often significantly increases sexual performance (Shelly et al. 2002a; Yuval et al. 2002; Gavriel et al. 2011), although the form of delivery and the ratio of protein to sugar requires fine-tuning for each species. Often in such insects the adults are adapted to ingesting bacteria as protein sources, generally a scarce resource under natural conditions (Lauzon et al. 2004). In tephritid flies this adaptation is reflected in mouthparts that allow the ingestion of only liquids and suspended particles less than 0.5 μm in size, such as bacteria of the Enterobacteriaceae

(Vijayasegaran et al. 1997; Coronado-Gonzales et al. 2008; Augustinos et al., this volume). Nevertheless, the importance of bacteria in the behavioural ecology of some target insects, for example their role in nitrogen fixation and by making other nutrients available through enzymatic degradation, is still not fully understood (Behar et al. 2005). Current mass-rearing practices may even be promoting non-beneficial or harmful bacteria. Thus, the inoculation of post-teneral diets with probiotic diets, i.e. a diet that contains beneficial gut micro-organisms, is an area with much potential to improve sterile male performance (Niyazi et al. 2004; Akami et al. 2019; Stathopoulou et al. 2021; Augustinos et al., this volume).

Combining hormone exposure and protein-enriched or probiotic adult diets results in synergistic interactions, thus increasing sterile male competitiveness many-fold when compared with either only protein or only hormone treatments (Teal et al. 2007; Haq and Hendrichs 2013; Haq et al. 2014). Such hormonal and nutritional therapies are affordable, easily incorporated into protocols followed at fly emergence and release facilities, and earlier release reduces costs associated with holding maturing sterile males (Pereira et al., this volume).

3.1.5. *Sterile Males as Carriers of Bioactive Materials*

There is also great potential to use semiochemical supplements or treatments to boost post-factory sterile male performance. Some species that are attracted to natural attractants sequester these as pheromone precursors into their pheromonal systems, and subsequently release them during courtship. For example, sterile males of *Bactrocera* spp. exposed to such components before release can significantly improve their mating competitiveness (Haq et al. 2018), and, in addition, such components can act as very potent allomones to deter predators (Tan 2000). Furthermore, pre-release exposure to such attractants can significantly reduce sterile male response to male-annihilation baits in the field (Shelly 1994). On the other hand, non-exposed wild males would still be attracted and killed by male annihilation technique (MAT) baits, thus potentially allowing a “male replacement” strategy, consisting of the simultaneous application of male annihilation and sterile male release. Modelling such a concurrent synergistic SIT/MAT implementation confirmed that it is an extremely powerful control strategy, enabling the sterile fly release rate to be reduced to only ca. 5% of that when the SIT is applied alone (Barclay et al. 2014).

The mating competitiveness of wild or mass-reared Mediterranean fruit fly males is similarly enhanced considerably by exposure of pre-release sterile males to ginger root oil and citrus peel oils (Papadopoulos et al. 2001; Shelly et al. 2002b; Kouloussis et al. 2013, 2017). This has great potential in increasing the cost-effectiveness of deploying sterile males, and even exposure to vapour has this effect on emerging males, thus enabling the development of an “aromatherapy” that has facilitated application in fly emergence and release facilities (Shelly et al. 2004a, b; Pereira et al., this volume). Another advantage of aromatherapy is that wild females mated initially with wild males are more likely to remate with ginger root oil-treated sterile males, and furthermore, initial matings with ginger root oil-treated sterile males reduce the likelihood of wild female remating with wild males (Shelly et al. 2004a, b). This is important since, in fruit flies, normally the final male partner fertilizes the majority of the eggs in multiple-mated females.

The identification of the stimulatory cuticular hydrocarbons in New World screwworm female flies, which elicit mating responses in screwworm males, opens the possibility of applying them to sterile females (Carlson et al. 2005). Commonly, after rearing a newly colonized screwworm strain for a few generations under mass-production conditions, screwworm females experience the loss of these cuticular pheromones, and only sterile males accept them as partners; wild males consistently reject them and mate only with wild females (Vreysen et al. 2006). Similar pheromonal compounds may exist in the ecological spheres of other pest species, and still await discovery for post-factory application to boost sterile insect performance.

Finally, there is a great potential to use sterile males or sterile females as carriers of various other bioactive materials (Knipling 1979). One possibility is autodissemination, in which sterile insects would be inoculated with electrostatically charged powder formulated with slow-acting insecticides or entomopathogens (Vickers et al. 2004), which would be spread throughout the pest population through intraspecific interactions (Howse et al. 2007; Bouyer and Lefrançois 2014). Large-scale field application against Mediterranean fruit flies showed that sterile males used as vectors or disseminators of *Beauveria bassiana* Vuillemin can result in effective area-wide suppression, with at least 57% of the wild fly population becoming infected in the treatment area, resulting in total population reduction of over 90%, compared with 0% infection in the non-treated area (Flores et al. 2013; Toledo et al. 2017). The additional cost when used in combination with the SIT appears low when compared with the benefit obtained from the complementary effect of using both techniques. Such a combined approach can obtain high levels of population suppression prior to the release of sterile insects, thus achieving favourable sterile-to-fertile ratios and sterility induction in the wild population (FAO/IAEA 2019).

Another possibility is autoconfusion, a type of mating disruption particularly suited for moth pests, in which released sterile males would carry on their bodies pheromone particles that attract wild males. This would transfer particles to them, and subsequently these males would contaminate other males, resulting in increased mating disruption (Knipling 1979; Howse et al. 2007). Since the few released males that would eventually encounter pheromone-calling females are sterile, autoconfusion and the SIT are clearly compatible. This approach may be more cost-effective for area-wide application than conventional mating disruption or even the establishment of a reduced number of dispensers in the field where wild males would get contaminated. A further possibility is simultaneous mating disruption of a moth pest and releases of another sterilized pest that is much cheaper to mass-rear (Suckling et al. 2011). The moth pheromone-inoculated sterile males, e.g. Mediterranean fruit flies, would significantly increase the mating disruption of the target moth pest population due to a higher overflooding ratio, and simultaneously suppress the local Mediterranean fruit fly population.

There are probably many other possibilities of applying bioactive materials to emerging sterile males, but so far these are mostly theoretical, and much research is required to explore the various possibilities and to assure the environment-friendliness of such approaches.

3.1.6. *Hybrid Vigour*

Hybrid vigour relies on the crossing of two inbred strains to produce an F_1 generation that exhibits increased fitness. It is a general phenomenon, used widely in plant breeding to improve the fitness of plants in the field. To be applied to the SIT, it would require that two inbred strains be reared in a facility, and a simple way exists to select males and females from both strains for directed mating to produce F_1 insects for sterilization and release. At present, in most species, it is not possible to do this automatically. However, it can be tested in the Mediterranean fruit fly, where genetic sexing strains are being mass-produced. In facilities using these strains, two separate sexing strains, both based on pupal colour and temperature sensitivity, could be maintained in separate production modules. In the generation before release, male pupae (brown) from one strain could be placed with female pupae (white) from the second strain, and vice versa, in adult cages and F_1 eggs collected. These could then be heat-treated, and the surviving males reared, sterilized, and released. This procedure could even be carried out without the need for a pupal colour sorter since there is a difference in the developmental time of white and brown pupae in the genetic sexing strains (Franz et al., this volume). Some protocols based on this principle have been evaluated (Mangan 1992; Zapater et al. 1996; McInnis et al. 2002; Gilchrist and Meats 2014; Ekechukwu et al. 2015; Sánchez-Rosario et al. 2017).

3.1.7. *Radiation Dose*

Radiation is one of the many contributing factors to reduced competitiveness of sterile insects; the higher the radiation dose, the more the competitiveness of the insect can be compromised. In most programmes, the radiation dose used produces almost full sterility (as measured by percentage egg hatch from a mating involving either an irradiated male or female insect). Even though this parameter is convenient, it is probably not the only one to use when deciding on the appropriate dose. Measuring egg hatch in the laboratory does not take into account factors such as male competitiveness and sperm competitiveness that, in the end, will combine to determine the hatchability of eggs produced by females of the wild population. These last two parameters will be the major factors in inducing sterility in the wild insects, and they must be taken into account when deciding on the radiation dose to be applied. If the dose is reduced below that which gives full egg sterility, a more competitive insect is released, which in the end induces more sterility in the wild population (Toledo et al. 2004; Rull et al. 2007; Yamada et al. 2014; Marec et al., this volume). Thus, an optimum radiation dose based on induced sterility should be identified for each particular species and strain (Rull et al. 2012). However, better decision-making tools are needed to be able to identify the optimum radiation dose (Parker and Mehta 2007). Of course, the rate of increase of the target population must be considered; it determines the effectiveness of various combinations of male sterility and release ratios in reducing the target population (Klassen and Crecch 1973).

3.2. *Improving Technology*

As well as improving the quality of sterile insects, progress will need to be made in all components of SIT implementation, from cage and facility design, to mass-production and other processes, and to programme planning and evaluation. Improved and more reliable air-handling systems in mass-rearing facilities can have a major impact on the efficiency and cost of rearing. Several facilities now have centralized systems where environmental conditions throughout the facility can be monitored and adjusted as necessary. This centralized system brings with it some disadvantages; other facilities have been built using a modular design (Tween 1987). Facility design, equipment needs, cost, and space allocation for the different production processes are key components. Interactive spreadsheets are now available to integrate the relevant data for various production volumes. This facilitates the planning, financing, construction, and equipping of rearing facilities that produce different numbers of sterile males (Cáceres et al. 2012), as well as managing operations and product quality based on the timely analysis of information (FAO/IAEA 2018b).

3.2.1. *Shelf-Life and Shipment*

In many areas of the world, pest problems are seasonal, and thus operational programmes need to release sterile insects only during specific times of the year. Nevertheless, insects can be produced year-round in a rearing facility; it makes economic sense to keep a facility productive for most of the year. In species that have a facultative diapause, this behavioural trait can be used to stockpile insects during the time when they are not required for release; this appears to have been achieved for the commercial production of the onion maggot. This trait can also be exploited when shipping sterile insects (Bloem et al. 1997). However, many tropical and subtropical pest species have no diapause, and these insects cannot be stored, although in some insects eggs can be stored indefinitely in a dry state, e.g. eggs of *Aedes* spp. Mosquitoes enter into dormancy after a drying process (Zheng et al. 2015). However, for temperate-climate species that have an obligate diapause, this prevents the development of mass-rearing technology and thus limits the application of the SIT. Research on developing strains of these species that do not enter diapause, or in which diapause termination can be induced, is therefore needed so as to overcome this barrier (Colinet et al. 2015; Chen et al. 2016; FAO/IAEA 2017b).

Sterile insects are often transported over long distances and across international borders (Dyck, Reyes Flores et al., this volume). This is usually done when the insect is in the late pupal stage, and some form of cooling and/or anoxia is used to prevent adult emergence during transit. This procedure can reduce insect quality, particularly if the transport period exceeds 48 hours. Pupae are usually shipped in cardboard containers containing cool packs to maintain a low temperature, and then the packing materials are discarded, sometimes creating a disposal problem. It would be desirable if durable equipment were designed that would maintain the correct temperature and atmospheric conditions, and increase the safety of insect shipments (Dowell et al., this volume). There are constraints in transporting biological material, both within and between some countries, and anything that can be done to overcome such constraints or improve the biosafety of shipments is valuable.

There is now another way to ship insects. Fertile eggs are being shipped from egg production facilities to larval rearing facilities that only rear, sterilize, and release sterile adults. The egg reception facilities do not need to maintain mother and large adult colonies for egg production, and this greatly simplifies their operational protocols. It has been demonstrated that Mediterranean fruit fly eggs can be shipped over long distances for extended periods of time without losing their viability and affecting the quality of the insects that are subsequently mass-reared. This concept permits the egg production facility to concentrate its efforts on the maintenance of a mother colony and a large colony for production purposes (section 3.1.2.). In principle, one large central facility could provide eggs to several satellite insect rearing and sterilizing facilities. This concept has already been put into practice in the Moscamed Programme in Guatemala and Mexico, where ca. 100 million heat-treated eggs from a genetic sexing strain maintained at the mass-rearing facility in Guatemala are shipped daily to the male-rearing facility in Tapachula, Mexico (Tween 2004).

3.2.2. *Rearing Systems*

The hardware used to produce sterile insects is continually being modified and improved. In countries with high labour costs and good maintenance practices, partial to full automation of mass-rearing systems will become common. Even in less developed countries, some degree of automation for key processes will be required to provide improved consistency in sterile insect quality (IAEA 2003). There are two key biological components, i.e. egg production and larval rearing, where new design components would have a major impact on mass-rearing efficiency. At present, the cages used for maintaining production colonies often allow females to produce only a portion of their full egg potential. This is caused mainly by males harassing the ovipositing females (cages have a high insect density) and a shortening of the female adult lifespan. New cage designs that address these issues will have an immediate positive impact on rearing efficiency and insect quality (Liedo et al. 2007).

Larvae are usually reared on an artificial diet held in a container. When the larval period is complete, larvae leave the diet or are separated from it and allowed to pupate. This process can be costly, wasteful, and in some cases unpredictable. In some species, the larvae pupate in the diet, and special procedures are required to obtain adult insects, i.e. by positive phototaxis (Dowell et al., this volume). The development of a defined liquid diet held in a type of fermenter, in which the larvae grow to maturation before being separated from the diet, would be a major change in rearing philosophy. Unless unexpected problems in rearing systems arise, the status quo tends to be maintained. Facility managers must ensure that innovations and new concepts are continually evaluated (Parker, Mamai et al., this volume).

3.2.3. *Sterilization*

Insects are usually sterilized by ionizing radiation produced from ^{60}Co or ^{137}Cs sources. These irradiators are effective, but have some disadvantages. They require a considerable amount of regulatory support. The radioactive source declines with time, and thus radiation times get longer; eventually the source has to be recharged, a

complicated and expensive procedure. It is becoming increasingly difficult to arrange for the international transport of this type of source to some parts of the world.

At present, exposure to ionizing radiation is the best way to sterilize insects. However, in view of the logistical difficulties associated with shipping and using radioactive sources, other options are being explored (Bakri et al., this volume). Electron beam technology to generate electrons, or photons such as X-rays, is an option. These systems are being encouraged because they do not involve any radioactive materials, only require access to electricity and cooling water, and can be switched on and off as needed (IAEA 2012). They require no complicated regulatory framework, and avoid the complex and costly shipment of radioactive sources across international borders. On the other hand, at present their availability is limited, they are relatively expensive to purchase and operate, and require substantial maintenance expertise. Nevertheless, increasingly, information on the development and use of alternative equipment to sterilize insects is becoming available (Mehta and Parker 2011; IAEA 2017).

Sterility can also be generated biologically through cytoplasmic incompatibility (CI), when different insect populations of the same species are mated that carry endosymbionts of the genus *Wolbachia* (Townson 2002; Robinson, this volume). *Wolbachia*-induced CI will be used increasingly as a tool for vector population suppression in a self-limiting way (IIT), in combination with radiation to avoid the release of any fertile females, as well as to avoid the transmission of animal, human or plant diseases (Bourtzis and Robinson 2006; Bourtzis et al. 2016; Lees et al., this volume), or as a self-sustaining drive system to deliberately replace a target population (Alphey 2014; Hoffmann et al. 2014). The development of molecular methods (Häcker et al., this volume) of sterilizing insects is discussed in section 4.3.

3.2.4. Release Technology

The area-wide approach to pest management requires that sterile insects be distributed as adults over large areas; this is usually done by aircraft, guided by the Global Positioning System (GPS) and Geographic Information Systems (GIS) technologies that have significantly improved the precision of sterile insect distribution (Bouyer et al., this volume).

Most programmes release chilled adult sterile insects into the wild by means of small fixed-wing aircraft. While this is the fastest method of release, providing uniform distribution over target areas, for some situations it is not the most appropriate or cost-effective release platform. Depending on the target insect and the scale of the programme, there are alternative types of aerial vehicles that should be assessed further since they have the potential for significant cost savings (Tan and Tan 2013). In recent years, technological developments related to Remotely Piloted Aircraft Systems (RPAS) have created opportunities for their use in releasing sterile insects under specific conditions and regulatory frameworks (Benavente-Sánchez et al. 2021). Their disadvantages, such as reduced payload and flight duration, are counterbalanced by advantages such as accuracy, increased safety, and cost-efficiency for small- and medium-scale operations, with the largest cost saving represented by eliminating the need for a pilot and related wages and fuel consumption. However, mandatory special operational permits are required from regulatory agencies, in

particular for operations beyond the visual line of sight (Benavente-Sánchez et al. 2021; Dowell et al., this volume). Cheaper alternatives to currently used aircraft will enable widening applicability of the SIT to smaller-scale programmes, for example mosquito suppression operations that are focused largely on urban and suburban areas.

The current practice is to release sterile insects via an auger or conveyor belt from a chilled container in the aircraft, removing the need to hold and release insects in bulky biodegradable containers, saving airplane space and thus greatly reducing flying time and hence costs. This procedure requires that adult insects, after emergence from pupae, be collected and placed in the chilled container. The logistics of this procedure for very large numbers of insects are daunting, given that insect quality must be maintained at as high a level as possible. The technology to hold insects at a low temperature for several hours in an aircraft, and to achieve the required distribution over a release area, has greatly improved (Leal Mubarqui et al. 2014). Nevertheless, there is still a major concern about the negative impact on insect quality from the stress of low temperature and high insect density in the release machine (Andress et al. 2012, 2013; Shelly et al. 2013). The low temperature, when combined with a high humidity, results in water condensation that makes the insects wet. In the dense holding columns such wet insects become stuck together, exiting the release machine in clumps instead of a continuous stream. Also, because the moving augers of the mechanical delivery system can damage the insects, conveyor belts will be increasingly preferred (Leal Mubarqui et al. 2014).

One indication of the future is new systems that are based on cryogenics (such as liquid CO₂ to make dry ice pellets) as the cooling component. Also, passive chilling systems like those based on the use of phase-change materials can maintain the required cool-temperature range in the insect-holding container at a low payload cost (Tween and Rendón 2007; Benavente-Sánchez et al. 2021). They simplify maintenance, eliminate the high electric load on the aircraft, and allow greater control over temperature and humidity, thus minimizing damage to the sterile insects. The cryogenic system is in use in Guatemala, can deliver up to 50 million sterile *C. capitata* males per flight, and enables the simultaneous release of several species of sterile insects and parasitoids (Tween and Rendón 2007). Computer software linked to a satellite-guided aerial navigation system is programmed to deliver an adjustable number of sterile insects (as needed in each release block), and to turn off the release machine when outside the target blocks. The performances of the pilot, aircraft, and machine are recorded, and can be analysed after each flight (Dowell et al., this volume).

3.2.5. *Field Monitoring*

The widespread use of GPS/GIS technologies has dramatically increased the accuracy and ease with which insect populations can be monitored before, during, and after the implementation of a programme (Bouyer et al., this volume). This increase in precision is enabling much better use of programme resources, and rapid decisions to modify programme activities are now possible. The use of bar-coded traps, and the ability to enter field data directly into hand-held computers for rapid downloading at the field centre, will increasingly make a major contribution to the accuracy and accessibility of data, and enable managers to monitor the efficiency of trapping

personnel. New technologies are also allowing the development of automated insect monitoring systems to provide real-time alerts of the presence and location of a pest, as conventional monitoring systems can result in delays in the information reaching decision-makers, potentially risking outbreaks and the loss of markets. Such technologies will increasingly be deployed in countries with high labour costs because manual checking of thousands of traps is labour intensive and also costly in terms of vehicles and logistics (Potamitis et al. 2017; Schellhorn and Jones 2021).

Field monitoring also requires effective methods to attract and trap insects of both sexes, and in many species there is active research on trapping methods. Data from traps are used to calculate the ratio of released to wild insects, and to directly monitor the relative size of the wild population (Vreysen, this volume). Traditionally, since females are responsible for population growth and hence damage levels, most emphasis has been placed on developing efficient female traps. However, when sterile insects are released, it is important to monitor males in the field. Nevertheless, when all-male releases of Mediterranean fruit flies are made, traps biased to preferentially collect females are useful to minimize the recapture of sterile males, and to facilitate discrimination between wild and recaptured sterile flies (Epsky et al. 2014).

During monitoring it is useful that released insects be easily and unequivocally differentiated from wild insects trapped in the field. (However, it is acknowledged that the New World screwworm eradication programme in Central America was implemented without marking the released sterile insects.) To accomplish this, usually larvae or pupae destined for release as sterile insects are marked with a dye (Dowell et al., this volume; Parker, Mamai et al., this volume; Vreysen, this volume). When trapped insects are brought in from the field, their origin is determined by examining them for the presence of the dye. This process is very labour intensive, expensive, and subject to significant errors of interpretation. Hagler and Jackson (2001) and Johnson et al. (2017) have described several new technologies that may be improvements on these dyes. Morphological markers have often been suggested as possible ways to monitor insects in the field, but usually they are associated with reduced competitiveness and therefore cannot be used effectively in the field. In the Mediterranean fruit fly, a phenotypic mutation has been isolated; it may be useful in the field since it does not appear to be associated with reduced competitiveness both during mass-rearing and in field-cage competition tests (Niyazi et al. 2005). Molecular methods offer a solution to marking for special situations, e.g. to identify the genetic origin of the sperm in spermatheca as an indicator of the degree of induced sterility in the target population (San Andrés et al. 2007; Juan-Blasco et al. 2013, 2014; Smidler et al. 2018; Häcker et al., this volume), but generally they are not practical for routine screening of large numbers of trapped insects. As indicated below (section 4.2.), markers involving the use of transgenic insects would involve regulatory problems.

3.2.6. *Suppression*

Before releasing sterile insects, it is essential to suppress the field pest population so as to facilitate achieving favourable sterile to wild insect overflooding ratios. Since the SIT must be carried out on an area-wide basis, suppression techniques are included in this same requirement (Mangan and Bouyer, this volume). The area-wide approach will often require that pest populations have to be suppressed in inaccessible areas and

also in areas where there is no human population. Although sterile insects can be successfully distributed by aircraft, the same is not always possible with a suppression “technology”, e.g. in many countries, it is not permitted to conduct aerial spraying with traditional insecticides, especially over organic crops or sensitive areas. This puts severe restrictions on the type of suppression technology that is appropriate for area-wide application.

Another difficulty involves suppression activities in urban areas, where many vector breeding sites or host plants may be located. The human populations in these areas need to be persuaded that what can be a very intrusive intervention will eventually benefit the health or economy of their community, but better and more selective suppression methods are needed. There is considerable interest in developing, for use in such sensitive areas, bait stations that both attract and kill insect populations (Piñero et al. 2014). Mass-trapping in urban areas significantly reduced *Ae. aegypti* density and disease prevalence in mosquitoes (Barrera 2017). Also, autodissemination stations from which adult mosquitoes transfer juvenile hormone analogue or insect growth regulators between resting and oviposition sites have been shown to be effective (Devine et al. 2009). The method has since been validated against various *Aedes* species, but is quite expensive because high densities of deployed dissemination stations are necessary (given their low attractiveness) to achieve a good suppression level.

Additional suppression technologies that can be applied by aircraft are highly desirable, and this has led to the identification of a new insecticide-bait formulation that is quite selective for fruit fly suppression. Spinosad is an insecticide developed from the bacterium *Saccharopolyspora spinosa* Mertz and Yao, and it has been incorporated into a protein bait spray that has been organically certified (Peck and McQuate 2000; USDA/APHIS/PPQ 2000; Mangan and Bouyer, this volume; Nagel and Peveling, this volume). However, this insecticide is not immune to the resistance problem; strains resistant to spinosad were rapidly selected in the house fly *Musca domestica* L. (Shono and Scott 2003).

Mating disruption of lepidopteran pests, and male annihilation for *Bactrocera* spp. fruit flies, are other effective and more compatible suppression methods that can also be delivered from the air (Vargas et al. 2014; Cardé 2021; Staten and Walters 2021). Normally, the MAT is applied before sterile males are released; however, the simultaneous implementation of the SIT/MAT is highly synergistic when sterile males have been exposed to the parapheromone methyl eugenol before release (Barclay et al. 2014).

Effective tsetse fly suppression is now possible using the sequential aerosol technique (SAT), where extremely low amounts of pyrethroid insecticide are delivered by low-flying aircraft at certain times when climatic conditions are optimal (Allsopp and Phillemon-Motsu 2002). Effective suppression technology, and an “environment” in which it can be used, will always be an absolutely essential component of AW-IPM programmes integrating the SIT.

4. TRANSGENESIS AND GENE EDITING

During the last 30 years, modern biotechnology has developed molecular technologies that allowed first to routinely introduce foreign genes (transgenesis) into the germ line of many pest species (Hoy 1992; Robinson and Franz 2000; Robinson et al. 2004), and more recently site-specific genome editing techniques such as the CRISPR/Cas system (clustered regularly interspaced short palindromic repeats) that permit the precise editing of genomes at pre-defined positions (Meccariello et al. 2017; Nielsen 2021). There are also RNA interference approaches under development that do not change genomic information, but instead influence gene expression (Baum and Roberts 2014).

All these modern molecular technologies have significantly increased, and will continue to increase, the potential for understanding and modifying the genetic information in support of SIT application (Häcker et al., this volume). They can be used for self-sustaining approaches that aim at population replacement strategies, e.g. using gene drives or *Wolbachia*, or for self-limiting approaches, such as the SIT, that require continuous releases but aim at leaving no ecological footprint. These molecular technologies may benefit operational programmes in the four areas outlined below (Handler 2002; Häcker et al., this volume).

There has been much investment and speculation in this area, but until now very few transgenic or gene-edited strains of pest insects have been produced and field tested in pilot projects. Their wider adoption has been slow because of the low public acceptance of such approaches (NAS 2002) and the regulatory requirements and approvals that are needed in most countries for their application (Reeves et al. 2012; EFSA 2013); however, paradoxically, the release of sterile transgenic insects is probably one of the lowest risk strategies for transgenic organisms (Hoy 1995, 2000; FAO/IAEA 2006).

4.1. Genetic Sexing

As described by Franz et al. (this volume), the SIT can often be made much more effective if only males are mass-reared and released (Rendón et al. 2004; Lance and McInnis, this volume). However, in mosquitoes and other vectors, where the females transmit disease, developing effective genetic sexing systems is essential (Lebon et al. 2018; Ndo et al. 2018). Traditionally, genetic sexing strains have been based on classical Mendelian genetics, and use a combination of selectable markers and male-linked translocations to achieve sex-linkage. These strains have disadvantages — the mass-reared colony is only 50% fertile, females in the colony are homozygous for the marker, and they show reduced viability. Most importantly, the systems are not transferable to other species. Considering that the Mendelian sexing systems can take many years to develop for a single species, this is probably the major disadvantage.

Genetic sexing using molecular approaches, although they have to overcome similar problems such as strain stability and competitiveness, are expected to be more generic, and thus transferable between different pest species. They can either be targeted towards killing females or transforming putative female zygotes into males, and both systems require conditionality to ensure maintenance of colonies. As in

conventional sexing strains, if females are targeted for killing, then lethality should be induced at an early stage, ideally in the egg, as very large numbers of zygotes can be treated together. This requires that early-acting sex-specific promoters are identified and placed under conditional control, as has been achieved for *Anastrepha suspensa* (Loew) (Schetelig and Handler 2012), and then transferred to *C. capitata* (Ogaugwu et al. 2013), *Lucilia cuprina* (Wiedemann) (Yan and Scott 2015), *Anastrepha ludens* (Loew) (Schetelig et al. 2016), and *C. hominivorax* (Concha et al. 2016). Also, much progress has been made recently in mosquitoes (Bourtzis and Tu 2018).

Transforming females into males has the advantage of doubling the male output, and in the future will increasingly be pursued. It requires a detailed knowledge of sex determination in the species under study (Meccariello et al. 2019). While the sex-determining factor appears to be highly conserved in Diptera, Lepidoptera, and Hymenoptera, its sequence is not, and there is considerable variation in the functional relationships (Shearman 2002; Verhulst et al. 2010). Nevertheless, sex reversion via a transient RNAi effect has already been performed successfully in *C. capitata* (Pane et al. 2002), *Bactrocera oleae* (Rossi) (Lagos et al. 2007), and *L. cuprina* (Concha and Scott 2009), resulting in fertile fully transformed XX males. The same sex transformation effects have been demonstrated in *C. capitata* following transgenesis (Saccone et al. 2005). Furthermore, a CRISPR/Cas-based X shredder, that shreds the X chromosome in sperm during spermatogenesis, or the targeted disruption of the expression of female fertility genes (resulting in sex ratio distortion), have been developed for the *Anopheles gambiae* complex (Galizi et al. 2016; Kyrou et al. 2018; Fasulo et al. 2021).

4.2. Marking

As indicated above, sterile insects for release are usually marked with fluorescent powders or other dyes (Dowell et al., this volume; Parker, Mamai et al., this volume; Vreysen, this volume); however, they are not reliable, and daily exposure during mass-rearing may be harmful to workers. Molecular techniques enable marking with fluorescent proteins. Using such genetic markers for released insects requires that the marker be dominant, and that it can be monitored even in dead adults, as insects are usually dead when removed from traps. Transgenic strains carrying fluorescent markers have already been developed in a wide variety of insect pests (Häcker et al., this volume), and their stability in dead specimens after trapping has been confirmed by fluorescence microscopy and PCR (Meza et al. 2011; Simmons et al. 2011). However, the fitness cost to the insect for the production of this exogenous protein is unknown. Also, no data are available on the effect of this marker on the behaviour of the insect in the field, or indeed on the response of conspecifics or predators to fluorescent insects. Assessing this is especially relevant since insects use the UV spectrum for vision.

Strains with fluorescent sperm can also be used to monitor the mating success of the released males by screening the spermathecae of wild females (Juan-Blasco et al. 2013). Furthermore, sex-specific expression of fluorescent markers can allow automated sexing, as shown for immature stages or adults of *Ae. aegypti* (Smith et al.

2007), *Anopheles stephensi* Liston (Catteruccia et al. 2005), or *An. gambiae* (Marois et al. 2012) using the Complex Object Parametric Analyser and Sorter (COPAS).

4.3. Sterilization

The use of ionizing radiation has proven to be an extremely effective way to sterilize insects for release in the field (Bakri et al., this volume). To reduce somatic damage, the procedure is done at as late a developmental stage as possible. This physical process is a fail-safe procedure when the correct protocols are followed. It is also not subject to the development of resistance, can be used on any strain, and does not interfere with the mass-rearing process. As with all the procedures to which released insects have to be subjected, radiation does have some negative effect on the competitiveness of treated insects. However, the detrimental effects of radiation have sometimes been exaggerated. The overall competitiveness of a released insect is determined by a whole combination of different factors related to factory adaptation, selection, mass-rearing, transport, and handling and release procedures, etc. Itô et al. (this volume) strongly make the case that reductions in competitiveness in the field are much more likely to be due to colonization and long-term mass-rearing effects, as well as post-factory handling, than to radiation.

Reproductive sterilization can also be achieved through genetic approaches, eliminating the need for irradiation. Any system to induce sterility in the field through dominant lethality must be conditional in some way so that efficient mass-rearing can be carried out. This conditionality can be achieved by using transcriptional activation or suppression systems based on the presence or absence of antibiotics in the larval diet (Heinrich and Scott 2000; Thomas et al. 2000). The permissive condition in the facility will require the presence of an antibiotic or its analogue during the mass-rearing of the colony, although not the male production for release. Following release of the transgenic males, the female progeny of a wild female mated with such a male would die in the absence of the antibiotic.

Horn and Wimmer (2003) engineered reproductive sterility in a *Drosophila* strain by transferring embryonically lethal transgenes, so that both male and female progeny die as embryos. Subsequently, this sterilization system was adapted and transferred to tephritid pests such as *C. capitata*, *A. ludens*, and *A. suspensa* (Schetelig et al. 2009, 2016; Schetelig and Handler 2012). Alternatively, transgenic strains developed based on the RIDL system (Release of Insects carrying a Dominant Lethal) (Thomas et al. 2000), are late-acting conditional lethality systems that kill progeny in late larval or pupal stages. They have been designed for *C. capitata* and *Ae. aegypti* (Gong et al. 2005; Phuc et al. 2007; Harris et al. 2012). However, some of these strains will require further refinement because they are not completely sterile in matings with wild females, and thus their genome may become incorporated into the target population (Evans et al. 2019). The lethality in the late larval stage is another disadvantage, resulting in damage from crop pests. Nevertheless, for *Ae. aegypti*, open-field trials with transgenic mosquitoes, carried out in Grand Cayman (Harris et al. 2012) and Brazil (Carvalho et al. 2015), achieved over 80% suppression of local populations. Open-field trials are also planned for a transgenic sexing strain of the New World screwworm in Panama (Concha et al. 2016).

A specific concern regarding these types of systems (that require the addition of a bioactive compound to very large volumes of larval diet) is the disposal of the diet, e.g. the Mediterranean fruit fly facility in Guatemala produces about 25 tons of diet per day. This is a real issue since mass-rearing facilities often sell spent larval diet as cattle feed. In addition, maintaining the appropriate concentration throughout the diet will not be easy. A third concern is the effect of the diet on the level of antibiotic. As the diet is a microcosm of bacterial and fungal growth, it will not be easy to standardize the exposure of the larvae to the antibiotic. It will also be important to choose the appropriate type of dominant lethality with which to kill the progeny in the field. Any genes involved in cell death mechanisms, or which show general cell toxicity, would probably not be suitable and/or would raise environmental concerns. Since effective sterilization of wild females remains the key to a successful programme that includes the release of sterile insects, there is no room for error in the sterilization procedure, and any proposed biological system must be extremely robust, controllable, and accurate.

There has been much argument about the relative merits of using either biological or physical methods to induce lethality in field populations (Alphey and Andreasen 2002). While it may appear that the molecular approach has some potential advantages, it is important to distinguish the characteristics of these two methods in producing sterility in a field population. Both approaches, to be effective, must act in a dominant manner and induce lethality in eggs produced by wild females following mating with released males. The key difference, and the one that will finally determine whether the molecular approach has any practical value, is that molecular sterility is based on a single dominant factor. Any variation in the expression of the sterility factor following interaction with the large and heterogeneous genome in the field population will very quickly lead to selection for non-sensitive females and loss of sterility induction. This is a major weakness of any molecular approach; there is no practical way to evaluate the possibility that this might happen without compromising the eventual use of the technology. One solution that has been proposed is to introduce several constructs into the same individual, but the fitness costs of such multiple insertions still has to be determined.

In contrast, sterility produced by radiation is based on the action of an almost infinite number of dominant lethal mutations, and every released male carries a different set of sterilizing factors (Robinson 2002); thus, it is very difficult for a field population to develop any sort of resistance (Whitten and Mahon, this volume). Radiation-induced sterility has built-in redundancy, and in spite of some disadvantages it remains the only proven and environment-friendly technique for the introduction of sterility into field populations of pest insects.

4.4. Paratransgenesis

A fourth area in which transgenic technology may benefit future programmes that include the release of sterile insects is manipulating their symbiotic organisms (Miller 2010; Augustinos et al., this volume; Häcker et al., this volume). Such mutualists are often required by some insect vectors for the transmission of important animal, human or plant diseases. Paratransgenesis of symbionts offers major possibilities to develop

vector strains that are refractory to transmission by introducing into the symbionts genes coding for proteins that prevent the vector from transmitting the pathogen. One example is *Rhodnius prolixus* Stål, an important insect vector of Chagas disease caused by *Trypanosoma cruzi*. *R. prolixus* is associated with the symbiont *Rhodococcus rhodnii*, which was modified to express proteins that are toxic to, or block the transmission of, *T. cruzi* (Beard et al. 2001). Another example is tsetse fly species, where the paratransgenesis has been developed to impede trypanosome transmission by blood-feeding sterile males (Abd-Alla et al. 2013; Demirbas-Uzel et al. 2018; De Vooght et al. 2018; Kariithi et al. 2018). Similar developments are in progress for several mosquito species using fungi, viruses, or bacteria (Wilke and Marrelli 2015).

An approach that is showing great potential is to harness, for paratransgenesis purposes, the *Wolbachia*-induced cytoplasmic incompatibility phenomenon that can be used to induce sterilization to suppress or modify natural populations (Zabalou et al. 2004; Bourtzis et al. 2016). Infection of *Ae. aegypti* by different *Wolbachia* strains can lead to several or different outcomes such as a shortened lifespan, resulting in: (1) a reduction in the female potential to transmit pathogens that must infect adult mosquitoes to complete their development cycle, (2) a limited susceptibility to infection with the dengue or chikungunya virus or the *Plasmodium* parasite, or (3) depending on the strain of *Wolbachia*, an induction of cytoplasmic incompatibility, apparently with no significant fitness cost and high horizontal transmission (Wilke and Marrelli 2015; Flores and O'Neill 2018). However, each specific vector situation endeavouring to use *Wolbachia* for paratransgenesis and cytoplasmic incompatibility will need to be assessed carefully (Hughes et al. 2014); also, there are cases where *Wolbachia* enhanced disease infection (Dodson et al. 2014).

Since 2011, the World Mosquito Program has been releasing successfully *Wolbachia*-infected *Ae. aegypti* males and females for population replacement in various pilot locations in Australia, and also more recently in Colombia and Indonesia (Flores and O'Neill 2018). Releases (for population suppression) of only male *Wolbachia*-infected *Ae. aegypti* are ongoing or planned in Brazil, China, Singapore, and the USA (Callaway 2016; Mains et al. 2016; Waltz 2017; Hacker et al., this volume; Lees et al., this volume).

4.5. Technical and Regulatory Constraints

Any transgenic strain proposed for sterile insect release will have to meet stringent criteria regarding the expression of the specific trait that it carries and the overall quality of the strain itself. These are not inconsiderable technical constraints, as has been demonstrated for the introduction of sexing strains based on classical approaches (Franz et al., this volume). The impact of large-scale rearing under extremely stressful conditions, and the number of individuals reared (often exceeding millions/week), can uncover significant genetic and biological events that can never be induced and studied under typical laboratory conditions, and for which the consequences are at present unknown. It is safe to say that extensive evaluations of transgenic strains are required before they can be used operationally in the field.

Major constraints in using transgenic strains for area-wide field release, even when sterilized, are the often considerable opposition from the public and special interest groups such as organic farmers (Reeves and Phillipson 2017), as well as the regulatory aspect (Lehane and Aksoy 2012). Whereas there are no real regulatory constraints for strains based on classical approaches (section 2.6.), for transgenic strains there are a number of regulatory approvals required (Reeves et al. 2012) (that represent major hurdles to be overcome in a majority of countries) before transgenic insects can be released. Furthermore, regulatory frameworks for transgenic insects are not yet harmonized nationally and internationally (NAPPO 2007; EFSA 2013), usually requiring lengthy case-by-case evaluations.

An elegant way to overcome such constraints has been proposed in Lepidoptera, where females are the heterogametic sex. Transformation to develop sexing strains using dominant conditional lethals, only expressed in females, would result in only colony females carrying the transgenic constructs, with the resulting sterile males being non-transgenic (Marec et al. 2005). Alternatively, besides classical genetic approaches, other rapidly evolving non-transgenic technologies can be pursued for self-limiting strategies, such as CRISPR/Cas and RNAi approaches (Beetham 2018), although in Europe strains resulting from genome editing are being subject to the same stringent regulations as transgenic organisms. Fortunately, in many other countries, including Japan and the USA, such editing that results in small insertions or deletions will not be treated as transgenic organisms (Nature 2018), permitting, for example, the removal of genes required for sex determination to create non-transgenic sexing strains (Meccariello et al. 2019). RNAi methods also offer a way to work without transgenic organisms, as long as the dsRNA is not conditionally or constitutively expressed in the insect, and if it does not persist in the environment (Häcker et al., this volume).

On the other hand, there is the great potential in the future of using CRISPR/Cas gene drives for self-replicating purposes, to spread rapidly beneficial traits through interbreeding pest and vector populations, thereby eliminating the need for continuous mass-rearing and release. However, even though such approaches hold great promise for high impact and low cost, they have raised a number of societal concerns and regulatory demands that will necessitate careful consideration (James et al. 2018; Nielsen 2021). There are also technical issues that will first need to be overcome, such as the emergence of resistance and other evolutionary processes, before the application of such a promising approach can become reality (Champer et al. 2017).

5. CONCLUSIONS

Global trends towards a cleaner agriculture and less aggressive pest control will increase the demand for more target-specific, biologically based, and sustainable suppression methods such as the release of sterile insects. Globalization is resulting in more incursions and outbreaks of invasive pests, requiring effective and environment-friendly eradication tools such as the SIT. Furthermore, international trade is creating an environment in which area-wide approaches to the management of insect pests have a comparative advantage. Increasingly, overcoming technical and scientific constraints, and introducing new technological innovations such as refractory vectors,

diapause termination, sterile-insect stockpiling, and post-factory performance-boosting treatments for sterile males will enable the SIT to be used against new target pests, and improve cost-effectiveness against current targets.

Nevertheless, these innovations and improvements are no guarantee for the successful implementation of the SIT as part of AW-IPM programmes. These programmes are management-intensive compared with most other control operations. Close coordination among many simultaneously occurring activities is required; their execution requires precision in time and space. Since the area-wide approach also depends on the cooperation and participation of all stakeholders in the target area, excellent management of communications with all stakeholders is indispensable, paying close attention to political, socio-economic, and environmental sensitivities. Therefore, effective programme implementation depends on the establishment of an efficient management structure that is semi-independent of normal government bureaucracy and corruption; mosquito abatement districts are good examples of effective and largely autonomous self-governing programmes (Foley IV et al. 2021). A common denominator of failed SIT projects has been the lack of such dynamic management structures and dedicated leadership to respond flexibly to changing situations. Equally important is transparency, external technical advice, and an effective oversight structure to maximize limited resources and to attract stakeholder funding (Dyck, Reyes Flores et al., this volume).

A major unexploited opportunity is the integration of sterile insects and augmentative biological control. As long as key pests are treated with insecticides, natural and augmentative biocontrol is disrupted, and hence there is only a limited potential for growth in the application of pollinators or natural enemies to deal with secondary pests. On the other hand, when a key pest is managed using biologically based tools (e.g. host resistance, mating disruption, sterile insects, etc.), natural and mass-reared biological control agents can complement these methods, and also play an important role in controlling less important pests. Thus, the existing augmentative biocontrol industry (ANBP 2018; IBMA 2018) and the SIT appear to be natural allies, and the expected gradual progress in commercializing the SIT will probably advance within this context. The private-sector biocontrol industry already has the technical “know how” to manage the mass-rearing, quality control, handling, and shipping of beneficials, and therefore could make significant contributions that improve and advance the SIT technology. Adding sterile insects to their products can provide a complete “biological package”. A pioneer in this approach is BioBee in Israel, which is expanding from greenhouse to field pest management (Bassi et al. 2007; Steinberg and Cayol 2009).

Also, there is much unexploited potential in applying sterility to natural enemy production and facilitation. This involves using sterile insects not to transfer sterile sperm but as sterile hosts/prey/vectors for parasitoids/predators/pathogens to facilitate the use, and enhance the efficacy, of biological control agents (Hendrichs et al. 2009). At present, considerable research is being conducted to exploit the many potential possibilities and applications to facilitate natural enemy production, handling, and shipment, and also to provide sterile hosts for the establishment and maintenance over critical periods, or early season build-up (or in border or trap crops), of natural enemy populations in the field.

6. REFERENCES

- Abd-Alla, A. M. M., M. Bergoin, A. G. Parker, N. K. Maniania, J. M. Vlak, K. Bourtzis, D. G. Boucias, and S. Aksoy. 2013. Improving sterile insect technique (SIT) for tsetse flies through research on their symbionts and pathogens. *Journal of Invertebrate Pathology* 112(Suppl. 1): S2–S10. <https://doi.org/10.1016/j.jip.2012.07.009>
- Adnan, S. M., I. Farhana, J. R. Inskeep, P. Rempoulakis, and P. W. Taylor. 2019. Accelerated sexual maturation in methoprene-treated sterile and fertile male Queensland fruit flies (Diptera: Tephritidae), and mosquito larvicide as an economical and effective source of methoprene. *Journal of Economic Entomology* 112: 2842–2849. <https://doi.org/10.1093/jee/toz214>
- Akani, M., A. A. Andongma, C. Zhengzhong, J. Nan, K. Khaeso, E. Jurkevitch, C.-Y. Niu, and B. Yuval. 2019. Intestinal bacteria modulate the foraging behavior of the oriental fruit fly *Bactrocera dorsalis* (Diptera: Tephritidae). *PLOS ONE* 14(1): e0210109. <https://doi.org/10.1371/journal.pone.0210109>
- Aker, H., and P. W. Taylor. 2018. Sexual inhibition of female Queensland fruit flies mated by males treated with raspberry ketone supplements as immature adults. *Journal of Applied Entomology* 142: 380–387. <https://doi.org/10.1111/jen.12484>
- Aker, H., V. Mendez, R. Morelli, J. Pérez, and P. W. Taylor. 2017. Raspberry ketone supplement promotes early sexual maturation in male Queensland fruit fly, *Bactrocera tryoni* (Diptera: Tephritidae). *Pest Management Science* 73: 1764–1770. <https://doi.org/10.1002/ps.4538>
- Allsopp, R., and T. K. Phillemon-Motsu. 2002. Tsetse control in Botswana – a reversal in strategy. *Pesticide Outlook* 13(2): 73–76. <http://dx.doi.org/10.1039/B202993F>
- Alphey, L. 2014. Genetic control of mosquitoes. *Annual Review of Entomology* 59: 205–224. <https://doi.org/10.1146/annurev-ento-011613-162002>
- Alphey, L., and M. Andreasen. 2002. Dominant lethality and insect population control. *Molecular and Biochemical Parasitology* 121(2): 173–178. [https://doi.org/10.1016/S0166-6851\(02\)00040-3](https://doi.org/10.1016/S0166-6851(02)00040-3)
- (ANBP) Association of Natural Biocontrol Producers. 2018. <http://www.anbp.org/>
- Andress, E., E. Jones, M. War, and T. Shelly. 2012. Effects of pre-release chilling on the flight ability of sterile males of the Mediterranean fruit fly (Diptera: Tephritidae). *Florida Entomologist* 95: 587–592. <https://journals.flvc.org/flaent/article/view/80371/77612>
- Andress, E., M. War, and T. Shelly. 2013. Effect of pre-release storage on the flight ability of sterile Mediterranean fruit flies (Diptera: Tephritidae). *Florida Entomologist* 96: 1615–1617. <https://journals.flvc.org/flaent/article/view/82808/79688>
- Barclay, H. J., D. McInnis, and J. Hendrichs. 2014. Modeling the area-wide integration of male annihilation and the simultaneous release of methyl eugenol-exposed *Bactrocera* spp. sterile males. *Annals of the Entomological Society of America* 107: 97–112. <https://doi.org/10.1603/AN13010>
- Barnes, B. N. 2007. Privatizing the SIT: a conflict between business and technology? pp. 449–456. *In* M. J. B. Vreysen, A. S. Robinson and J. Hendrichs (eds.), *Area-wide control of insect pests. From research to field implementation*. Springer, Dordrecht, The Netherlands. <https://www.iaea.org/sites/default/files/area-wide-control-insect-pests-book.pdf>
- Barrera, R. 2017. City-wide control of *Aedes aegypti* and Zika virus using mass-trapping in Puerto Rico, pp. 28–29. *In* Book of Abstracts. FAO/IAEA International Conference on Area-Wide Management of Insect Pests: Integrating the Sterile Insect and Related Nuclear and Other Techniques, 22–26 May 2017, Vienna, Austria. Poster. Abstract ID 250. IAEA-CN-248. IAEA, Vienna, Austria. <http://www-naweb.iaea.org/naweb/ipc/AWM-Book-of-Abstracts.pdf>
- Bassi, Y., S. Steinberg, and J. P. Cayol. 2007. Private sector investment in Mediterranean fruit fly mass-production and SIT operations — the “sheep” of the private sector among the “wolves” of the public good? pp. 457–472. *In* M. J. B. Vreysen, A. S. Robinson and J. Hendrichs (eds.), *Area-wide control of insect pests. From research to field implementation*. Springer, Dordrecht, The Netherlands. <https://www.iaea.org/sites/default/files/area-wide-control-insect-pests-book.pdf>
- Baum, J. A., and J. K. Roberts. 2014. Chapter Five -- Progress towards RNAi-mediated insect pest management, pp. 249–295. *In* T. S. Dhadialla and S. S. Gill (eds.), *Insect midgut and insecticidal proteins. Advances in Insect Physiology* 47. <https://doi.org/10.1016/B978-0-12-800197-4.00005-1>
- Beard, C. B., E. M. Dotson, P. M. Pennington, S. Eichler, C. Cordon-Rosales, and R. V. Durvasula. 2001. Bacterial symbiosis and paratransgenic control of vector-borne Chagas disease. *International Journal for Parasitology* 31: 621–627. [https://doi.org/10.1016/S0020-7519\(01\)00165-5](https://doi.org/10.1016/S0020-7519(01)00165-5)
- Beetham, P. 2018. GMOs are not agriculture's future - biotech is. *Scientific American*, September 5, 2018. <https://blogs.scientificamerican.com/observations/gmos-are-not-agricultures-future-biotech-is/>

- Behar, A., B. Yuval, and E. Jurkevitch. 2005.** Enterobacteria-mediated nitrogen fixation in natural populations of the fruit fly *Ceratitis capitata*. *Molecular Ecology* 14: 2637–2643. <https://doi.org/10.1111/j.1365-294X.2005.02615.x>
- Beier, J., R. S. Lees, D. D. Chadee, and J. R. L. Gilles (eds.). 2014.** Biology and behaviour of male mosquitoes in relation to new approaches to control disease transmitting mosquitoes. *Acta Tropica* 132(Suppl.): S1–S188. <https://www.sciencedirect.com/journal/acta-tropica/vol/132/suppl/S>
- Bellini, R., A. Albieri, F. Balestrino, M. Carrieri, D. Porretta, S. Urbanelli, M. Calvitti, R. Moretti, and S. Maini. 2010.** Dispersal and survival of *Aedes albopictus* (Diptera: Culicidae) males in Italian urban areas and significance for sterile insect technique application. *Journal of Medical Entomology* 47: 1082–1091. <https://doi.org/10.1603/ME09154>
- Bello-Rivera, A., R. Pereira, W. Enkerlin, S. Bloem, K. Bloem, S. D. Hight, J. E. Carpenter, H. G. Zimmermann, H. M. Sanchez-Anguiano, R. Zetina-Rodriguez, and F. J. Trujillo-Arriaga. 2021.** Successful area-wide programme that eradicated outbreaks of the invasive cactus moth in Mexico, pp. 561–580. *In* J. Hendrichs, R. Pereira and M. J. B. Vreysen (eds.), *Area-wide integrated pest management. Development and field application*. CRC Press, Boca Raton, FL, USA.
- Benavente-Sánchez, D., J. Moreno-Molina, and R. Argilés-Herrero. 2021.** Prospects for remotely piloted aircraft systems in area-wide integrated pest management programmes, pp. 903–916. *In* J. Hendrichs, R. Pereira and M. J. B. Vreysen (eds.), *Area-wide integrated pest management. Development and field application*. CRC Press, Boca Raton, FL, USA.
- Bloem, S., K. A. Bloem, and L. S. Fielding. 1997.** Mass-rearing and storing codling moth larvae in diapause: a novel approach to increase production for sterile insect release. *Journal of the Entomological Society of British Columbia* 94: 75–81. <https://journal.entsocbc.ca/index.php/journal/article/view/467>
- Bloem, S., K. A. Bloem, and A. L. Knight. 1998.** Oviposition by sterile codling moths, *Cydia pomonella* (Lepidoptera: Tortricidae) and control of wild populations with combined releases of sterile moths and egg parasitoids. *Journal of the Entomological Society of British Columbia* 95: 99–109. <https://journal.entsocbc.ca/index.php/journal/article/view/479>
- Bloem, K., S. Bloem, J. Carpenter, S. Hight, J. Floyd, and H. Zimmermann. 2007.** Don't let cactus blast us: development of a bi-national plan to stop the spread of the cactus moth *Cactoblastis cactorum* in North America, pp. 337–344. *In* M. J. B. Vreysen, A. S. Robinson and J. Hendrichs (eds.), *Area-wide control of insect pests. From research to field implementation*. Springer, Dordrecht, The Netherlands. <https://www.iaea.org/sites/default/files/area-wide-control-insect-pests-book.pdf>
- Boersma, N. 2021.** The suppression of the false codling moth in South Africa using an AW-IPM approach with a SIT component, pp. 93–109. *In* J. Hendrichs, R. Pereira and M. J. B. Vreysen (eds.), *Area-wide integrated pest management. Development and field application*. CRC Press, Boca Raton, FL, USA.
- Boller, E. F., and D. L. Chambers. 1977.** Quality control: an idea book for fruit fly workers. International Organization for Biological Control of Noxious Animals and Plants, West Palearctic Regional Section. 162 pp.
- Bourtzis, K., and A. S. Robinson. 2006.** Insect pest control using *Wolbachia* and/or radiation, pp. 225–246. *In* K. Bourtzis and T. Miller (eds.), *Insect symbiosis*, vol. 2. CRC Press, Boca Raton, FL, USA.
- Bourtzis, K., and Z. J. Tu. 2018.** Joint FAO/IAEA Coordinated Research Project on “Exploring genetic, molecular, mechanical and behavioural methods of sex separation in mosquitoes” – an introduction. *Parasites and Vectors* 11(Suppl. 2): 653. <https://doi.org/10.1186/s13071-018-3206-9>
- Bourtzis, K., R. S. Lees, J. Hendrichs, and M. J. B. Vreysen. 2016.** More than one rabbit out of the hat: radiation, transgenic and symbiont-based approaches for sustainable management of mosquito and tsetse fly populations. *Acta Tropica* 157: 115–130. <https://doi.org/10.1016/j.actatropica.2016.01.009>
- Bouyer, J., and T. Lefrançois. 2014.** Boosting the sterile insect technique to control mosquitoes. *Trends in Parasitology* 30: 271–273. <https://doi.org/10.1016/j.pt.2014.04.002>
- Briceño, R. D., and W. G. Eberhard. 2002.** Decisions during courtship by male and female medflies (Diptera: Tephritidae): correlated changes in male behavior and female acceptance criteria in mass-reared flies. *Florida Entomologist* 85: 14–31. <http://www.fcla.edu/FlaEnt/fe85p14.pdf>
- Caceres, C., J. P. Cayol, W. Enkerlin, G. Franz, J. Hendrichs, and A. S. Robinson. 2004.** Comparison of Mediterranean fruit fly (*Ceratitis capitata*) (Tephritidae) bisexual and genetic sexing strains: development, evaluation and economics, pp. 367–381. *In* B. N. Barnes (ed.), *Proceedings, Symposium: 6th International Symposium on Fruit Flies of Economic Importance, 6–10 May 2002, Stellenbosch, South Africa*. Isteg Scientific Publications, Irene, South Africa. https://nucleus.iaea.org/sites/naipc/twd/Documents/6thISFFEI_Proceedings/6th-ISFFEI-Proceedings-2004-Stellenbosch-South-Africa.pdf

- Cáceres, C., D. McInnis, T. Shelly, E. Jang, A. Robinson, and J. Hendrichs. 2007. Quality management systems for fruit fly (Diptera: Tephritidae) sterile insect technique. *Florida Entomologist* 90: 1–9. <http://journals.fcla.edu/flaent/article/view/75606>
- Cáceres, C., P. Rendón, and A. Jessup. 2012. The FAO/IAEA spreadsheet for designing and operation of insect mass rearing facilities. *Procedures Manual*. FAO Plant Production and Protection Paper 205. Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture. Rome, Italy. <https://www.iaea.org/resources/manual/the-fao/iaea-spreadsheet-for-designing-and-operation-of-insect-mass-rearing-facilities>
- Cagnotti, C. L., M. M. Viscarret, M. B. Riquelme, E. N. Botto, L. Z. Carabajal, D. F. Segura, and S. N. López. 2012. Effects of X-rays on *Tuta absoluta* for use in inherited sterility programmes. *Journal of Pest Science* 85: 413–421. <https://doi.org/10.1007/s10340-012-0455-9>
- Callaway, E. 2016. Infected mosquitoes fight Zika. South America hosts largest trials yet of *Wolbachia*-infected insects to combat viruses. *Nature* 539(7627): 17–18. DOI: 10.1038/nature.2016.20878 https://www.nature.com/polopoly_fs/1.20878!/menu/main/topColumns/topLeftColumn/pdf/nature.2016.20878.pdf
- Calvitti, M., P. C. Remotti, and U. Cirio. 2000. The sterile insect technique in the integrated pest management of whitefly species in greenhouses, pp. 185–192. In K. H. Tan (ed.), *Proceedings: Area-Wide Control of Fruit Flies and Other Insect Pests*. International Conference on Area-Wide Control of Insect Pests, and the 5th International Symposium on Fruit Flies of Economic Importance, 28 May–5 June 1998, Penang, Malaysia. Penerbit Universiti Sains Malaysia, Pulau Pinang, Malaysia. http://www-naweb.iaea.org/nafa/ipc/public/Area-wide-control_73-229.pdf
- Cardé, R. T. 2021. Mating disruption with pheromones for control of moth pests in area-wide management programmes, pp. 779–794. In J. Hendrichs, R. Pereira and M. J. B. Vreysen (eds.), *Area-wide integrated pest management. Development and field application*. CRC Press, Boca Raton, FL, USA.
- Carlson, D. A., K. Akasaka, T. Ohtaki, H. Ohrui, K. Mori, and D. Berkebile. 2005. Identification of natural sex stimulant pheromones in New World screwworm via comparison with synthetic diastereomeric isomers using chiral HPLC, pp. 292–293. In *Book of Extended Synopses*. FAO/IAEA International Conference on Area-Wide Control of Insect Pests: Integrating the Sterile Insect and Related Nuclear and Other Techniques, 9–13 May 2005, Vienna, Austria. Poster number IAEA-CN-131/178P. IAEA, Vienna, Austria.
- Carpenter, J. E., H. Hidayani, and W. Sheenan. 1996. Compatibility of F₁ sterility and a parasitoid, *Cotesia marginiventris* (Hymenoptera: Braconidae), for managing *Spodoptera exigua* (Lepidoptera: Noctuidae): acceptability and suitability of hosts. *Florida Entomologist* 79: 289–295. <http://www.fcla.edu/FlaEnt/fe79p289.pdf>
- Carpenter, J., S. Bloem, and H. Hofmeyr. 2007. Area-wide control tactics for the false codling moth *Thaumotobia leucotreta* in South Africa: a potential invasive species, pp. 351–359. In M. J. B. Vreysen, A. S. Robinson and J. Hendrichs (eds.), *Area-wide control of insect pests. From research to field implementation*. Springer, Dordrecht, The Netherlands. <https://www.iaea.org/sites/default/files/area-wide-control-insect-pests-book.pdf>
- Carvalho, D. O., A. R. McKemey, L. Garziera, R. Lacroix, C. A. Donnelly, L. Alphey, A. Malavasi, and M. L. Capurro. 2015. Suppression of a field population of *Aedes aegypti* in Brazil by sustained release of transgenic male mosquitoes. *PLOS Neglected Tropical Diseases* 9(7): e0003864. <https://doi.org/10.1371/journal.pntd.0003864>
- Catteruccia, F., J. P. Benton, and A. Crisanti. 2005. An *Anopheles* transgenic sexing strain for vector control. *Nature Biotechnology* 23: 1414–1417. <https://doi.org/10.1038/nbt1152>
- Cayol, J. P. 2000a. Changes in sexual behavior and life history traits of tephritid species caused by mass-rearing processes, pp. 843–860. In M. Aluja and A. L. Norrbom (eds.), *Fruit flies (Tephritidae): phylogeny and evolution of behavior*. CRC Press, Boca Raton, FL, USA.
- Cayol, J. P. 2000b. World-wide sexual compatibility in medfly, *Ceratitis capitata* (Wied.), and its implications for SIT, pp. 657–665. In K. H. Tan (ed.), *Proceedings: Area-Wide Control of Fruit Flies and Other Insect Pests*. International Conference on Area-Wide Control of Insect Pests, and the 5th International Symposium on Fruit Flies of Economic Importance, 28 May–5 June 1998, Penang, Malaysia. Penerbit Universiti Sains Malaysia, Pulau Pinang, Malaysia. http://www-naweb.iaea.org/nafa/ipc/public/Area-wide-control_651-Back-Cover.pdf
- Champer, J., R. Reeves, S. Y. Oh, C. Liu, J. Liu, A. G. Clark, and P. W. Messer. 2017. Novel CRISPR/Cas9 gene drive constructs reveal insights into mechanisms of resistance allele formation and drive efficiency in genetically diverse populations. *PLOS Genetics* 13(7): e1006796.

- <https://doi.org/10.1371/journal.pgen.1006796>
- Chan, Jr., H. T., H. Ako, R. Y. Niño-Duponte, J. R. Carpenter, and E. B. Jang. 2000.** Composition of Mediterranean fruit fly third instar larvae (Diptera: Tephritidae) and diet: nutrient balance studies on amino acids, minerals and nutrient composition in fresh and spent mass rearing diets, pp. 567–576. *In* K. H. Tan (ed.), Proceedings: Area-Wide Control of Fruit Flies and Other Insect Pests. International Conference on Area-Wide Control of Insect Pests, and the 5th International Symposium on Fruit Flies of Economic Importance, 28 May–5 June 1998, Penang, Malaysia. Penerbit Universiti Sains Malaysia, Pulau Pinang, Malaysia. http://www.naweb.iaea.org/nafa/ipc/public/Area-wide-control_491-650.pdf
- Charmillot, P. J., D. Pasquier, and D. Hofer. 2002.** Control of codling moth *Cydia pomonella* by autosterilization. Bulletin IOBC/SROP 25: 117–120.
- Chen, Z. Z., Y. C. Dong, Y. H. Wang, A. A. Andongma, M. A. Rashid, P. Krutmuang, and C. Y. Niu. 2016.** Pupal diapause termination in *Bactrocera minax*: an insight on 20-hydroxyecdysone induced phenotypic and genotypic expressions. Scientific Reports 6: 27440. <https://doi.org/10.1038/srep27440>
- Cohen, A. C. 2015.** Insect diets: science and technology. Second edition. CRC Press, Boca Raton, FL, USA. 473 pp. <https://www.crcpress.com/Insect-Diets-Science-and-Technology-Second-Edition/Cohen/p/book/9781466591943>
- Colinet, H., B. J. Sinclair, P. Vernon, and D. Renault. 2015.** Insects in fluctuating thermal environments. Annual Review of Entomology 60: 123–140. <https://doi.org/10.1146/annurev-ento-010814-021017>
- Concha, C., and M. J. Scott. 2009.** Sexual development in *Lucilia cuprina* (Diptera, Calliphoridae) is controlled by the transformer gene. Genetics 182: 785–798. <https://doi.org/10.1534/genetics.109.100982>
- Concha, C., A. Palavesam, F. D. Guerrero, A. Sagel, F. Li, J. A. Osborne, Y. Hernandez, T. Pardo, G. Quintero, M. Vasquez, G. P. Keller, P. L. Phillips, J. B. Welch, W. O. McMillan, S. R. Skoda, and M. J. Scott. 2016.** A transgenic male-only strain of the New World screwworm for an improved control program using the sterile insect technique. BMC Biology 14: 72. <https://doi.org/10.1186/s12915-016-0296-8>
- Coronado-Gonzalez, P. A., S. Vijayasegaran, and A. S. Robinson. 2008.** Functional morphology of the mouthparts of the adult Mediterranean fruit fly, *Ceratitis capitata*. Journal of Insect Science 8: 73. <https://doi.org/10.1673/031.008.7301>
- Demirbas-Uzel, G., L. De Vooght, A. G. Parker, M. J. B. Vreysen, R. L. Mach, J. Van Den Abbeele, and A. M. M. Abd-Alla. 2018.** Combining paratransgenesis with SIT: impact of ionizing radiation on the DNA copy number of *Sodalis glossinidius* in tsetse flies. BMC Microbiology 18(Suppl. 1): 160. <https://doi.org/10.1186/s12866-018-1283-8>
- Devine, G. J., E. Z. Perea, G. F. Killeen, J. D. Stancil, S. J. Clark, and A. C. Morrison. 2009.** Using adult mosquitoes to transfer insecticides to *Aedes aegypti* larval habitats. Proceedings of the National Academy of Sciences of the USA. 106(28): 11530–11534. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2702255/>
- Devorshak, C. 2007.** Area-wide integrated pest management programmes and agricultural trade: challenges and opportunities for regulatory plant protection, pp. 407–415. *In* M. J. B. Vreysen, A. S. Robinson and J. Hendrichs (eds.), Area-wide control of insect pests. From research to field implementation. Springer, Dordrecht, The Netherlands. <https://www.iaea.org/sites/default/files/area-wide-control-insect-pests-book.pdf>
- De Vooght, L., S. Van Keer, and J. Van Den Abbeele. 2018.** Towards improving tsetse fly paratransgenesis: stable colonization of *Glossina morsitans morsitans* with genetically modified *Sodalis*. BMC Microbiology 18(Suppl. 1):165. <https://doi.org/10.1186/s12866-018-1282-9>
- Dodson, B. L., G. L. Hughes, O. Paul, A. C. Matarachiero, L. D. Kramer, and J. L. Rasgon. 2014.** *Wolbachia* enhances West Nile virus (WNV) infection in the mosquito *Culex tarsalis*. PLOS Neglected Tropical Diseases 8: e2965. <https://doi.org/10.1371/journal.pntd.0002965>
- Dowell, R. V., I. A. Siddiqui, F. Meyer, and E. L. Spaugy. 2000.** Mediterranean fruit fly preventative release programme in southern California, pp. 369–375. *In* K. H. Tan (ed.), Proceedings: Area-Wide Control of Fruit Flies and Other Insect Pests. International Conference on Area-Wide Control of Insect Pests, and the 5th International Symposium on Fruit Flies of Economic Importance, 28 May–5 June 1998, Penang, Malaysia. Penerbit Universiti Sains Malaysia, Pulau Pinang, Malaysia. http://www.naweb.iaea.org/nafa/ipc/public/Area-wide-control_351-490.pdf
- (EFSA) European Food Safety Authority. 2013.** Guidance on the environmental risk assessment of genetically modified animals. Panel on Genetically Modified Organisms (GMO), EFSA, Parma, Italy. EFSA Journal 11(5): 3200. <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2013.3200>

- Ekechukwu, N. E., R. Baeshen, S. F. Traorè, M. Coulibaly, A. Diabate, F. Catteruccia, and F. Tripet. 2015. Heterosis increases fertility, fecundity, and survival of laboratory-produced F₁ hybrid males of the malaria mosquito *Anopheles coluzzii*. G3: Genes, Genomes, Genetics 5(12): 2693–2709. <https://doi.org/10.1534/g3.115.021436>
- Enkerlin, W. R., and M. M. Quinlan. 2004. Development of an international standard to facilitate the transboundary shipment of sterile insects, pp. 203–212. In B. N. Barnes (ed.), Proceedings, Symposium: 6th International Symposium on Fruit Flies of Economic Importance, 6–10 May 2002, Stellenbosch, South Africa. Iste Scientific Publications, Irene, South Africa. https://nucleus.iaea.org/sites/naipc/twd/Documents/6thISFFEI_Proceedings/6th-ISFFEI-Proceedings-2004-Stellenbosch-South-Africa.pdf
- (EPA) United States Environmental Protection Agency. 2017. EPA registers the *Wolbachia* ZAP strain in live male Asian tiger mosquitoes. For release November 7, 2017. EPA-HQ-OPP-2016-0205.
- Epsky, N. D., P. E. Kendra, and E. Q. Schnell. 2014. History and development of food-based attractants, pp. 75–118. In T. Shelly, N. Epsky, E. B. Jang, J. Reyes-Flores and R. Vargas (eds.), Trapping and the detection, control, and regulation of tephritid fruit flies. Springer Science+Business Media, Dordrecht, The Netherlands.
- Evans, B. R., P. Kotsakiozi, A. L. Costa-da-Silva, R. S. Ioshino, L. Garziera, M. C. Pedrosa, A. Malavasi, J. F. Virginio, M. L. Capurro, and J. R. Powell. 2019. Transgenic *Aedes aegypti* mosquitoes transfer genes into a natural population. Scientific Reports 9: 13047. <https://doi.org/10.1038/s41598-019-49660-6>
- Everaarts, T. C. 2016. Uienvlieg houdt van luwte en zand. Uienkoerier Mei 2016. <http://www.hlbv.nl/images/pdf/uienkoerier%20mei%202016.pdf>
- (FAO) Food and Agriculture Organization of the United Nations. 1992. The New World screwworm eradication programme. North Africa 1988–1992. FAO, Rome, Italy. <https://www.iaea.org/sites/default/files/18/03/ipc-screwworm-libya-1992-full.pdf>
- (FAO) Food and Agriculture Organization of the United Nations. 1996. Code of conduct for the import and release of exotic biological control agents. International Standard for Phytosanitary Measures (ISPM) Number 3, International Plant Protection Convention (IPPC). FAO, Rome, Italy.
- (FAO) Food and Agriculture Organization of the United Nations. 2016a. Requirements for the establishment of pest free places of production and pest free production sites. International Standard for Phytosanitary Measures (ISPM) Number 10, adopted 1999, published 2016. International Plant Protection Convention (IPPC). FAO, Rome, Italy.
- (FAO) Food and Agriculture Organization of the United Nations. 2016b. Requirements for the establishment of areas of low pest prevalence. International Standard for Phytosanitary Measures (ISPM) Number 22, adopted 2005, published 2016. International Plant Protection Convention (IPPC). FAO, Rome, Italy.
- (FAO) Food and Agriculture Organization of the United Nations. 2017a. Requirements for the establishment of pest free areas. International Standard for Phytosanitary Measures (ISPM) Number 4, adopted 2005, published 2017. International Plant Protection Convention (IPPC). FAO, Rome, Italy.
- (FAO) Food and Agriculture Organization of the United Nations. 2017b. Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms. International Standard for Phytosanitary Measures (ISPM) Number 3, adopted 2005, published 2017. International Plant Protection Convention (IPPC). FAO, Rome, Italy.
- (FAO) Food and Agriculture Organization of the United Nations. 2017c. Glossary of phytosanitary terms (as adopted by CPM-11 and CPM-12 ink amendments). International Standard for Phytosanitary Measures (ISPM) Number 5, adopted 2016, published 2017. International Plant Protection Convention (IPPC). FAO, Rome, Italy.
- (FAO/IAEA) Food and Agriculture Organization of the United Nations/International Atomic Energy Agency. 2006. Status and risk assessment of the use of transgenic arthropods in plant protection. Proceedings of a technical meeting held in Rome, 8–12 May 2002. IAEA-TECDOC-1483. FAO/IAEA, Vienna, Austria. 155 pp. <https://www.iaea.org/resources/technical-report/status-and-risk-assessment-of-the-use-of-transgenic-arthropods-in-plant-protection>
- (FAO/IAEA) Food and Agriculture Organization of the United Nations/International Atomic Energy Agency. 2008. Model business plan for a sterile insect production facility. IAEA, Vienna, Austria. <https://www.iaea.org/publications/7130/model-business-plan-for-a-sterile-insect-production-facility>
- (FAO/IAEA) Food and Agriculture Organization of the United Nations/International Atomic Energy Agency. 2017a. Guideline for packing, shipping, holding and release of sterile flies in area-wide fruit fly

- control programmes. J. L. Zavala-López and W. R. Enkerlin (eds.). Second edition. Rome, Italy. 140 pp.
<http://www-naweb.iaea.org/nafa/ipc/public/Guideline-for-Packing-Sept2017.pdf>
- (FAO/IAEA) Food and Agriculture Organization of the United Nations/International Atomic Energy Agency. 2017b.** Dormancy management to enable mass-rearing and increase efficacy of sterile insects and natural enemies. Report of the Third Research Coordination Meeting of an FAO/IAEA CRP held 29 May–2 June 2017 in Vienna, Austria. IAEA, Vienna, Austria. 63 pp.
<http://www-naweb.iaea.org/nafa/ipc/crp/RCM3DormancyReport.pdf>
<http://www-naweb.iaea.org/nafa/ipc/crp/ipc-dormancy-mass-rearing.html>
- (FAO/IAEA) Food and Agriculture Organization of the United Nations/International Atomic Energy Agency. 2018a.** History of transboundary shipment of sterile insects. IAEA, Vienna, Austria.
<https://nucleus.iaea.org/sites/naipc/dirsit/SitePages/HISTORY%20OF%20TRANSBOUNDARY%20SHIPMENTS%20OF%20STERILE%20INSECTS.aspx?WikiPageMode=Edit&InitialTabId=Ribbon.EditingTools.CPEditTab&VisibilityContext=WSSWikiPage>
- (FAO/IAEA) Food and Agriculture Organization of the United Nations/International Atomic Energy Agency. 2018b.** “The dashboard” for managers of sterile insect technique production facilities. A mass-rearing metrics and monitoring tool. P. Rendón. A. Aldana and C Cáceres (eds.). FAO/IAEA, Vienna, Austria. 18 pp.
https://www.iaea.org/sites/default/files/23_mar_2018_corrected_version_mmb_-_19_march_2018_-_b5.pdf
- (FAO/IAEA) Food and Agriculture Organization of the United Nations/International Atomic Energy Agency. 2019.** Use of entomopathogenic fungi for fruit fly control in area-wide SIT programmes. A. Villaseñor, S. Flores, S. E. Campos, J. Toledo, P. Montoya, P. Liedo and W. Enkerlin (eds.). Vienna, Austria. 43 pp. <https://www.iaea.org/sites/default/files/10072019-eng.pdf>
- (FAO/IAEA/USDA) Food and Agriculture Organization of the United Nations/International Atomic Energy Agency/United States Department of Agriculture. 2019.** Product quality control for sterile mass-reared and released tephritid fruit flies. Version 7.0. IAEA, Vienna, Austria. 148 pp.
<https://www.iaea.org/sites/default/files/qcv7.pdf>
- Fasulo, B., A. Meccariello, P. A. Papathanos, and N. Windbichler. 2021.** Synthetic sex ratio distorters based on CRISPR for the control of harmful insect populations, pp. 843–855. *In* J. Hendrichs, R. Pereira and M. J. B. Vreysen (eds.), Area-wide integrated pest management. Development and field application. CRC Press, Boca Raton, FL, USA.
- Fatima, B., N. Ahmad, R. M. Memon, M. Bux, and Q. Ahmad. 2009.** Enhancing biological control of sugarcane shoot borer, *Chilo infuscatellus* (Lepidoptera: Pyralidae), through use of radiation to improve laboratory rearing and field augmentation of egg and larval parasitoids. *Biocontrol Science and Technology* 19(S1): 277–290. http://www-naweb.iaea.org/nafa/ipc/crp/Biocontrol_final.pdf
- Fisher, K., and C. Caceres. 2000.** A filter rearing system for mass reared genetic sexing strains of Mediterranean fruit fly (Diptera: Tephritidae), pp. 543–550. *In* K. H. Tan (ed.), Proceedings: Area-Wide Control of Fruit Flies and Other Insect Pests. International Conference on Area-Wide Control of Insect Pests, and the 5th International Symposium on Fruit Flies of Economic Importance, 28 May–5 June 1998, Penang, Malaysia. Penerbit Universiti Sains Malaysia, Pulau Pinang, Malaysia.
http://www-naweb.iaea.org/nafa/ipc/public/Area-wide-control_491-650.pdf
- Flores, H. A., and S. L. O'Neill. 2018.** Controlling vector-borne diseases by releasing modified mosquitoes. *Nature Reviews Microbiology* 16: 508–518. <https://doi.org/10.1038/s41579-018-0025-0>
- Flores, S., S. Campos, A. Villaseñor, A. Valle, W. Enkerlin, J. Toledo, P. Liedo, and P. Montoya. 2013.** Sterile males of *Ceratitidis capitata* (Diptera: Tephritidae) as disseminators of *Beauveria bassiana* conidia for IPM strategies. *Biocontrol Science and Technology* 23: 1186–1198.
<https://www.tandfonline.com/doi/abs/10.1080/09583157.2013.822473>
- Foley IV, E. W., R. L. Morreale, D. F. Hoel, and A. M. Lloyd. 2021.** Area-wide mosquito management in Lee County, Florida, USA, pp. 319–338. *In* J. Hendrichs, R. Pereira and M. J. B. Vreysen (eds.), Area-wide integrated pest management. Development and field application. CRC Press, Boca Raton, FL, USA.
- Follett, P. A., and J. J. Duan (eds.). 2000.** Non-target effects of biological control. Springer Science+Business Media, LLC, New York, USA. 316 pp.
- Fournier, F. 2014.** Développement d’une stratégie de lutte intégrée de la mouche de l’oignon ‘sans pesticides au semis’ financièrement accessible aux producteurs maraîchers. Rapport final. 49 pp.
<https://prisme.ca/wp-content/uploads/2016/11/Rapport-final-PCAA-mouches-steriles-2014.pdf>

- Galizi, R., A. Hammond, K. Kyrou, C. Taxiarchi, F. Bernardini, S. M. O'Loughlin, P. A. Papatianos, T. Nolan, N. Windbichler, and A. Crisanti. 2016.** A CRISPR-Cas9 sex-ratio distortion system for genetic control. *Scientific Reports* 6: 31139. DOI: 10.1038/srep31139
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4971495/>
- Gavriel, S., E. Jurkevitch, Y. Gazit, and B. Yuval. 2011.** Bacterially enriched diet improves sexual performance of sterile male Mediterranean fruit flies. *Journal of Applied Entomology* 135: 564–573.
<https://doi.org/10.1111/j.1439-0418.2010.01605.x>
- Gilchrist, A. S., and A. W. Meats. 2014.** An evaluation of outcrossing to improve mass-reared strains of the Queensland fruit fly *Bactrocera tryoni*. *International Journal of Tropical Insect Science* 34(S1): S35–S44. <https://doi.org/10.1017/S1742758414000216>
- Gong, P., M. J. Epton, G. Fu, S. Scaife, A. Hiscox, K. C. Condon, G. C. Condon, N. I. Morrison, D. W. Kelly, T. Dafa'alla, P. G. Coleman, and L. Alphey. 2005.** A dominant lethal genetic system for autocidal control of the Mediterranean fruitfly. *Nature Biotechnology* 23: 453–456.
<https://doi.org/10.1038/nbt1071>
- Hagler, J. R., and C. G. Jackson. 2001.** Methods for marking insects: current techniques and future prospects. *Annual Review of Entomology* 46: 511–543. <https://doi.org/10.1146/annurev.ento.46.1.511>
- Hamm, J. J., and J. E. Carpenter. 1997.** Compatibility of nuclear polyhedrosis viruses and inherited sterility for control of corn earworm and fall armyworm (Lepidoptera: Noctuidae). *Journal of Entomological Science* 32: 148–153. <https://doi.org/10.18474/0749-8004-32.2.148>
- Handler, A. M. 2002.** Prospects for using genetic transformation for improved SIT and new biocontrol methods. *Genetica* 116: 137–149. <https://doi.org/10.1023/A:1020924028450>
- Haq, I., and J. Hendrichs. 2013.** Pre-release feeding on hydrolysed yeast and methoprene treatment enhances male *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae) longevity. *Journal of Applied Entomology* 137(Issue s1): 99–102. <https://doi.org/10.1111/j.1439-0418.2011.01617.x>
- Haq, I. U., M. J. B. Vreysen, P. E. A. Teal, and J. Hendrichs. 2014.** Methoprene application and diet protein supplementation to male melon fly, *Bactrocera cucurbitae*, modifies female remating behavior. *Insect Science* 21: 637–646. <https://doi.org/10.1111/1744-7917.12073>
- Haq, I. U., C. Cáceres, J. S. Meza, J. Hendrichs, and M. J. B. Vreysen. 2018.** Different methods of methyl eugenol application enhance the mating success of male oriental fruit fly (Diptera: Tephritidae). *Scientific Reports* 8: 6033. <https://doi.org/10.1038/s41598-018-24518-5>
- Harris, A. F., A. R. McKemey, D. Nimmo, Z. Curtis, I. Black, S. A. Morgan, M. N. Oviedo, R. Lacroix, N. Naish, N. I. Morrison, A. Collado, J. Stevenson, S. Scaife, T. Dafa'alla, G. Fu, C. Phillips, A. Miles, N. Raduan, N. Kelly, C. Beech, C. A. Donnelly, W. D. Petrie, and L. Alphey. 2012.** Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. *Nature Biotechnology* 30: 828–830. <https://doi.org/10.1038/nbt.2350>
- Heinrich, J. C., and M. J. Scott. 2000.** A repressible female-specific lethal genetic system for making transgenic insect strains suitable for a sterile-release program. *Proceedings of the National Academy of Sciences of the USA* 97(15): 8229–8232. <https://doi.org/10.1073/pnas.140142697>
- Hendrichs, J., V. Wornoayporn, B. I. Katsoyannos, and K. Gaggli. 1993.** First field assessment of the dispersal and survival of mass reared sterile Mediterranean fruit fly males of an embryonal, temperature sensitive genetic sexing strain, pp. 453–462. *In* Proceedings: Management of Insect Pests: Nuclear and Related Molecular and Genetic Techniques. FAO/IAEA International Symposium, 19–23 October 1992, Vienna, Austria. STI/PUB/909. IAEA, Vienna, Austria.
<https://www.iaea.org/publications/3782/management-of-insect-pests-nuclear-and-related-molecular-and-genetic-techniques>
- Hendrichs, J., G. Franz, and P. Rendon. 1995.** Increased effectiveness and applicability of the sterile insect technique through male-only releases for control of Mediterranean fruit flies during fruiting seasons. *Journal of Applied Entomology* 119: 371–377.
<https://doi.org/10.1111/j.1439-0418.1995.tb01303.x>
- Hendrichs, J., A. S. Robinson, J. P. Cayol, and W. Enkerlin. 2002.** Medfly areawide sterile insect technique programmes for prevention, suppression or eradication: the importance of mating behavior studies. *Florida Entomologist* 85: 1–13. <http://journals.fcla.edu/flaent/article/view/75040>
- Hendrichs, J., K. Bloem, G. Hoch, J. E. Carpenter, P. Greany, and A. S. Robinson. 2009.** Improving the cost-effectiveness, trade and safety of biological control for agricultural insect pests using nuclear techniques. *Biocontrol Science and Technology* 19(Suppl. 1): 3–22.
<https://www.iaea.org/resources/scientific-paper/improving-the-cost-effectiveness-trade-and-safety-of-biological-control-for-agricultural-insect-pests-using-nuclear-techniques>

- Hendrichs, M. A., V. Wornoayporn, B. Katsoyannos, and J. Hendrichs. 2007.** Quality control method to measure predator evasion in wild and mass-reared Mediterranean fruit flies (Diptera: Tephritidae). *Florida Entomologist* 90: 64–70. <http://journals.fcla.edu/flaent/article/view/75615/73273>
- Henneberry, T. J. 2007.** Integrated systems for control of the pink bollworm *Pectinophora gossypiella* in cotton, pp. 567–579. *In* M. J. B. Vreysen, A. S. Robinson and J. Hendrichs (eds.), *Area-wide control of insect pests. From research to field implementation*. Springer, Dordrecht, The Netherlands. <https://www.iaea.org/sites/default/files/area-wide-control-insect-pests-book.pdf>
- Higgs, S. 2018.** It is official: vector-borne and zoonotic diseases in the United States and territories on the rise—now what? *Vector-Borne and Zoonotic Diseases* 18(6): 285. <https://www.liebertpub.com/doi/10.1089/vbz.2018.29002.hig>
- Hoffmann, A. A., and P. A. Ross. 2018.** Rates and patterns of laboratory adaptation in (mostly) insects. *Journal of Economic Entomology* 111: 501–509. <https://doi.org/10.1093/jeetoy024>
- Hoffmann, A. A., I. Iturbe-Ormaetxe, A. G. Callahan, B. L. Phillips, K. Billington, J. K. Axford, B. Montgomery, A. P. Turley, and S. L. O'Neill. 2014.** Stability of the wMel *Wolbachia* infection following invasion into *Aedes aegypti* populations. *PLOS Neglected Tropical Diseases* 8(9): e3115. <https://doi.org/10.1371/journal.pntd.0003115>
- Horn, C., and E. A. Wimmer. 2003.** A transgene-based, embryo-specific lethality system for insect pest management. *Nature Biotechnology* 21: 64–70. <https://www.nature.com/articles/nbt769>
- Horner, R. M., J. T. S. Walker, D. J. Rogers, P. L. Lo, and D. M. Suckling. 2016.** Use of the sterile insect technique in New Zealand: benefits and constraints. *New Zealand Plant Protection* 69: 296–304. https://nzpps.org/nzpp_abstract.php?paper=692960
- Howse, P., C. Armsworth, and I. Baxter. 2007.** Autodissemination of semiochemicals and pesticides: a new concept compatible with the sterile insect technique, pp. 275–281. *In* M. J. B. Vreysen, A. S. Robinson and J. Hendrichs (eds.), *Area-wide control of insect pests. From research to field implementation*. Springer, Dordrecht, The Netherlands. <https://www.iaea.org/sites/default/files/area-wide-control-insect-pests-book.pdf>
- Hoy, M. A. 1992.** Genetic engineering of predators and parasitoids for pesticide resistance, pp. 37–49. *In* I. Denholm, A. L. Devonshire and D. W. Hollomon (eds.), *Resistance 91: achievements and developments in combating pesticide resistance*. Elsevier Applied Science, London, UK.
- Hoy, M. A. 1995.** Impact of risk analyses on pest-management programs employing transgenic arthropods. *Parasitology Today* 11: 229–232. [https://doi.org/10.1016/0169-4758\(95\)80086-7](https://doi.org/10.1016/0169-4758(95)80086-7)
- Hoy, M. A. 2000.** Deploying transgenic arthropods in pest management programs: risks and realities, pp. 319–334. *In* A. A. James and A. M. Handler (eds.), *Insect transgenesis – methods and applications*. CRC Press, Boca Raton, FL, USA.
- Hughes, G. L., B. L. Dodson, R. M. Johnson, C. C. Murdock, H. Tsujimoto, Y. Suzuki, A. A. Patt, L. Cui, C. W. Noss, R. M. Barry, J. M. Sakamoto, E. A. Hornett, and J. L. Rasgon. 2014.** Native microbiome impedes vertical transmission of *Wolbachia* in *Anopheles* mosquitoes. *Proceedings of the National Academy of Sciences of the USA* 111(34): 12498–12503. <https://doi.org/10.1073/pnas.1408888111>
- (IAEA) International Atomic Energy Agency. 2002.** Evaluation of Lepidoptera population suppression by radiation induced sterility. IAEA-TECDOC 1283. ISSN 1011-4289. Vienna, Austria. 120 pp. <https://www.iaea.org/publications/6426/evaluation-of-lepidoptera-population-suppression-by-radiation-induced-sterility>
- (IAEA) International Atomic Energy Agency. 2003.** Automation for tsetse mass rearing for use in sterile insect technique programmes. Final report of a co-ordinated research project 1995–2001. IAEA-TECDOC-1353. IAEA, Vienna, Austria. <https://www.iaea.org/publications/6813/automation-for-tsetse-mass-rearing-for-use-in-sterile-insect-technique-programmes>
- (IAEA) International Atomic Energy Agency. 2012.** Developing alternatives to gamma irradiation for the sterile insect technique, pp. 81–91. *In* *Nuclear Technology Review* 2012. Vienna, Austria. 170 pp. <https://www.iaea.org/resources/scientific-paper/developing-alternatives-to-gamma-irradiation-for-the-sterile-insect-technique>
- (IAEA) International Atomic Energy Agency. 2017.** Technical specification for an X-ray system for the irradiation of insects for the sterile insect technique and other related technologies. Vienna, Austria. <https://www.iaea.org/resources/manual/technical-specification-for-an-x-ray-system-for-the-irradiation-of-insects-for-the-sterile-insect-technique-and-other-related-technologies>
- (IBMA) International Biocontrol Manufacturers Association. 2018.** <http://www.ibma-global.org/>
- Itô, Y., M. Yamagishi, and H. Kuba. 1993.** Mating behaviour of the melon fly. Sexual selection and sperm competition, pp. 441–452. *In* *Proceedings: Management of Insect Pests: Nuclear and Related*

- Molecular and Genetic Techniques. FAO/IAEA International Symposium, 19–23 October 1992, Vienna, Austria. STI/PUB/909. IAEA, Vienna, Austria.
<https://www.iaea.org/publications/3782/management-of-insect-pests-nuclear-and-related-molecular-and-genetic-techniques>
- Itô, Y., H. Kakinohana, M. Yamagishi, and T. Kohama. 2003.** Eradication of the melon fly, *Bactrocera cucurbitae*, from Okinawa, Japan, by means of the sterile insect technique, with special emphasis on the role of basic studies. *Journal of Asia-Pacific Entomology* 6: 119–129.
[https://doi.org/10.1016/S1226-8615\(08\)60177-6](https://doi.org/10.1016/S1226-8615(08)60177-6)
- James, S., F. H. Collins, P. A. Welkhoff, C. Emerson, H. C. J. Godfray, M. Gottlieb, B. Greenwood, S. W. Lindsay, C. M. Mbogo, F. O. Okumu, H. Quemada, M. Savadogo, J. A. Singh, K. H. Tountas, and Y. T. Touré. 2018.** Pathway to deployment of gene drive mosquitoes as a potential biocontrol tool for elimination of malaria in sub-Saharan Africa: recommendations of a scientific working group. *The American Journal of Tropical Medicine and Hygiene* 98(6 Suppl.): 1–49.
<https://doi.org/10.4269/ajtmh.18-0083>
- Johnson, B. J., S. N. Mitchell, C. J. Paton, J. Stevenson, K. M. Staunton, N. Snoch, N. Beebe, B. J. White, and S. A. Ritchie. 2017.** Use of rhodamine B to mark the body and seminal fluid of male *Aedes aegypti* for mark-release-recapture experiments and estimating efficacy of sterile male releases. *PLOS Neglected Tropical Diseases* 11(9): e0005902. <https://doi.org/10.1371/journal.pntd.0005902>
- Juan-Blasco, M., A. Urbaneja, V. San Andrés, P. Castañera, and B. Sabater-Muñoz. 2013.** Improving the sterile sperm identification method for its implementation in the area-wide sterile insect technique program against *Ceratitis capitata* (Diptera: Tephritidae) in Spain. *Journal of Economic Entomology* 106: 2541–2547. <https://doi.org/10.1603/EC13064>
- Juan-Blasco, M., B. Sabater-Muñoz, I. Pla, R. Argilés, P. Castañera, J. A. Jacas, M. V. Ibáñez-Gual, and A. Urbaneja. 2014.** Estimating SIT-driven population reduction in the Mediterranean fruit fly, *Ceratitis capitata*, from sterile mating. *Bulletin of Entomological Research* 104: 233–242.
<https://doi.org/10.1017/S0007485313000692>
- Kariithi, H. M., I. K. Meki, D. I. Schneider, L. De Vooght, F. M. Khamis, A. Geiger, G. Demirbaş-Uzel, J. M. Vlak, I. A. Ince, S. Kelm, F. Njiokou, F. N. Wamwiri, I. I. Malele, B. L. Weiss, and A. M. M. Abd-Alla. 2018.** Enhancing vector refractoriness to trypanosome infection: achievements, challenges and perspectives. *BMC Microbiology* 18(Suppl. 1): 179.
<https://doi.org/10.1186/s12866-018-1280-y>
- Kaspi, R., and M. P. Parrella. 2006.** Improving the biological control of leafminers (Diptera: Agromyzidae) using the sterile insect technique. *Journal of Economic Entomology* 99: 1168–1175.
<https://doi.org/10.1093/jee/99.4.1168>
- Kaspi, R., and M. P. Parrella. 2008.** Synergistic interaction between parasitoids and sterile insects. *Integrated Control in Protected Crops. Temperate Climate, International Organization of Biological Control/Western Palearctic Region Section Bulletin* 32: 99–102.
http://www.naweb.iaea.org/nafa/ipc/crp/Biocontrol_final.pdf
- Kean, J. M., A. E. A. Stephens, S. L. Wee, and D. M. Suckling. 2007.** Optimizing strategies for eradication of discrete-generation lepidopteran pests using inherited sterility, pp. 211–220. *In* M. J. B. Vreysen, A. S. Robinson and J. Hendrichs (eds.), *Area-wide control of insect pests. From research to field implementation*. Springer, Dordrecht, The Netherlands.
<https://www.iaea.org/sites/default/files/area-wide-control-insect-pests-book.pdf>
- Klassen, W., and J. F. Creech. 1973.** Population suppression with dominant and conditional lethal mutations. Some important considerations and approaches, pp. 65–79. *In* *Proceedings, Panel: Computer Models and Application of the Sterile-Male Technique*. Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture, 13–17 December 1971, Vienna, Austria. STI/PUB/340. IAEA, Vienna, Austria.
https://www.iaea.org/sites/default/files/el_computer_models.pdf
- Knipling, E. F. 1979.** The basic principles of insect population suppression and management. *Agriculture Handbook Number 512*. SEA, USDA, Washington, DC, USA.
- Knipling, E. F. 1992.** Principles of insect parasitism analysed from new perspectives: practical implications for regulating insect populations. *Agricultural Handbook Number 693*. United States Department of Agriculture, Washington, DC, USA.
- Knols, B. G. J., B. N. Njiru, E. M. Mathenge, W. R., Mukabana, J. C. Beier, and G. F. Killeen. 2002.** MalariaSphere: a greenhouse-enclosed simulation of a natural *Anopheles gambiae* (Diptera: Culicidae) ecosystem in western Kenya. *Malaria Journal* 1: 1–19. <https://doi.org/10.1186/1475-2875-1-19>

- Kouloussis, N. A., B. I. Katsoyannos, N. T. Papadopoulos, C. S. Ioannou, and I. V. Iliadis. 2013. Enhanced mating competitiveness of *Ceratitis capitata* males following exposure to citrus compounds. *Journal of Applied Entomology* 137(Issue s1): 30–38. <https://doi.org/10.1111/j.1439-0418.2010.01535.x>
- Kouloussis, N. A., C. D. Gerofotis, C. S. Ioannou, I. V. Iliadis, N. T. Papadopoulos, and D. S. Koveos. 2017. Towards improving sterile insect technique: exposure to orange oil compounds increases sexual signalling and longevity in *Ceratitis capitata* males of the Vienna 8 GSS. *PLOS ONE* 12(11): e0188092 <https://doi.org/10.1371/journal.pone.0188092>
- Kyritsis, G. A., A. A. Augustinos, C. Cáceres, and K. Bourtzis. 2017. Medfly gut microbiota and enhancement of the sterile insect technique: similarities and differences of *Klebsiella oxytoca* and *Enterobacter* sp. AA26 probiotics during the larval and adult stages of the VIENNA 8^{D53+} genetic sexing strain. *Frontiers in Microbiology* 8: 2064. <https://doi.org/10.3389/fmicb.2017.02064>
- Kyrou, K., A. M. Hammond, R. Galizi, N. Kranjc, A. Burt, A. K. Beaghton, T. Nolan, and A. Crisanti. 2018. A CRISPR–Cas9 gene drive targeting *doublesex* causes complete population suppression in caged *Anopheles gambiae* mosquitoes. *Nature Biotechnology* 36: 1062–1066. <https://doi.org/10.1038/nbt.4245>
- Lagos, D., M. Koukidou, C. Savakis, and K. Komitopoulou. 2007. The *transformer* gene in *Bactrocera oleae*: the genetic switch that determines its sex fate. *Insect Molecular Biology* 16: 221–230. <https://doi.org/10.1111/j.1365-2583.2006.00717.x>
- Lanouette, G., J. Brodeur, F. Fournier, V. Martel, M. Vreysen, C. Cáceres, and A. Firlej. 2017. The sterile insect technique for the management of the spotted wing drosophila, *Drosophila suzukii*: establishing the optimum irradiation dose. *PLOS ONE* 12(9): e0180821. <https://doi.org/10.1371/journal.pone.0180821>
- Launzon, C. R., S. Potter, S. McCombs, N. Niyazi, and T. Shelly. 2004. Maternal spread of *Enterobacter agglomerans* and *Klebsiella pneumoniae* through populations of the Mediterranean fruit fly: another piece of the symbiotic puzzle. Poster, Proceedings XXII International Congress of Entomology, 15–21 August 2004, Brisbane, Queensland, Australia.
- Leal Mubbarqui, R. L., R. C. Perez, R. Angulo Kladt, J. L. Zavala Lopez, A. Parker, M. T. Seck, B. Sall, and J. Bouyer. 2014. The smart aerial release machine, a universal system for applying the sterile insect technique. *PLOS ONE* 9(7): e103077. <https://doi.org/10.1371/journal.pone.0103077>
- Lebon, C., A. Benlali, C. Atyame, P. Mavingui, and P. Tortosa. 2018. Construction of a genetic sexing strain for *Aedes albopictus*: a promising tool for the development of sterilizing insect control strategies targeting the tiger mosquito. *Parasites and Vectors* 11(Suppl. 2): 658. <https://doi.org/10.1186/s13071-018-3212-y>
- Lees, R. S., J. R. L. Gilles, J. Hendrichs, M. J. B. Vreysen, and K. Bourtzis. 2015. Back to the future: the sterile insect technique against mosquito disease vectors. *Current Opinion in Insect Science* 10: 156–162. <https://doi.org/10.1016/j.cois.2015.05.011>
- Lehane, M. J., and S. Aksoy. 2012. Control using genetically modified insects poses problems for regulators. *PLOS Neglected Tropical Diseases* 6(1): e1495. <https://doi.org/10.1371/journal.pntd.0001495>
- Leopold, R. A. 2007. Colony maintenance and mass-rearing: using cold storage technology for extending the shelf-life of insects, pp. 149–162. *In* M. J. B. Vreysen, A. S. Robinson and J. Hendrichs (eds.), *Area-wide control of insect pests. From research to field implementation*. Springer, Dordrecht, The Netherlands. <https://www.iaea.org/sites/default/files/area-wide-control-insect-pests-book.pdf>
- Leopold, R. A., W. B. Wang, D. R. Berkebile, and T. P. Freeman. 2001. Cryopreservation of embryos of the New World screwworm *Cochliomyia hominivorax* (Diptera: Calliphoridae). *Annals of the Entomological Society of America* 94: 695–701. [https://doi.org/10.1603/0013-8746\(2001\)094\[0695:COEOTN\]2.0.CO;2](https://doi.org/10.1603/0013-8746(2001)094[0695:COEOTN]2.0.CO;2)
- Liedo, P., S. Salgado, A. Oropeza, and J. Toledo. 2007. Improving mating performance of mass-reared sterile Mediterranean fruit flies (Diptera: Tephritidae) through changes in adult holding conditions: demography and mating competitiveness. *Florida Entomologist* 90: 33–40. <http://journals.fcla.edu/flaent/article/view/75611/73269>
- Lindquist, D. A., M. Abusowa, and M. J. R. Hall. 1992. The New World screwworm fly in Libya: a review of its introduction and eradication. *Medical and Veterinary Entomology* 6: 2–8. <https://doi.org/10.1111/j.1365-2915.1992.tb00027.x>
- Loosjes, M. 2000. The sterile insect technique for commercial control of the onion fly, pp. 181–184. *In* K. H. Tan (ed.), *Proceedings: Area-Wide Control of Fruit Flies and Other Insect Pests*. International Conference on Area-Wide Control of Insect Pests, and the 5th International Symposium on Fruit Flies

- of Economic Importance, 28 May–5 June 1998, Penang, Malaysia. Penerbit Universiti Sains Malaysia, Pulau Pinang, Malaysia. http://www-naweb.iaea.org/nafa/ipc/public/Area-wide-control_73-229.pdf
- Mackauer, M.** 1976. Genetic problems in the production of biological control agents. *Annual Review of Entomology* 21: 369–385. <https://doi.org/10.1146/annurev.en.21.010176.002101>
- Mahon, R. J., and H. Ahmad.** 2000. Mass rearing the Old World screw-worm fly, *Chrysomya bezziana*, pp. 325–328. *In* K. H. Tan (ed.), *Proceedings: Area-Wide Control of Fruit Flies and Other Insect Pests. International Conference on Area-Wide Control of Insect Pests, and the 5th International Symposium on Fruit Flies of Economic Importance, 28 May–5 June 1998, Penang, Malaysia.* Penerbit Universiti Sains Malaysia, Pulau Pinang, Malaysia. http://www-naweb.iaea.org/nafa/ipc/public/Area-wide-control_230-350.pdf
- Mains, J. W., C. L. Brelsfoard, R. I. Rose, and S. L. Dobson.** 2016. Female adult *Aedes albopictus* suppression by *Wolbachia*-infected male mosquitoes. *Scientific Reports* 6: 33846. <https://doi.org/10.1038/srep33846>
- Mangan, R. L.** 1992. Evaluating the role of genetic change in insect colonies maintained for pest management, pp. 269–288. *In* T. E. Anderson and N. C. Leppla (eds.), *Advances in insect rearing for research and management.* Westview Press, Boulder, CO, USA.
- Maniania, N. K., and S. Ekesi.** 2013. The use of entomopathogenic fungi in the control of tsetse flies. *Journal of Invertebrate Pathology* 112(Suppl. 1): S83–S88. <https://doi.org/10.1016/j.jip.2012.07.019>
- Mannion, C. M., J. E. Carpenter, and H. R. Gross.** 1995. Integration of inherited sterility and a parasitoid, *Archytas marmoratus* (Diptera: Tachinidae), for managing *Helicoverpa zea* (Lepidoptera: Noctuidae): acceptability and suitability of hosts. *Environmental Entomology* 24: 1679–1684. <https://doi.org/10.1093/ee/24.6.1679>
- Marec, F., L. G. Neven, A. S. Robinson, M. Vreysen, M. R. Goldsmith, J. Nagaraju, and G. Franz.** 2005. Development of genetic sexing strains in Lepidoptera: from traditional to transgenic approaches. *Journal of Economic Entomology* 98: 248–259. <https://doi.org/10.1093/jee/98.2.248>
- Marois, E., C. Scali, J. Soichot, C. Kappler, E. A. Levashina, and F. Catteruccia.** 2012. High-throughput sorting of mosquito larvae for laboratory studies and for future vector control interventions. *Malaria Journal* 11: 302. <https://doi.org/10.1186/1475-2875-11-302>
- McDonald, I. C.** 1976. Ecological genetics and the sampling of insect populations for laboratory colonization. *Environmental Entomology* 5: 815–820. <https://doi.org/10.1093/ee/5.5.815>
- McInnis, D. O., T. E. Shelly, and J. Komatsu.** 2002. Improving male mating competitiveness and survival in the field for medfly, *Ceratitis capitata* (Diptera: Tephritidae) SIT programs. *Genetica* 116: 117–124. <https://link.springer.com/article/10.1023%2FA%3A1020919927542>
- Meccariello, A., S. M. Monti, A. Romanelli, R. Colonna, P. Primo, M. G. Inghilterra, G. Del Corsano, A. Ramaglia, G. Iazzetti, A. Chiarore, F. Patti, S. D. Heinze, M. Salvemini, H. Lindsay, E. Chiavacci, A. Burger, M. D. Robinson, C. Mosimann, D. Bopp, and G. Saccone.** 2017. Highly efficient DNA-free gene disruption in the agricultural pest *Ceratitis capitata* by CRISPR-Cas9 ribonucleoprotein complexes. *Scientific Reports* 7: 10061. <https://doi.org/10.1038/s41598-017-10347-5>
- Meccariello, A., M. Salvemini, P. Primo, B. Hall, P. Koskinioti, M. Dalíková, A. Gravina, M. A. Gucciardino, F. Forlenza, M.-E. Gregoriou, D. Ippolito, S. M. Monti, V. Petrella, M. M. Perrotta, S. Schmeing, A. Ruggiero, F. Scolari, E. Giordano, K. T. Tsoumani, F. Marec, N. Windbichler, K. P. Arunkumar, K. Bourtzis, K. D. Mathiopoulos, J. Ragoussis, L. Vitagliano, Z. Tu, P. A. Papathanos, M. D. Robinson, and G. Saccone.** 2019. *Maleness-on-the-Y (MoY)* orchestrates male sex determination in major agricultural fruit fly pests. *Science* 365(6460): 1457–1460. DOI: 10.1126/science.aax1318 <https://science.sciencemag.org/content/365/6460/1457/tab-pdf>
- Mehta, K., and A. Parker.** 2011. Characterization and dosimetry of a practical X-ray alternative to self-shielded gamma irradiators. *Radiation Physics and Chemistry* 80: 107–113. <https://doi.org/10.1016/j.radphyschem.2010.08.011>
- Meza, J. S., X. Nirmala, G. J. Zimowska, C. S. Zepeda-Cisneros, and A. M. Handler.** 2011. Development of transgenic strains for the biological control of the Mexican fruit fly, *Anastrepha ludens*. *Genetica* 139: 53–62. <https://www.ncbi.nlm.nih.gov/pubmed/20737195>
- Miller, H., and J. Cochrane.** 2018. Viewpoint: how politics pollutes the FDA’s genetically modified animal regulations and stifles innovation. 30 March 2018. Genetic Literacy Project. <https://geneticliteracyproject.org/2018/03/30/viewpoint-how-politics-pollutes-the-fdas-genetically-modified-animal-regulations-and-stifles-innovation/>
- Miller, T. A.** 2010. Paratransgenesis as a potential tool for pest control: review of applied arthropod symbiosis. *Journal of Applied Entomology* 135: 474–478.

- <https://doi.org/10.1111/j.1439-0418.2010.01600.x>
- Miyatake, T., and D. Haraguchi. 1996.** Mating success in *Bactrocera cucurbitae* (Diptera: Tephritidae) under different rearing densities. *Annals of the Entomological Society of America* 89: 284–289. <https://doi.org/10.1093/aesa/89.2.284>
- Miyatake, T., and M. Yamagishi. 1993.** Active quality control in mass reared melon flies. Quantitative genetic aspects, pp. 201–213. *In* Proceedings: Management of Insect Pests: Nuclear and Related Molecular and Genetic Techniques. FAO/IAEA International Symposium, 19–23 October 1992, Vienna, Austria. STI/PUB/909. IAEA, Vienna, Austria. <https://www.iaea.org/publications/3782/management-of-insect-pests-nuclear-and-related-molecular-and-genetic-techniques>
- Miyatake, T., M. Yamagishi, and H. Kakinohana. 1997.** Quality control in mass-reared melon flies after the eradication from Japan: results from artificial selection experiments. *Bulletin Okinawa Agricultural Experiment Station* 18: 9–17.
- Moeri, O. E., J. P. Cuda, W. A. Overholt, S. Bloem, and J. E. Carpenter. 2009.** F₁ sterile insect technique: a novel approach for risk assessment of *Episimus unguiculus* (Lepidoptera: Tortricidae), a candidate biological control agent of *Schinus terebinthifolius* in the continental USA. *Biocontrol Science and Technology* 19(S1): 303–315. http://www-naweb.iaea.org/nafa/ipc/crp/Biocontrol_final.pdf
- Mutika, G. N., E. Opiyo, and A. S. Robinson. 2001.** Assessing mating performance of male *Glossina pallidipes* (Diptera: Glossinidae) using a walk-in field cage. *Bulletin of Entomological Research* 91: 281–287. <https://doi.org/10.1079/BER2001102>
- (NAPPO) North American Plant Protection Organization. 2007.** Guidelines for importation and confined field release of transgenic arthropods in NAPPO member countries. NAPPO Regional Standards for Phytosanitary Measures (RSPM), RSPM No. 27. <https://www.napppo.org/files/1814/3753/9399/RSPM27-e.pdf>
- (NAS) National Academy of Sciences. 2002.** Animal biotechnology: identifying science based concerns.
- Nature. 2018.** Foreign research funds, Australia's science minister and gene editing in Japan. *The week in science*: 24–30 August 2018. <https://www.nature.com/articles/d41586-018-06041-9>
- Navarro-Llopis, V., S. Vacas, J. Sanchis, J. Primo, and C. Alfaro. 2011.** Chemosterilant bait stations coupled with sterile insect technique: an integrated strategy to control the Mediterranean fruit fly (Diptera: Tephritidae). *Journal of Economic Entomology* 104: 1647–1655. <https://doi.org/10.1603/EC10448>
- Navarro-Llopis, V., L. Ayala, J. Sanchis, J. Primo, and P. Moya. 2015.** Field efficacy of a *Metarhizium anisopliae*-based attractant-contamination device to control *Ceratitis capitata* (Diptera: Tephritidae). *Journal of Economic Entomology* 108: 1570–1578. <https://doi.org/10.1093/jee/tov157>
- Ndo, C., Y. Poumachu, D. Metitsi, H. P. Awono-Ambene, T. Tchuinkam, J. L. R. Gilles, and K. Bourtzis. 2018.** Isolation and characterization of a temperature-sensitive lethal strain of *Anopheles arabiensis* for SIT-based application. *Parasites and Vectors* 11(Suppl. 2): 659. <https://doi.org/10.1186/s13071-018-3216-7>
- Nielsen, K. M. 2021.** New molecular genetic techniques: regulatory and societal considerations, pp. 645–654. *In* J. Hendrichs, R. Pereira and M. J. B. Vreysen (eds.), *Area-wide integrated pest management. Development and field application*. CRC Press, Boca Raton, FL, USA.
- Nikolouli, K., H. Colinet, D. Renault, T. Enriquez, L. Mouton, P. Gibert, F. Sassu, C. Cáceres, C. Stauffer, R. Pereira, and K. Bourtzis. 2018.** Sterile insect technique and *Wolbachia* symbiosis as potential tools for the control of the invasive species *Drosophila suzukii*. *Journal of Pest Science* 91: 489–503. <https://doi.org/10.1007/s10340-017-0944-y>
- Niyazi, N., C. R. Lauzon, and T. E. Shelly. 2004.** Effect of probiotic adult diets on fitness components of sterile male Mediterranean fruit flies (Diptera: Tephritidae) under laboratory and field cage conditions. *Journal of Economic Entomology* 97: 1570–1580. <https://doi.org/10.1603/0022-0493-97.5.1570>
- Niyazi, N., C. Cáceres, A. Delprat, V. Wornoayporn, E. Ramirez Santos, G. Franz, and A. S. Robinson. 2005.** Genetics and mating competitiveness of *Ceratitis capitata* (Diptera: Tephritidae) strains carrying the marker *Sergeant*, *Sr²*. *Annals of the Entomological Society of America* 98: 119–125. [https://doi.org/10.1603/0013-8746\(2005\)098\[0119:GAMCOC\]2.0.CO;2](https://doi.org/10.1603/0013-8746(2005)098[0119:GAMCOC]2.0.CO;2)
- Ogaugwu, C. E., M. F. Schetelig, and E. A. Wimmer. 2013.** Transgenic sexing system for *Ceratitis capitata* (Diptera: Tephritidae) based on female-specific embryonic lethality. *Insect Biochemistry and Molecular Biology* 43: 1–8. <https://doi.org/10.1016/j.ibmb.2012.10.010>

- Pane, A., M. Salvemini, P. D. Bovi, C. Polito, and G. Saccone. 2002. The *transformer* gene in *Ceratitis capitata* provides a genetic basis for selecting and remembering the sexual fate. *Development* 129: 3715–3725. <http://dev.biologists.org/content/129/15/3715>
- Papadopoulos, N. T., B. I. Katsoyannos, N. A. Kouloussis, and J. Hendrichs. 2001. Effect of orange peel substances on mating competitiveness of male *Ceratitis capitata*. *Entomologia Experimentalis et Applicata* 99: 253–261. <https://doi.org/10.1046/j.1570-7458.2001.00824.x>
- Parker, A., and K. Mehta. 2007. Sterile insect technique: a model for dose optimization for improved sterile insect quality. *Florida Entomologist* 90: 88–95. <http://journals.fcla.edu/flaent/article/view/75618/73276>
- Peck, S. L., and G. T. McQuate. 2000. Field tests of environmentally friendly malathion replacements to suppress wild Mediterranean fruit fly (Diptera: Tephritidae) populations. *Journal of Economic Entomology* 93: 280–289. <https://doi.org/10.1603/0022-0493-93.2.280>
- Pereira, R., B. Yuval, P. Liedo, P. E. A. Teal, T. E. Shelly, D. O. McInnis, and J. Hendrichs. 2013. Improving sterile male performance in support of programmes integrating the sterile insect technique against fruit flies. *Journal of Applied Entomology* 137(Issue s1): 178–190. <https://doi.org/10.1111/j.1439-0418.2011.01664.x>
- Phuc, H. K., M. H. Andreassen, R. S. Burton, C. Vass, M. J. Epton, G. Pape, G. Fu, K. C. Condon, S. Scaife, C. A. Donnelly, P. G. Coleman, H. White-Cooper, and L. Alphey. 2007. Late-acting dominant lethal genetic systems and mosquito control. *BMC Biology* 5: 11. <https://doi.org/10.1186/1741-7007-5-11>
- Piñero, J. C., W. Enkerlin, and N. D. Epsky. 2014. Recent developments and applications of bait stations for integrated pest management of tephritid fruit flies, pp. 457–492. *In* T. Shelly, N. Epsky, E. B. Jang, J. Reyes-Flores and R. Vargas (eds.), *Trapping and the detection, control, and regulation of tephritid fruit flies*. Springer Science+Business Media, Dordrecht, The Netherlands.
- Potamitis, I., P. Eliopoulos, and I. Rigakis. 2017. Automated remote insect surveillance at a global scale and the internet of things. *Robotics* 6(3): 19. <https://doi.org/10.3390/robotics6030019>
- Qadda, A., A. El Yaegoubi, and J. Wadjinny. 2017. Expanding area-wide Mediterranean fruit fly management in Morocco through the implementation of a mass-rearing facility, p. 175. *In* Book of Abstracts. FAO/IAEA International Conference on Area-Wide Management of Insect Pests: Integrating the Sterile Insect and Related Nuclear and Other Techniques, 22–26 May 2017, Vienna, Austria. Abstract ID 275, IAEA-CN-248. IAEA, Vienna, Austria. <http://www-naweb.iaea.org/nafe/ipc/AWM-Book-of-Abstracts.pdf>
- Reeves, R. G., and M. Phillipson. 2017. Mass releases of genetically modified insects in area-wide pest control programs and their impact on organic farmers. *Sustainability* 9: 59. <https://doi.org/10.3390/su9010059>
- Reeves, R. G., J. A. Denton, F. Santucci, J. Bryk, and F. A. Reed. 2012. Scientific standards and the regulation of genetically modified insects. *PLOS Neglected Tropical Diseases* 6(1): e1502. <https://doi.org/10.1371/journal.pntd.0001502>
- Rendón, P., D. McInnis, D. Lance, and J. Stewart. 2004. Medfly (Diptera: Tephritidae) genetic sexing: large-scale field comparison of males-only and bisexual sterile fly releases in Guatemala. *Journal of Economic Entomology* 97: 1547–1553. <https://doi.org/10.1603/0022-0493-97.5.1547>
- Robinson, A. S. 2002. Mutations and their use in insect control. *Mutation Research/Reviews in Mutation Research* 511: 113–132. [https://doi.org/10.1016/S1383-5742\(02\)00006-6](https://doi.org/10.1016/S1383-5742(02)00006-6)
- Robinson, A. S., and G. Franz. 2000. The application of transgenic insect technology in the sterile insect technique, pp. 307–319. *In* A. M. Handler and A. A. James (eds.), *Insect transgenesis: methods and applications*. CRC Press, Boca Raton, FL, USA.
- Robinson, A. S., G. Franz, and K. Fisher. 1999. Genetic sexing strains in the medfly, *Ceratitis capitata*: development, mass rearing and field application. *Trends in Entomology* 2: 81–104. www.researchtrends.net/tia/abstract.asp?in=0&vn=2&tid=20&aid=684&pub=1999&type=
- Robinson, A. S., G. Franz, and P. W. Atkinson. 2004. Insect transgenesis and its potential role in agriculture and human health. *Insect Biochemistry and Molecular Biology* 34: 113–120. <https://doi.org/10.1016/j.ibmb.2003.10.004>
- Robinson, J. 2018. Sterile insect technique promising in fight against greenhouse pepper pest. 24 April 2018. AGInnovation, Agri-Technology Commercialization Centre. Guelph, ON, Canada.
- Rull, J., and A. Barreda-Landa. 2007. Colonization of a hybrid strain to restore male *Anastrepha ludens* (Diptera: Tephritidae) mating competitiveness for sterile insect technique programs. *Journal of Economic Entomology* 100: 752–758. <https://doi.org/10.1093/jee/100.3.752>

- Rull, J., F. Diaz-Fleisher, and J. Arredondo. 2007. Irradiation of *Anastrepha ludens* (Diptera: Tephritidae) revisited: optimizing sterility induction. *Journal of Economic Entomology* 100: 1153–1159. <https://doi.org/10.1093/jee/100.4.1153>
- Rull, J., A. Birke, R. Ortega, P. Montoya, and L. López. 2012. Quantity and safety vs. quality and performance: conflicting interests during mass rearing and transport affect the efficiency of sterile insect technique programs. *Entomologia Experimentalis et Applicata* 142: 78–86. <http://onlinelibrary.wiley.com/doi/10.1111/j.1570-7458.2011.01196.x/abstract>
- Saccone, G., A. Pane, A. De Simone, M. Salvemini, A. Milano, L. Annunziata, U. Mauro, and L. C. Polito. 2007. New sexing strains for Mediterranean fruit fly *Ceratitis capitata*: transforming females into males, pp. 95–102. In M. J. B. Vreysen, A. S. Robinson and J. Hendrichs (eds.), *Area-wide control of insect pests. From research to field implementation*. Springer, Dordrecht, The Netherlands. <https://www.iaea.org/sites/default/files/area-wide-control-insect-pests-book.pdf>
- San Andrés, V., A. Urbaneja, B. Sabater-Muñoz, and P. Castañera. 2007. A novel molecular approach to assess mating success of sterile *Ceratitis capitata* (Diptera: Tephritidae) males in sterile insect technique programs. *Journal of Economic Entomology* 100: 1444–1449. <https://doi.org/10.1093/jee/100.4.1444>
- Sánchez-Rosario, M., D. Pérez-Staples, J. Toledo, J. Valle-Mora, and P. Liedo. 2017. Artificial selection on mating competitiveness of *Anastrepha ludens* for sterile insect technique application. *Entomologia Experimentalis et Applicata* 162(2): 133–147. <https://doi.org/10.1111/eea.12540>
- Schellhorn, N. A., and L. K. Jones. 2021. Real-time insect detection and monitoring: breaking barriers to area-wide integrated management of insect pests, pp. 889–902. In J. Hendrichs, R. Pereira and M. J. B. Vreysen (eds.), *Area-wide integrated pest management. Development and field application*. CRC Press, Boca Raton, FL, USA.
- Schetelig, M. F., and A. M. Handler. 2012. A transgenic embryonic sexing system for *Anastrepha suspensa* (Diptera: Tephritidae). *Insect Biochemistry and Molecular Biology* 42: 790–795. <https://doi.org/10.1016/j.ibmb.2012.07.007>
- Schetelig, M. F., C. Caceres, A. Zacharopoulou, G. Franz, and E. A. Wimmer. 2009. Conditional embryonic lethality to improve the sterile insect technique in *Ceratitis capitata* (Diptera: Tephritidae). *BMC Biology* 7: 4. <https://doi.org/10.1186/1741-7007-7-4>
- Schetelig, M. F., A. Targovska, J. S. Meza, K. Bourtzis, and A. M. Handler. 2016. Tetracycline-suppressible female lethality and sterility in the Mexican fruit fly, *Anastrepha ludens*. *Insect Molecular Biology* 25: 500–508. <https://doi.org/10.1111/imb.12238>
- Schneider, J. C. (ed.). 2009. *Principles and procedures for rearing high quality insects*. Mississippi State University, Mississippi State, MS, USA. 352 pp. https://openlibrary.org/books/OL25114831M/Principles_and_procedures_for_rearing_high_quality_insects
- Seth, R. K., and T. K. Barik. 2009. Assessment of infective behaviour and reproductive potential over successive generations of entomopathogenic nematodes, *Steinernema glaseri* (Rhabditida: Steinernematidae), reared within radiosterilized host larvae, towards *Spodoptera litura* (Lepidoptera: Noctuidae). *Biocontrol Science and Technology* 19(S1): 111–125. http://www-naweb.iaea.org/nafa/ipc/crp/Biocontrol_final.pdf
- Shearman, D. C. A. 2002. The evolution of sex determination systems in dipteran insects other than *Drosophila*. *Genetica* 116: 25–43. <https://doi.org/10.1023/A:1020955507978>
- Shelly, T. E. 1994. Consumption of methyl eugenol by male *Bactrocera dorsalis* (Diptera: Tephritidae): low incidence of repeat feeding. *Florida Entomologist* 77: 201–208. <http://journals.fcla.edu/flaent/article/view/59039>
- Shelly, T. E., S. S. Kennelly, and D. O. McInnis. 2002a. Effect of adult diet on signalling activity, mate attraction, and mating success in male Mediterranean fruit flies (Diptera: Tephritidae). *Florida Entomologist* 85: 150–155. <http://journals.fcla.edu/flaent/article/view/75056>
- Shelly, T. E., A. S. Robinson, C. Caceres, V. Wornoyaporn, and A. Islam. 2002b. Exposure to ginger root oil enhances mating success of male Mediterranean fruit flies (Diptera: Tephritidae) from a genetic sexing strain. *Florida Entomologist* 85: 440–445. <http://journals.fcla.edu/flaent/article/view/75121>
- Shelly, T. E., J. Edu, and E. Pahio. 2004a. Sterile males of the Mediterranean fruit fly exposed to ginger root oil induce female remating: implications for the sterile insect technique (Diptera: Tephritidae). *Florida Entomologist* 87: 628–629. <http://journals.fcla.edu/flaent/issue/view/3578>
- Shelly, T. E., D. O. McInnis, E. Pahio, and J. Edu. 2004b. Aromatherapy in the Mediterranean fruit fly (Diptera: Tephritidae): sterile males exposed to ginger root oil in prerelease storage boxes display increased mating competitiveness in field-cage trials. *Journal of Economic Entomology* 97: 846–853.

- <https://doi.org/10.1093/jee/97.3.846>
- Shelly, T. E., J. Edu, and J. Nishimoto. 2013.** Chilling and flight ability and mating competitiveness of sterile males of the Mediterranean fruit fly. *Journal of Applied Entomology* 137(Issue s1): 11–18. <https://doi.org/10.1111/j.1439-0418.2010.01532.x>
- Shono, T., and J. G. Scott. 2003.** Spinosad resistance in the housefly, *Musca domestica*, is due to a recessive factor on autosome 1. *Pesticide Biochemistry and Physiology* 75: 1–7. [https://doi.org/10.1016/S0048-3575\(03\)00011-7](https://doi.org/10.1016/S0048-3575(03)00011-7)
- Simmons, G. S., A. R. McKemey, N. I. Morrison, S. O'Connell, B. E. Tabashnik, J. Claus, G. Fu, G. Tang, M. Sledge, A. S. Walker, C. E. Phillips, E. D. Miller, R. I. Rose, R. T. Staten, C. A. Donnelly, and L. Alphey. 2011.** Field performance of a genetically engineered strain of pink bollworm. *PLOS ONE* 6(9): e24110. <https://doi.org/10.1371/journal.pone.0024110>
- Smidler, A. L., S. N. Scott, E. Mameli, W. R. Shaw, and F. Catteruccia. 2018.** A transgenic tool to assess *Anopheles* mating competitiveness in the field. *Parasites and Vectors* 11(Suppl. 2): 651. <https://doi.org/10.1186/s13071-018-3218-5>
- Smith, R. C., M. F. Walter, R. H. Hice, D. A. O'Brochta, and P. W. Atkinson. 2007.** Testis-specific expression of the $\beta 2$ tubulin promoter of *Aedes aegypti* and its application as a genetic sex-separation marker. *Insect Molecular Biology* 16: 61–71. <https://doi.org/10.1111/j.1365-2583.2006.00701.x>
- Soemori, H., S. Tsukaguchi, and H. Nakamori. 1980.** Comparison of mating ability and mating competitiveness between mass-reared and wild strains of the melon fly, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae). *Japanese Journal of Applied Entomology and Zoology* 24: 246–250. (in Japanese, English summary).
- Staten, R. T., and M. L. Walters. 2021.** Technology used by field managers for pink bollworm eradication with its successful outcome in the United States and Mexico, pp. 51–92. *In* J. Hendrichs, R. Pereira and M. J. B. Vreysen (eds.), *Area-wide integrated pest management. Development and field application*. CRC Press, Boca Raton, FL, USA.
- Stathopoulou, P. M., E. Asimakis, and G. Tsiamis. 2021.** *Enterobacter*: one bacterium multiple functions, pp. 917–945. *In* J. Hendrichs, R. Pereira and M. J. B. Vreysen (eds.), *Area-wide integrated pest management. Development and field application*. CRC Press, Boca Raton, FL, USA.
- Steinberg, S., and J. P. Cayol. 2009.** Synergism between biological control and sterile insect technique: can commercial mass production of biocontrol agents and sterile insects be integrated within the same industrial entity? *Biocontrol Science and Technology* 19(Suppl. 1): 271–275. <https://www.iaea.org/resources/scientific-paper/synergism-between-biological-control-and-sterile-insect-technique-can-commercial-mass-production-of-biocontrol-agents-and-sterile-insects-be-integrated-within-the-same-industrial-entity>
- Suckling, D. M. 2003.** Applying the sterile insect technique for biosecurity: benefits and constraints. *New Zealand Pl. Prot.* 56: 21–26. <http://www.hortnet.co.nz/publications/nzpps/proceedings/03/03-021.pdf>
- Suckling, D. M., B. Woods, V. J. Mitchell, A. Twidle, I. Lacey, E. B. Jang, and A. R. Wallace. 2011.** Mobile mating disruption of light-brown apple moths using pheromone-treated sterile Mediterranean fruit flies. *Pest Management Science* 67: 1004–1014. <https://doi.org/10.1002/ps.2150>
- Sultan, M., R. Buitenhuis, G. Murphy, and C. D. Scott-Dupree. 2017.** Development of a mechanical sexing system to improve the efficacy of an area-wide sterile insect release programme to control American serpentine leafminer (Diptera: Agromyzidae) in Canadian ornamental greenhouses. *Pest Management Science* 73: 830–837. <https://doi.org/10.1002/ps.4494>
- Tabashnik, B. E., M. S. Sisterson, P. C. Ellsworth, T. J. Dennehy, L. Antilla, L. Liesner, M. Whitlow, R. T. Staten, J. A. Fabrick, G. C. Unnithan, A. J. Yelich, C. Ellers-Kirk, V. S. Harpold, X. Li, and Y. Carrière. 2010.** Suppressing resistance to *Bt* cotton with sterile insect releases. *Nature Biotechnology* 28: 1304–1307. <https://doi.org/10.1038/nbt.1704>
- Tan, K. H. 2000.** Behaviour and chemical ecology of *Bactrocera* flies, pp. 647–656. *In* K. H. Tan (ed.), *Proceedings: Area-Wide Control of Fruit Flies and Other Insect Pests. International Conference on Area-Wide Control of Insect Pests, and the 5th International Symposium on Fruit Flies of Economic Importance, 28 May–5 June 1998, Penang, Malaysia*. Penerbit Universiti Sains Malaysia, Pulau Pinang, Malaysia. http://www.naweb.iaea.org/nafa/ipc/public/Area-wide-control_491-650.pdf http://www.naweb.iaea.org/nafa/ipc/public/Area-wide-control_651-Back-Cover.pdf
- Tan, L. T., and K. H. Tan. 2013.** Alternative air vehicles for sterile insect technique aerial release. *Journal of Applied Entomology* 137(Issue s1): 126–141. <https://doi.org/10.1111/j.1439-0418.2011.01649.x>
- Taret, G., M. Sevilla, V. Wornoayporn, A. Islam, S. Ahmad, C. Caceres, A. S. Robinson, and M. J. B. Vreysen. 2010.** Mating compatibility among populations of codling moth *Cydia pomonella* Linnaeus

- (Lepidoptera: Tortricidae) from different geographic origins. *Journal of Applied Entomology* 134: 207–215. <https://doi.org/10.1111/j.1439-0418.2008.01375.x>
- Teal, P. E. A., Y. Gomez-Simuta, and A. T. Proveaux. 2000.** Mating experience and juvenile hormone enhance sexual signaling and mating in male Caribbean fruit flies. *Proceedings of the National Academy of Sciences of the USA* 97(7): 3708–3712. <https://doi.org/10.1073/pnas.97.7.3708>
- Teal, P. E. A., Y. Gomez-Simuta, B. D. Dueben, T. C. Holler, and S. Olson. 2007.** Improving the efficacy of the sterile insect technique for fruit flies by incorporation of hormone and dietary supplements into adult holding protocols, pp. 163–173. *In* M. J. B. Vreysen, A. S. Robinson and J. Hendrichs (eds.), *Area-wide control of insect pests. From research to field implementation*. Springer, Dordrecht. <https://www.iaea.org/sites/default/files/area-wide-control-insect-pests-book.pdf>
- Thomas, D. D., C. A. Donnelly, R. J. Wood, and L. S. Alphey. 2000.** Insect population control using a dominant, repressible, lethal genetic system. *Science* 287(5462): 2474–2476. <http://science.sciencemag.org/content/287/5462/2474>
- Thomas, J. K., G. J. Fadul, G. P. Keller, and M. F. Chaudhury. 2018.** The use of dried bovine hemoglobin and plasma for mass rearing New World screwworm. *Journal of Insect Science* 18: 12. <https://doi.org/10.1093/jisesa/iey052>
- Toledo, J., J. Rull, A. Oropeza, E. Hernández, and P. Liedo. 2004.** Irradiation of *Anastrepha obliqua* (Diptera: Tephritidae) revisited: optimizing sterility induction. *Journal of Economic Entomology* 97: 383–389. <https://doi.org/10.1093/jee/97.2.383>
- Toledo, J., S. Flores, S. Campos, A. Villaseñor, W. Enkerlin, P. Liedo, A. Valle, and P. Montoya. 2017.** Pathogenicity of three formulations of *Beauveria bassiana* and efficacy of autoinoculation devices and sterile fruit fly males for dissemination of conidia for the control of *Ceratitidis capitata*. *Entomologia Experimentalis et Applicata* 164: 340–349. <https://doi.org/10.1111/eea.12608>
- Townson, H. 2002.** *Wolbachia* as a potential tool for suppressing filarial transmission. *Annals of Tropical Medicine and Parasitology* 96(Suppl. 2): S117–S127. (Now known as *Pathogens and Global Health*) <https://www.ncbi.nlm.nih.gov/pubmed/?term=Townson+2002+wolbachia#>
- Tweddle, N., and R. J. Mahon. 2000.** Insurance against an Old World screw-worm fly invasion of Australia, pp. 95–99. *In* K. H. Tan (ed.), *Proceedings: Area-Wide Control of Fruit Flies and Other Insect Pests. International Conference on Area-Wide Control of Insect Pests, and the 5th International Symposium on Fruit Flies of Economic Importance, 28 May–5 June 1998, Penang, Malaysia*. Penerbit Universiti Sains Malaysia, Pulau Pinang, Malaysia. http://www.naweb.iaea.org/nafa/ipc/public/Area-wide-control_73-229.pdf
- Tween, G. 1987.** A modular approach to fruit fly production facilities for the Mediterranean fruit fly Central America program, pp. 283–291. *In* A. P. Economopoulos (ed.), *Proceedings, Symposium: Fruit Flies 2nd International Symposium, 16–21 September 1986, Crete, Greece*. Printer: G. Tsiveriotis Ltd., Athens, Greece. Distributor: Elsevier Science Publishers, Amsterdam, The Netherlands. <http://www.naweb.iaea.org/nafa/ipc/public/FF-Proc-Intl-Symp-cvr-144.pdf>
- Tween, G. 2004.** MOSCAMED-Guatemala — An evolution of ideas, pp. 119–126. *In* B. N. Barnes (ed.), *Proceedings, Symposium: 6th International Symposium on Fruit Flies of Economic Importance, 6–10 May 2002, Stellenbosch, South Africa*. Isteg Scientific Publications, Irene, South Africa. https://nucleus.iaea.org/sites/naipc/twd/Documents/6thISFFEI_Proceedings/6th-ISFFEI-Proceedings-2004-Stellenbosch-South-Africa.pdf
- Tween, G., and P. Rendón. 2007.** Current advances in the use of cryogenics and aerial navigation technologies for sterile insect delivery systems, pp. 229–238. *In* M. J. B. Vreysen, A. S. Robinson and J. Hendrichs (eds.), *Area-wide control of insect pests. From research to field implementation*. Springer, Dordrecht. <https://www.iaea.org/sites/default/files/area-wide-control-insect-pests-book.pdf>
- (USDA/APHIS/PPQ) United States Department of Agriculture/Animal and Plant Health Inspection Service/Plant Protection and Quarantine. 2000.** Effectiveness of SUCCESS 0.02 CB™ for the control of fruit flies. Report. Methods Development Station, Guatemala.
- Vargas, R. I., L. Leblanc, J. C. Piñero, and K. M. Hoffman. 2014.** Male annihilation, past, present, and future, pp. 493–507. *In* T. Shelly, N. Epsky, E. B. Jang, J. Reyes-Flores and R. Vargas (eds.), *Trapping and the detection, control, and regulation of tephritid fruit flies*. Springer, Dordrecht, The Netherlands.
- Venter, J.-H., C. W. L. Baard, and B. N. Barnes. 2021.** Area-wide management of Mediterranean fruit fly with the sterile insect technique in South Africa: new production and management techniques pay dividends, pp. 129–141. *In* J. Hendrichs, R. Pereira and M. J. B. Vreysen (eds.), *Area-wide integrated pest management. Development and field application*. CRC Press, Boca Raton, FL, USA.
- Verhulst, E. C., L. van de Zande, and L. W. Beukeboom. 2010.** Insect sex determination: it all evolves around *transformer*. *Current Opinion in Genetics and Development* 20: 376–383.

- <https://doi.org/10.1016/j.gde.2010.05.001>
- Vickers, R. A., M. J. Furlong, A. White, and J. K. Pell. 2004.** Initiation of fungal epizootics in diamondback moth populations within a large field cage: proof of concept for auto-dissemination. *Entomol. Experimentalis et Applicata* 111: 7–17. <https://doi.org/10.1111/j.0013-8703.2004.00140.x>
- Vijayasegaran, S., G. H. Walter, and R. A. I. Drew. 1997.** Mouthpart structure, feeding mechanisms, and natural food sources of adult *Bactrocera* (Diptera: Tephritidae). *Annals of the Entomological Society of America* 90: 184–201. <https://doi.org/10.1093/aesa/90.2.184>
- Vreysen, M. J. B., H. J. Barclay, and J. Hendrichs. 2006.** Modeling of preferential mating in areawide control programs that integrate the release of strains of sterile males only or both sexes. *Ann. Entomol. Soc. Amer.* 99: 607–616. [https://doi.org/10.1603/0013-8746\(2006\)99\[607:MOPMIA\]2.0.CO;2](https://doi.org/10.1603/0013-8746(2006)99[607:MOPMIA]2.0.CO;2)
- Vreysen, M. J. B., J. E. Carpenter, and F. Marec. 2010.** Improvement of the sterile insect technique for codling moth *Cydia pomonella* (Linnaeus) (Lepidoptera Tortricidae) to facilitate expansion of field application. *J. Appl. Entomology* 134: 165–181. <https://doi.org/10.1111/j.1439-0418.2009.01430.x>
- Vreysen, M. J. B., W. Klassen, and J. E. Carpenter. 2016.** Overview of technological advances toward greater efficiency and efficacy in sterile insect-inherited sterility programs against moth pests. *Florida Entomologist* 99 (Special Issue 1): 1–12. <http://journals.fcla.edu/flaent/article/view/88480/84948>
- Walker, C. S. 2012.** The application of sterile insect technique against the tomato leafminer *Liriomyza bryoniae*. PhD thesis, Imperial College, London, UK. 122 pp.
- Waltz, E. 2017.** US government approves 'killer' mosquitoes to fight disease. *Nature News*, 6 November. <https://www.nature.com/news/us-government-approves-killer-mosquitoes-to-fight-disease-1.22959>
- Weidhaas, D. E., S. G. Breeland, C. S. Lofgren, D. A. Dame, and P. Kaiser. 1974.** Release of chemosterilized males for the control of *Anopheles albimanus* in El Salvador. IV. Dynamics of the test population. *Amer. J. Trop. Med. Hygiene* 23(2): 298–308. <https://doi.org/10.4269/ajtmh.1974.23.298>
- (WHO) World Health Organization. 2017.** Global vector control response 2017–2030. Geneva, Switzerland. <http://apps.who.int/iris/bitstream/handle/10665/259205/9789241512978-eng.pdf;jsessionid=0B4BB7D7C8C5C56F52C5E21B9322F63C?se>
- Wilke, A. B. B., and M. T. Marrelli. 2015.** Paratransgenesis: a promising new strategy for mosquito vector control. *Parasites and Vectors* 8: 342. <https://doi.org/10.1186/s13071-015-0959-2>
- Wu, K. M. 2010.** No refuge for insect pests. The sterile insect technique offers an alternative to the refuge strategy for managing resistance to *Bt* toxins. *Nature Biotechnology* 28: 1273–1275. <https://doi.org/10.1038/nbt.1718>
- Yamada, H., A. G. Parker, C. F. Oliva, F. Balestrino, and J. R. L. Gilles. 2014.** X-ray-induced sterility in *Aedes albopictus* (Diptera: Culicidae) and male longevity following irradiation. *Journal of Medical Entomology* 51: 811–816. <https://doi.org/10.1603/ME13223>
- Yan, Y., and M. J. Scott. 2015.** A transgenic embryonic sexing system for the Australian sheep blow fly *Lucilia cuprina*. *Scientific Reports* 5: 16090. <https://www.nature.com/articles/srep16090>
- Yuval, B., R. Kaspi, S. A. Field, S. Blay, and P. Taylor. 2002.** Effects of post-teneral nutrition on reproductive success of male Mediterranean fruit flies (Diptera: Tephritidae). *Florida Entomologist* 85: 165–170. <https://journals.flvc.org/flaent/article/view/75058/72716>
- Zabalou, S., M. Riegler, M. Theodorakopoulou, C. Stauffer, C. Savakis, and K. Bourtzis. 2004.** *Wolbachia*-induced cytoplasmic incompatibility as a means for insect pest population control. *Proc. Nat. Acad. Sciences of the USA* 101(42): 15042–15045. <https://doi.org/10.1073/pnas.0403853101>
- Zapater, M. C., C. Banchemo, M. Battista, and H. Rizzo. 1996.** Heterosis in *Ceratitis capitata*, pp. 243–251. In B. A. McPherson and G. J. Steck (eds.), *Fruit fly pests. A world assessment of their biology and management*. St. Lucie Press, Delray Beach, FL, USA.
- Zheng, M. L., D. J. Zhang, D. D. Damiens, R. S. Lees, and J. R. L. Gilles. 2015.** Standard operating procedures for standardized mass rearing of the dengue and chikungunya vectors *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) - II - Egg storage and hatching. *Parasites and Vectors* 8: 348. <https://doi.org/10.1186/s13071-015-0951-x>
- Zheng, X., D. Zhang, Y. Li, C. Yang, Y. Wu, X. Liang, Y. Liang, X. Pan, L. Hu, Q. Sun, X. Wang, Y. Wei, J. Zhu, W. Qian, Z. Yan, A. G. Parker, J. R. L. Gilles, K. Bourtzis, J. Bouyer, M. Tang, B. Zheng, J. Yu, J. Liu, J. Zhuang, Z. Hu, M. Zhang, J. T. Gong, X. Y. Hong, Z. Zhang, L. Lin, Q. Liu, Z. Hu, Z. Wu, L. A. Baton, A. A. Hoffmann, and Z. Xi. 2019.** Incompatible and sterile insect techniques combined eliminate mosquitoes. *Nature* 572: 56–61. <https://doi.org/10.1038/s41586-019-1407-9>
- Zimmermann, H., S. Bloem, and H. Klein. 2004.** Biology, history, threat, surveillance and control of the cactus moth, *Cactoblastis cactorum*. IAEA/FAO-BSC/CM. IAEA, Vienna, Austria. http://www-naweb.iaea.org/nafa/ipc/public/faobsc_web.pdf