

**The value of existing regulatory frameworks for the environmental risk assessment of agricultural pest control using gene drives**

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## Abstract

The application of (synthetic) gene drives is a powerful tool to control populations of insects that are agricultural pests, vectors of diseases, or a threat to biodiversity potentially leading to the local or global eradication of a species. The potential use of gene drive organisms has triggered a heated discussion regarding their environmental impacts and regulatory oversight. However, experience exists in assessing the environmental impacts of a number of established agricultural pest control methods that require the release of living organisms, that provide high levels of area-wide control and that might be irreversible. This includes classical biological control, the sterile insect technique, the incompatible insect technique that is based on the cytoplasmic incompatibility caused by *Wolbachia* endosymbionts, and genetically modified insects containing self-limiting traits. The different technologies are described, the regulatory practice and experience is summarized and pathways through which these control technologies could harm valued ecosystem services are presented. With a focus on the application of gene drives in agriculture, using the invasive *Drosophila suzukii* (Diptera: Drosophilidae) as a case study we then discuss to what extent the existing frameworks could assist the risk assessment of insects carrying gene drives. We suggest that drawing on existing practices, experiences and legislative frameworks will provide a pragmatic and proportionate approach to evaluate the environmental risks of novel solutions based on gene drive technologies.

**Keywords:** environmental risk assessment, regulation, pathway to harm, problem formulation, protection goals, SIT, IIT, genetically modified organisms, biological control, non-target effects.

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## Abbreviations

SIT - Sterile insect technique

IIT – Incompatible insect technique

ERA – Environmental risk assessment

BCA - Biological control agents

52 CBC – Classical biological control  
53 CI - Cytoplasmic incompatibility  
54 HGT - Horizontal gene transfer  
55 HEG - Homing endonuclease gene  
56 PTH - Pathways to harm  
57 GMO – Genetically modified organism  
58 TPP - Target product profiles  
59

## 60 1. INTRODUCTION

61 While estimates of the number of species on earth vary, insects are by far the  
62 dominating class among the animals (Mora et al., 2011). Some species are a threat  
63 to agricultural production causing significant losses at the pre- and post-harvest stage  
64 (Culliney, 2014; Oerke, 2006; Savary et al., 2019), affect human or animal health by  
65 transmitting diseases (Lounibos, 2002), or have direct or indirect effects on native  
66 biodiversity (Kenis et al., 2009). Consequently, humans have always aimed to control  
67 populations of such harmful species. Increasing challenges such as the invasion of  
68 non-native insect species, e.g., as a consequence of increasing trade (Hulme, 2009)  
69 or climate change (Hulme, 2017), and increasing resistance to commonly used  
70 chemical insecticides (Borel, 2017) require the development and deployment of novel  
71 insect-control techniques. Genetics-based methods provide possible solutions  
72 (Alphey, 2014; Alphey and Bonsall, 2018).

73 Insect control strategies aim to reduce population size and even eliminate damaging  
74 species (Myers et al., 1998). The Global Eradication and Response Database  
75 (GERDA) currently contains incursion response and eradication programmes against  
76 a total of 183 arthropod species (Kean et al., 2019). Control strategies include the  
77 use of chemical or biological insecticides, resistant crop varieties (bred conventionally  
78 or by genetic engineering), biological control, and genetic control methods such as  
79 the sterile insect technique (SIT) or the incompatible insect technique (IIT) that is  
80 based on the cytoplasmic incompatibility caused by *Wolbachia* endosymbionts.  
81 Prominent examples of successful suppression or eradication of the target species  
82 are provided in Box 1. While it is recognized that the removal of one species can  
83 have adverse effects on the environment, for example, through food-web effects  
84 (Fang, 2010), only some of the technologies used for insect control are regulated and  
85 need to pass an environmental (and human and animal health) risk assessment

before being implemented.

----- place Box 1 approximately here -----

It is in this context of currently used insect pest or vector management practices that, through a comparative approach, the application of (synthetic) gene drives has to be considered. The term gene drive, first used by Hamilton (1967), describes the phenomenon of genetic elements spreading through populations because they are transmitted with a non-Mendelian (greater than 50%) rate to the next generation (Burt and Trivers, 2006). Natural gene drives or selfish DNA elements such as transposable elements, meiotic drive elements, endonucleases, B chromosomes, homing endonuclease genes and *Wolbachia* endosymbionts have been described (Burt and Trivers, 2006; Curtis and Sinkins, 1998; Lindholm et al., 2016; Sinkins and Gould, 2006). The development and adoption of gene drive constructs for insect pest management is not new and dates back to the 1940s (e.g., Curtis, 1968; Hastings, 1994; Hickey and Craig, 1966a,b; Serebrovsky, 1940). For instance, in ground-breaking work, Curtis (1968) illustrated that high threshold drives (based on chromosome inversions) could be used for insect vector management and work through the 1970s developed, implemented and monitored modified mosquitoes in field studies (Lorimer, 1981; Lorimer et al., 1976; McDonald et al., 1977). More recently, Burt (2003) proposed the idea of engineering such genes to target host DNA sequences and thus manipulate natural populations. The power of gene drives lies in the fact that they spread genetic elements through populations even though they are detrimental to the organism. While the concept of gene drives is not new, discussion about the use and potential risks of synthetic gene drives (= engineered gene drive) has evolved in recent years and a better understanding of the underlying mechanisms can help in the design of effective and safe synthetic drive systems (Lindholm et al., 2016; Sinkins and Gould, 2006).

The advent of molecular biology has allowed these sorts of genetic systems to be engineered in novel and unique ways. Methods such as chromosome inversions, the use of endonucleases and most recently the application of CRISPR/Cas9-based gene editing approaches (Sternberg and Doudna, 2015) have allowed genomes to be manipulated to bias inheritance away from standard Mendelian patterns. This has the potential to provide novel gene drive tools in various organisms (Champer et al.,

2016; Esvelt et al., 2014; Gantz and Bier, 2015; NASEM, 2016). Currently foreseen approaches for using CRISPR/Cas9-based synthetic gene drives fall into two broad categories: suppression and modification drives (Alphey, 2014; Champer et al., 2016). Suppression drives are designed to reduce or eradicate the target organism populations, like the multitude of other control methods currently applied. Replacement or modification drives are designed to spread a genetic modification throughout a population.

The emerging potential of synthetic gene drives has triggered a discussion regarding environmental and health concerns (Esvelt et al., 2014) as well as ethical considerations (Caplan et al., 2015; Thompson, 2018). There have been wide ranging calls for control and regulation of gene drive research and applications (Adelman et al., 2017; Akbari et al., 2015; Ledford, 2015; Oye et al., 2014 ) and for consent amongst all communities likely affected by these technologies to be involved in decisions (Kofler et al., 2018). As a consequence of this debate, a consortium of NGOs has called for a moratorium on any gene drive research; this has been rejected by the UN Convention on Biodiversity on several occasions since given the potential of gene drives it seemed disproportional to block further research (Callaway, 2016, 2018). Redford et al. (2019) provide a good review on the discussion within the International Union for Conservation of Nature (IUCN) in this respect.

All of this notwithstanding, it is necessary to consider risk assessment frameworks for synthetic gene drive technologies. Most of the current research on the application of synthetic gene drives in insects is focused on vector control of mosquitoes (e.g., malaria-transmitting *Anopheles*) to reduce human disease burden (James et al., 2018). Gene drives could equally play a role in the eradication of invasive species, disease transmitting vectors, and agricultural pests (Scott et al., 2018; Redford et al., 2019), and in this article we concentrate on the latter.

Here, we present environmental risk assessment practices and experience with other agricultural insect pest control technologies that require the release of living insects (see Box 1) and discuss to what extent these frameworks could assist the risk assessment of insects carrying gene drives. We focus on the application of gene drives in agriculture, using *Drosophila suzukii* (Diptera: Drosophilidae) as a case study.

## 2. EXPERIENCE WITH EXISTING FRAMEWORKS FOR ARTHROPOD CONTROL

Several established agricultural pest control options require the release of living organisms. Below we provide an overview of the use, existing regulations and experiences with environmental risk assessment (ERA) of selected control technologies. This summary is not intended to be comprehensive.

The initial stage in ERA is problem formulation. At this stage the scope of the assessment is defined, taking into account the policy protection goals (Gray, 2012; Raybould, 2006; Wolt et al., 2010). This includes the identification of specific or operational protection goals and the constructions of pathways by which the planned intervention could harm the entities to be protected (Devos et al., 2015, 2019). This approach thus ensures that the focus is put on hazards that could potentially lead to harm. Based on this, risk hypotheses are developed that can subsequently be tested.

Environmental protection goals, as laid down in regulations, describe what elements of the environment are valued and should be protected from harm. Such policy protection goals, however, are typically very broad and vague and thus need to be translated into operational or specific protection goals that “delineate the environmental components that need to be protected, where and over what time period, and the maximum impacts that can be tolerated” (Devos et al., 2015; Sanvido et al., 2012). A policy protection goal that typically applies to arthropod pest control methods is that of biodiversity, which covers important ecosystem services (Millenium Ecosystem Assessment; MEA, 2005). For the purpose of this paper we will focus on regulatory risk frameworks for assessing protection goals around the ecosystem services of biological control provided by natural enemies (such as predators and parasitoids).

At the outset, it is important to emphasize that some of the information requested by regulatory authorities on a particular organism/product aim to characterize the regulated entity or product and are not required for ERA. The differentiation is often difficult to make.

### **2.1. Classical Biological Control**

#### **2.1.1. Background**

Releasing biological control agents (BCA) such as predators and parasitoids to control arthropod pests is an important pest management tool. There are two principal applications of BCA (Heimpel and Mills, 2017). In augmentative biological

control, native or exotic species are mass-reared and repeatedly released in the field or the greenhouse; establishment and dispersal is not intended. The aim is a short-term or season-long suppression of the target pest. In the case of Classical Biological Control (CBC), natural enemies of invasive arthropod pests are (typically) introduced from the pest's area of origin. They are released with the aim to establish and to provide long-term control of the target pest, potentially even leading to the eradication of the exotic pest (Box 1). Consequently, potential environmental effects caused by this release are likely to be irreversible (Barratt and Ehlers, 2017). The application of CBC could thus serve as a model for ERA of arthropods with gene drives (Webber et al., 2015).

The story of CBC started with a spectacular success when in 1888/89 the ladybird beetle *Rodolia cardinalis* (Coleoptera: Coccinellidae) was introduced to California from New Zealand to control the cottony cushion scale *Icerya purchasi* (Hemiptera: Monophlebidae) (Caltagirone and Doult, 1989). Since then (until 2010) a total of 6158 introductions have been recorded, using 2384 different natural enemies against 588 pest species in 148 countries for classical biological control of plant and arthropod pests worldwide (BIOCAT database; Cock et al., 2016; Greathead and Greathead, 1992). In 32.6% of cases, the introduced species established in the new environment. Despite this long history, the prevalence of the risk associated with CBC only came to light in the 1980s following a series of publications that emphasized the potential harmful effects of exotic natural enemies (Howarth, 1983, 1991; Simberloff and Stiling, 1996). Numerous hazards of releasing exotic BCA to non-target organisms have been identified (Barratt and Ehlers, 2017; Heimpel and Mills, 2017; van Lenteren et al., 2003). These potential effects could be direct, e.g., the exotic species attacks native species, or indirect, caused e.g., by competition with, or displacement of, native natural enemies, or by hybridization with closely related native species. Therefore, an ERA should precede the release of exotic biocontrol agents. Over the years, regulators have become more risk-averse and, as a consequence, it is expected today that CBC programmes do follow appropriate risk evaluations and focus on only deploying highly specialist natural enemies.

### 2.1.2. Regulations

Guidelines for ERA procedures and data requirements concerning the use of exotic BCA have been produced by various international organizations including the Food

and Agriculture Organization of the United Nations (FAO, 2005), the Organisation for Economic Co-operation and Development (OECD, 2004), the European and Mediterranean Plant Protection Organization (EPPO, 2014), the North American Plant Protection Organization (NAPPO, 2015) and the Western Palaearctic Regional Section of the International Organization for Biological Control of Noxious Animals and Plants (IOBC/WPRS) (Bigler et al., 2005). While these guidelines have been transcribed into national legislation to varying degrees, several countries still have no specific types of regulations for biological control agents (Mason et al., 2017). ERA procedures and data requirements vary significantly. For example, many countries in Europe do not regulate the use and release of BCAs as long as they are not GMO. However, for CBC agents a harmonised approach is warranted, as has been achieved for the NAPPO region (Canada, USA, and Mexico) (Hunt et al., 2008; Mason et al., 2017), since biological control organisms released in this region are intended to establish and potentially disperse across the Americas.

### *2.1.3 Experience with Environmental Risk Assessment*

The organism under consideration needs to be characterized in detail. In many jurisdictions, this includes a statement on the strain and biotype that is used, as it has become increasingly evident that the success (and thus also the potential risk) of a BCA differs between strains and/or biotypes as they can, for example, differ in their ability to diapause (Barratt and Ehlers, 2017; Barratt et al., 2010).

Exotic BCAs used in CBC programmes are intended to establish in the area of introduction and the focus of the ERA is on non-target effects within the receiving ecosystem(s). Three elements are important to assess this risk (Barratt and Ehlers, 2017): (i) information on the host/prey specificity of the BCA in its native range of distribution, (ii) information on host/prey specificity and non-target effects available from other countries/areas where the same BCA has already been introduced, and (iii) information from host/prey specificity testing.

Arthropod agents that are regarded as potentially useful based on the current knowledge of their host/prey-range (elements (i) and (ii) above) can then be introduced into a quarantine facility in the country designated for release (Barratt et al., 2010; Hunt et al., 2008). Under quarantine conditions, the host/prey range of the agent is subsequently tested using non-target species from the area of planned release. Once host/prey-specificity data have been collected, an application for full



environmental release can be submitted.

For host/prey-specificity testing, non-target species are selected that (i) have phylogenetic/taxonomic affinities to the target(s) of the BCA, (ii) have ecological similarities (e.g., occupy the same habitat or niche) with the target(s), or (iii) are of particular value (e.g., species of economic or iconic value, species that are threatened or endangered, keystone species) (Barratt and Ehlers, 2017; Kuhlmann et al., 2006). The non-target species list compiled has to be approved (or at least acknowledged) by the regulatory authority in some jurisdictions (Hunt et al., 2008). In recent CBC programmes for arthropod control, between 12 and 25 non-target species have been tested for a single BCA (De Clercq et al., 2011).

In the first instance, no-choice experiments are conducted (van Lenteren et al., 2006). Such tests are considered worst-case and establish the physiological host range of the BCA. They often overestimate the ecological host range (resulting in false positives) since the ecological host range is also affected by numerous other factors that include species distribution, phenology and ecoclimatic tolerance (Babendreier et al., 2005; Jenner et al., 2014; Louda et al., 2003). This overestimation is desirable in an ERA since it minimizes the occurrence of false-negative results and the chance that potentially harmful organisms are released. However, on the other hand, false-positive results may lead to the unnecessary rejection of potentially safe agents. Thus, *“One of the main challenges for future research is to develop techniques for carrying out more ‘natural’ laboratory bioassays which will inform the ecological host range investigations from within the confines of quarantine facilities, and which may also be designed to better allow the expression of natural host finding and acceptance behaviors of the insects in confinement, ...”* (Barratt et al., 2010).

In cases where non-target species are successfully attacked by the BCA under (worst-case) laboratory conditions, the ecological host/prey range needs to be established. Experimentally this can, for example, involve the use of choice experiments where the extent to which the BCA prefers the non-target species over the target species is tested (van Lenteren et al., 2006). While there is no guidance on the use of semi field or restricted field releases, experiments may be conducted in the area of origin of the CBC agent, if applicable (Heimpel and Mills, 2017).

In addition to host-range testing, the quarantine phase is important to confirm the species identity and quality control of cultures, including evidence that the reared

population is free of contaminants such as other antagonists (e.g., hyperparasitoids) or diseases (e.g., Bigler et al., 2005; EPPO, 2014; Mason et al., 2017).

Unique to the regulation of BCAs is the fact that a risk/benefit analysis is conducted as part of the ERA in some jurisdictions (e.g., New Zealand, HSNO Act 1996; recommended also in the new EPPO standard PM6/4) as well as the effects of alternative pest control methods (Barratt and Ehlers, 2017; Bigler et al., 2005; EPPO, 2014; Mason et al., 2017; OECD, 2004).

#### *2.1.4 Risk Management Considerations*

Post-release monitoring is a pre-requisite for the release of exotic BCAs in some jurisdictions (e.g., NAPPO, 2015) and also recommended in others (EPPO, 2014).

The aim of this monitoring is to assess the efficacy of the CBC programme as well as the complex indirect, ecological interactions that may emerge. This includes, for example, monitoring the spread of the BCA and population establishment, attack on target and non-target organisms, and changes in target/non-target population and community level processes/structures. This information plays an important role in improving the predictions of the impacts of candidate control agents in the future. Reversibility of the release is not specifically considered in any CBC programme. It has, however, been suggested that the impact of a CBC agent could be reversed by introducing an additional natural enemy (Heimpel and Mills, 2017).

### **2.2. Sterile Insect Technique (SIT)**

#### *2.2.1. Background*

The SIT involves the repetitive release of mass-produced sterilized insects (typically males) (Dyck et al., 2005; Krafur, 1998). The target species is mass-reared, sexes are separated (where practical), males are sterilized and subsequently released. Offspring of wild females that have mated with the released sterilized males are nonviable.

SIT was first implemented in pest-management programmes against the New World screwworm (*Cochliomya hominivorax*; Diptera: Calliphoridae) in the USA in the 1950s (see Box 1). Since then a number of species have been targeted; SIT has been most successful in controlling lepidopteran and dipteran pests (Klassen and Curtis, 2005; Krafur, 1998). Most SIT programmes have been directed against populations of exotic (invasive) species targeting the population in the area of

introduction rather than the whole natural range (Nagel and Peveling, 2005). In the context of our work here, we focus on the most common approach of sterilization, i.e. using ionizing radiation, which causes dominant lethal mutations in the sperm (Bakri et al., 2005). In Lepidopterans, induced inherited sterility, where the radiation-induced deleterious effects are inherited by the F1 generation, has been found to be the optimal strategy (Carpenter et al., 2005). It requires a lower dose of radiation than when causing immediate sterility and has less effects on the fitness and mating competitiveness of the released males with wild type males. Sterility, however, can also be obtained by other means, including chemical sterilants, genetic engineering (Alphey, 2014; Phuc et al., 2007; see 2.4), by incompatible insect technique such as that created by *Wolbachia* infections (Nikolouli et al., 2018; see 2.3), or through hybrid sterility (Robinson, 2005). The success of any SIT programme depends on a number of factors (Nikolouli et al., 2018, Suckling, 2003): (i) the target population must be present at low levels; (ii) good knowledge of the genetics, biology and ecology of the target species (e.g., migration and mating behaviour, host range); (iii) availability of mass-rearing facilities to produce large numbers of high quality insects; (iv) an efficient sexing tool should be available; and, (v) established technology for release and monitoring of the sterile insects over large areas, covering the whole target population should be available. Effectiveness of SIT is associated with the fitness of the sterilized male insects as related to their dispersal ability, longevity, and ability to compete with wild males for mating with wild females. These parameters are evaluated not only under laboratory conditions but also in field cages and open field experiments (Calkins and Parker, 2005). In general, chemical or radiation techniques cause significant fitness decrease in sterilized males (Bakri et al., 2005; Calkins and Parker, 2005) and it is essential to select a dose that optimizes fitness and sterility (Suckling, 2003). The competitiveness of the sterilized insects is impacted by various factors including the strain used, the rearing system, the sterilization process, the handling, shipping and release methods. It is thus important that quality control and assurance protocols are developed (e.g., for fruit flies; FAO/IAEA/USDA, 2003) to ensure that the released insects meet minimum quality standards. For SIT programmes to be effective it is important that the target population is overflooded with sterilized insects in sufficient numbers to outmatch natural population growth. While the ratio of sterile to wild insects to be released varies among species, it can reach ratios of more than 100:1

(Nagel and Peveling, 2005) as for example recommended for codling moth control in New Zealand (Horner et al., 2016). The efficiency of SIT increases with decreasing target pest population. A prerequisite for successful SIT application is that the target pest population is already reduced by other control means and the technology is best applied within an area-wide integrated pest management programme (Hendrichs et al., 2005; Klassen, 2005; Mangan, 2005).

SIT programmes require repeated releases of sterilized insects, typically over long timescales. Successful programmes can lead to area-wide eradications of the target pests (see Box 1). SIT is also used as a preventative strategy to block the invasion of pest species, as for example in a buffer zone between North and South America to prevent the reinvasion of screwworms (Scott et al., 2017).

### *2.2.2. Regulations*

There is some uncertainty regarding the regulatory oversight of the release of sterilized insects as part of SIT programmes. Historically, sterile insects were not considered to be biological control agents since they are not self-replicating (Robinson and Hendrichs, 2005). It took until the 2005 revision of the Guidelines for the Export, Shipment, Import and Release of Biological Control Agents and other Beneficial Organisms: International Standard for Phytosanitary Measures 3, for sterile insects to be included as “beneficials” (FAO, 2005). Recently, in France, the High Council of Public Health has released recommendations related to the authorization of the release of SIT mosquitoes for the purpose of vector control (HCSP, 2018).

There, the most important ecological risks identified include species replacement, the residual fertility of irradiated mosquitoes and the impact on mosquito predators such as bats. Beyond this (to our knowledge), sterilized insects are not specifically addressed in any other regulatory documents or guidelines. Any regulation of SIT is triggered by (i) the fact that the species to be introduced and mass-produced is exotic; or (ii) the use of particular radioactive isotopes for sterilization or (iii) by the use of genetic engineering (for the latter see section 2.4.).

### *2.2.3. Experience with Environmental Risk Assessment*

It is generally acknowledged that sterile insects pose a low risk to the environment (Krafsur, 1998; Nagel and Peveling, 2005). The SIT works through mating, so the induced sterility is directed exclusively to the target species, and no direct adverse

effects on non-target species are expected or have been reported (Nagel and Peveling, 2005). A number of indirect environmental risks related to SIT are discussed (Nagel and Peveling, 2005). These include, first, risks related to the handling of the sterilants: the use of radioactive isotopes as sterilants requires that facilities are operated according to nuclear safety standards. Second, the release of large numbers of sterilized insects and the suppression of the target pest can lead to changes in interactions among species and food webs. While this is difficult to assess, the impact is generally regarded as being negligible as the effects caused by the mass-release of sterilized insects are short-lived since the insects cannot reproduce and the pests are typically invasive (non-native) in the area in which they are targeted. A third risk is that of unintentional releases or escapes of fertile insects (Parker, 2005). Consequently, setting up mass-rearing facilities within the potential habitats of the target pest population requires adherence to strict quarantine guidelines during production and shipping (Nagel and Peveling, 2005). In some cases, production is only allowed in regions where the pest is already established or where environmental conditions prevent establishment. An example is sterile fruit fly production in the USA (USDA, 2001). In addition, it is recommended that responsible authorities verify the effectiveness of the sterilizing treatments prior to the release (FAO, 2005). A fourth risk of SIT programmes relates to adverse effects caused by conventional control techniques (e.g., insecticides) that are used for population suppression prior to the release of sterilized insects (Nagel and Peveling, 2005). In general there is no formal ERA procedures in place for SIT.

#### *2.2.4. Risk Management Considerations*

Monitoring the effectiveness of a SIT release is an integral part of any programme (Parker, 2005). The FAO (2005) standard mentions, in particular, the marking of the sterile insects to differentiate them from wild insects to be able to monitor the release of the organisms “in order to evaluate and, as necessary, respond to the impact on the target and non-target organisms”. Each batch is monitored for radiation dose to ensure reasonable levels of sterilization occur. Otherwise, no additional risk management schemes are deployed after release, given that the sterilized insects are not able to reproduce, and thus any adverse impact would be highly localized and could (if required) be controlled using conventional (e.g., insecticide) methods.

## **2.3. *Wolbachia*-infected Insects**

### **2.3.1. Background**

*Wolbachia* (type species *W. pipientis*, Rickettsiales: Rickettsiaceae) are intracellular, maternally inherited endosymbionts that manipulate the reproduction of their host in various ways to favour their own maternal transmission (Werren et al., 2008). They are estimated to be present in 40 - 60% of insect species (Hilgenboecker et al., 2008; Weinert et al., 2015; Zug and Hammerstein, 2012). In addition to vertical transmission, they can also transmit horizontally to infect new host species (Werren et al., 1995, 2008), however, this appears to be a very rare event (Sinkins and Gould, 2006). While the transfer of *Wolbachia* between arthropod herbivores and their predators has not been observed (Hurst et al., 2012; Popovici et al., 2010) it does occur in parasitoid-host relationships both from host to parasitoid and also between parasitoids attacking the same host (Ahmed et al., 2015; Heath et al., 1999; Pattabhiramaiah et al., 2011; Stouthamer et al., 1999). There is evidence that horizontal gene transfer occurs between *Wolbachia* and its host (Choi et al., 2015; Klasson et al., 2009).

*Wolbachia* strains are regarded as selfish genetic elements that are deployed in incompatible insect technique (IIT) programs (Alphey, 2014). Cytoplasmic incompatibility (CI) (i.e., sperm-egg incompatibility) is the most common drive system of *Wolbachia* (O'Neill et al., 1997; Werren et al., 2008). The molecular basis of CI was recently described by LePage et al. (2017) and Lindsey et al. (2018). Under natural conditions, for example, the rapid spread of *Wolbachia* *w*Ri-infected strains has been observed in *Drosophila simulans* in California (Turelli and Hoffmann, 1991; Weeks et al., 2007) and in Australia (Kriesner et al., 2013).

Unidirectional CI occurs between *Wolbachia*-infected and uninfected populations. Uninfected females only produce viable offspring when they mate with uninfected males but not when they mate with infected males. Infected females, however, can successfully mate with infected and uninfected males and thus have a reproductive advantage. Consequently, the *Wolbachia* infection will spread through the population (Alphey, 2014; O'Neill et al., 1997). Bidirectional CI is found in populations that are infected with different (incompatible) *Wolbachia* strains. In this case, only matings between females and males carrying the same *Wolbachia* strain will result in offspring.

CI caused by *Wolbachia* can be deployed for the control or alteration of pest

populations in two main ways. First, the release of *Wolbachia*-infected males that are incompatible with the resident (uninfected) females could lead to population suppression (as though the males were sterilized) (termed incompatible insect technique, IIT). As males are dead-end hosts for the *Wolbachia*, the introduced strain does not establish in the target population. The first success of this approach was achieved in the 1960s (Laven, 1967) (see Box 1). At that time, however, the role of *Wolbachia* was unknown. More recently, control of natural *Ae. albopictus* populations by the release of males infected with the *Wolbachia* ZAP (also known as *wPip*) strain has been reported from the USA and a product (ZAP Males®) was approved by the US EPA in November 2017 (US EPA, 2017; Waltz 2017). As for SIT, large numbers of infected males have to be released. For example, a release ratio of 10 *Wolbachia*-infected males per 1 wild male is recommended (releases twice per week) for ZAP Males® (US EPA, 2017). Recently, Zheng et al. (2019) demonstrated that the release of *wPip*-infected *Ae. albopictus* (that were exposed to low-dose radiation to sterilize females prior to release) caused more than 90% population suppression on two islands in a river in Guongzhou, China. Second, *Wolbachia* can also be deployed to alter/replace a population of the target species (Bourtzis et al., 2014). This could for example be used to cause disease resistance in an insect vector population as certain strains of *Wolbachia* are known to limit their hosts' ability to transmit mosquito-borne pathogens (Iturbe-Ormaeche et al., 2011). The feasibility of this approach has been documented in semi-field experiments (Walker et al., 2011) and in the open field in Australia, where the *wMel* *Wolbachia* infection introduced into the Dengue vector mosquito *Aedes aegypti* (Diptera: Culicidae) was found to have successfully invaded the natural *Ae. aegypti* populations reaching near-fixation within a few months (Hoffmann et al., 2011, 2014; Schmidt et al., 2017). The possibility of transferring *Wolbachia* mechanically into novel hosts (transinfection) to create associations not restricted by mating barriers has vastly increased the possibilities for application of this technology (Hughes and Rasgon, 2014). The most widespread and successful technique is that of microinjection of early embryos (Hughes and Rasgon, 2014). For example, using this technique, *Wolbachia* have been successfully introduced into a naturally uninfected pest, the Mediterranean fruit fly *Ceratitidis capitata* (Diptera: Tephritidae) (Zabalou et al., 2004), and the mosquitoes *Ae. aegypti* (Xi et al. 2005) and *Anopheles stephensi* (Bian et al., 2013). In the case of *C. capitata*, strong unidirectional CI causes crosses between

infected males and uninfected females to produce virtually no viable offspring, resulting in population suppression in cage experiments (Zabalou et al., 2004, 2009). The use of novel *Wolbachia*-host associations for pest control requires the selection of an appropriate *Wolbachia* strain, successful transinfection and adaptations, investigations to detect potential fitness costs, and that the original host effects associated with a particular *Wolbachia* strain persist after transfer to the new host (Hoffmann et al., 2015; Hughes and Rasgon, 2014). Continuous selection is key to establish a stable transinfected line. When population suppression is the goal, it is advantageous to select a strain with a low invasion threshold (i.e., a strain with a low fitness cost associated with the *Wolbachia* infection) to limit the number of infected insects that need to be released (Hoffmann et al., 2015). As with SIT, it is advantageous to reduce the target populations, for example by insecticides, prior to the releases to reach higher levels of efficacy.

### 2.3.2. Regulations

Regulatory experience with the assessment of *Wolbachia*-infected insects has so far only been gained with mosquitoes. In the US, EPA has been regulating *Wolbachia* as a biopesticide since 2011. This includes experimental use permits for *Ae. polynesiensis*, *Ae. albopictus*, and *Ae. aegypti* (Dobson et al., 2016). In November 2017, US EPA approved the release of *Wolbachia*-infected *Aedes albopictus* males (ZAP Males®) to control this important vector in the USA (US EPA, 2017; Waltz, 2017). The agency evaluated the *Wolbachia* ZAP strain as a new microbial pesticide under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and its use in male *Ae. albopictus*. In Australia, the case involved the release of *Aedes aegypti*. Since both the mosquitoes and the *Wolbachia* strain (wMel) used occur naturally in Australia, this “novel organism association” did not fall under the various legislation that regulates the release of species in Australia (De Barro et al., 2011). After circulating through the various Federal agencies it was finally assessed in a similar manner to veterinary chemical products, i.e., considering *Wolbachia* as a “substance”, by the Australian Pesticides and Veterinary Medicines Authority (APVMA) (De Barro et al., 2011). In Europe, both the bacterium and the host arthropod are regarded and regulated as biocidal products and regulated accordingly, while their combination is not (EU, 2018).



### 2.3.3. Experience with Environmental Risk Assessment

In the case of *Wolbachia*-infected mosquitoes, the risk assessments conducted focused on the (i) effects on humans, and (ii) effects on the environment. Effects on humans were regarded as negligible given the fact that exposure is close to zero since only infected males are released, and male mosquitoes do not bite. It is important, however, that no *Wolbachia*-infected females are released since this could lead to the spread of the infection and consequently to population replacement rather than the intended suppression (Bourtzis et al., 2014). Consequently, a highly efficient sex-separation tool needs to be available or the methodology has to be combined with other tools such as low-dose irradiation to sterilize any females present (Nikolouli et al., 2018). For example, in the population suppression application of *Wolbachia* ZAP Males®, the sex separation technique applied is so efficient that the ratio at release is 1 infected female to 250,000 infected males (US EPA, 2017). In respect of environmental impacts, the assessments focused on the horizontal transfer of *Wolbachia*, the impact on non-target species that consume the infected mosquitoes, and on food-web effects (Murphy et al., 2010; Murray et al., 2016; US EPA, 2017). In general, horizontal transfers of *Wolbachia* are possible but appear to be very uncommon and may pose no new threat given that *Wolbachia* is naturally very common in arthropods and nematodes (O'Neill et al., 1997; Sinkins and Gould, 2006; Werren et al., 2008). Given that only males are released, which cannot transmit the *Wolbachia* vertically, exposure is limited to the lifespan of the males (O'Connor et al., 2012). Also, direct effects on mammals, birds and insects after ingestion of the *Wolbachia*-infected mosquitoes were considered to be negligible. The environmental risk of *Wolbachia*-infected mosquitoes has been assessed for *Ae. aegypti* in Australia (Murphy et al., 2010; Murray et al., 2016) and in Vietnam (Vietnam Eliminate Dengue Project, 2011). In the case of *Ae. aegypti* in Australia, food-web effects were regarded as negligible given the fact that the species was not considered to be an important component of the environment and the fact that alternative (current) control measures deliberately reduce the population.

### 2.3.4 Risk Management Considerations

Monitoring is recommended to inform on the effectiveness and success of the release, but no formal risk management options are required. There appears to be no formal requirement to collect information during the post-release phase to confirm the

ERA assumptions and conclusions.

## **2.4. Genetically Modified Insects**

### **2.4.1. Background**

Insects are genetically modified to introduce new traits, among them traits to suppress target insect populations (Slade and Morrison, 2014). Current applications of genetically modified (GM) insects are restricted to insects containing a self-limiting trait (Beech and Miller, 2013), that result in non-viable offspring due to the introduction of a repressible lethal genetic system (Phuc et al., 2007). As with SIT, this control strategy is based on repetitive release of mass-produced males carrying the lethal trait, which mate with females resulting in offspring that die before adulthood (Beech et al., 2009). The second generation of genetically modified insects was developed in such a way that male offspring survive thereby allowing additional mating cycles to reduce the pest population (Slade and Morrison, 2014).

This self-limiting technology has been used in open releases with the first generation mosquito *Aedes aegypti* OX513A to suppress local *Ae. aegypti* populations, vector of viruses causing diseases such as dengue and Zika (Alphey and Beech, 2012) resulting in more than 90% population suppression. OX513A has been tested since 2009 in trials in Malaysia (Lacroix et al., 2012; Lee et al., 2013), the Cayman Islands (Harris et al., 2011, 2012), Brazil (Carvalho et al., 2015), and Panama (Gorman et al., 2015), and activities are planned in Florida (USA) and India. In 2018, field trials with the second generation RIDL *Ae. aegypti* were also started in Brazil.

This self-limiting technology is also being developed to control arthropod pest species such as the fall army worm (*Spodoptera frugiperda*), diamondback moth (*Plutella xylostella*), olive fly (*Bactrocera olea*), Mediterranean fruit fly (*Ceratitis capitata*) and the spotted wing drosophila (*Drosophila suzukii*) (www.oxitec.com). Small scale field trials took place in 2017 in Geneva (NY, USA) with the OX4319L diamondback moth to test dispersal, survival and efficacy in suppression of the local diamondback moth population on cotton (Bolton et al., 2019). Previous glasshouse experiments have demonstrated the effectiveness of this approach (Harvey-Samuel et al., 2015).

The only larger scale field releases with GM agricultural pests were performed with radiation-sterilized pink bollworms genetically modified to express the fluorescent marker DsRed2. About 20 million insects were released and demonstrated field performance similar to that of a standard strain (Simmons et al., 2007, 2011).

#### 2.4.2. Regulations

GM insects are regulated in almost all jurisdictions under specific GMO legislation and regulatory experience has therefore been gained in countries where actual releases have taken place. In all jurisdictions an ERA is performed before GM insects can be released in the open, to assess potential adverse effects on the environment, including effects on human health. Guidance documents have been developed over the last few years that are specific to the use of GM insects. The World Health Organisation (WHO) has been at the forefront of developing guidance on the use of GM mosquitoes for many years, setting up projects for a harmonized framework of assessment (WHO, 2014). In 2016 additional guidance was published for living modified (LM) insects that act as vector of human and animal diseases, under the Cartagena Protocol on Biosafety (CBD, 2016). Regional approaches for GM insects have also been developed: NAPPO Standard RSPM 27 (NAPPO, 2007) and European Food Safety Authority Risk Assessment Guidance for GM Animals (EFSA, 2013) (Alphey and Beech, 2012, Beech and Miller, 2013; Wentworth, 2014). These guidelines do not differ greatly with respect to the ERA approach, but they do have different scopes. The WHO guidance is applicable for GM mosquitoes in all stages of release in the context of human health, the NAPPO standard (2007) is meant for importation and confined field release of GM arthropods in a phytosanitary context and the EFSA Guidance (2013) covers unconfined releases of all GM insects in the context of environmental safety. Other differences are the baseline used for comparison, the inclusion (or not) of efficacy measurement, and consideration of potential benefits of the application. Benefits are considered only in the WHO guidance, not in the other guidance mentioned.

An important aspect in these guidance documents is a phased approach for testing new technologies such as GM insects (Beech et al., 2012). A step-wise approach is advised to assess their potential risks, from laboratory to open release. Steps between the laboratory and open releases include ecologically (e.g., release in regions where organisms cannot overwinter) or physically (e.g., releases on islands) confined field trials and staged open field trials (WHO, 2014). During these steps the exposure to the environment is increased and containment of trials is reduced in a step-wise manner, but only if evaluation of the earlier steps indicates that the next step can be taken in terms of environmental safety (Glandorf and Breyer, 2016).

#### 2.4.3. Experience with Environmental Risk Assessment

Experience with the ERA over the last few years involved *Ae. aegypti* OX513A and was focused on direct and indirect effects on (i) human health and (ii) the environment. For GM insects the ERA focusses on hazards related to the newly introduced traits (or expressed metabolites) in the insect (Mumford, 2012). Most recent ERAs of *Ae. aegypti* OX513A are for an open release in Florida (FDA, 2016) and for a potential release on the island of Saba (Glandorf, 2017). With respect to the environment, the risk assessment focused on effects resulting from, for example, any fitness increase in the GM mosquito due to the introduced trait, consequences of horizontal gene transfer (HGT) of the introduced genes, interactions with non-target organisms after ingestion of GM insects, and effects on the food web resulting from lack of food due to suppression the natural *Aedes* populations. No fitness increase due to the newly introduced trait was observed. The probability of HGT was considered to be very unlikely, given the stable integration of the newly introduced genes in the genome of the GM mosquito. Even if HGT did occur, no adverse effects would be expected based on the nature of the transferred traits. Potential negative effects on non-target organism that consume GM *Aedes* were considered unlikely, based on feeding studies and the non-toxic and non-allergenic nature of the newly expressed proteins. Effects on the food web were considered to be negligible, since *Ae. aegypti* is not a keystone species as it occupies a narrow anthropophilic niche. In the context of the current vector control, potential adverse effects of GM *Ae. aegypti* on human health and the environment are therefore considered to be negligible (FDA, 2016; Glandorf, 2017), thereby confirming earlier conclusions of Brazil from 2014 (CTNBio, 2014).

#### 2.4.4 Risk Management Considerations

If risks are identified in the ERA, risk management measures (confinement measures) can be imposed on the release. As with SIT, monitoring of the insects and their efficacy is part of any programme (Parker, 2005). Monitoring of GM insects is enabled by a specific genetic marker to differentiate them from their wild counterpart, such as fluorescence based on the DsREd marker (Phuc et al., 2007) carried by all of Oxitec's genetically modified insects. The primary goal of monitoring GM insects is to detect potential adverse effects on human health and the environment (CBD, 2016) and is part of most field trials. Recently, hybridization between the released

population of OX513A *Ae. aegypti* and the native population has been reported in Brazil (Evans et al., 2019). The hybrid offspring, however, did not contain any transgenes and no evidence for environmental harm resulting from transferred background genes was provided.

In the EU, post-market monitoring for GM insects is obligatory but this may not be the case in all jurisdictions. In case of adverse effects, the releases of GM insects can be terminated, after which their number is expected to decline given their limited survival. Treatment with insecticides is another option to manage populations.

## **2.5. Defining Pathways to Harm**

The ecosystem service of biological pest control (i.e., the regulation of herbivores by arthropod predators and parasitoids) is an environmental component that is valued (in terms of human wellbeing) and should not be harmed by agricultural interventions (MEA, 2005). Across the different technologies described in section 2 that aim to suppress target pest(s), some principal pathways can be identified through which control interventions might harm biological control (Fig. 1):

1) The intended target pest population suppression could result in a lack of food for natural enemies and thus to a reduction in their populations with consequences for biological control. This indirect effect is an inevitable consequence of the intended effect, whatever the method used, and thus is typically accepted (Romeis et al., 2008) (#1 in Fig. 1).

2) The fitness of naturally occurring natural enemies might be adversely affected when consuming the released (SIT, *Wolbachia*-infected, GM) organisms. This could be due to the fact that the organisms have reduced quality as prey/hosts or caused directly by the intended modification (i.e., toxic effects of a gene drive construct or expressed proteins). This effect could be aggravated if the less fit released organisms were easier to catch or because they were released in large masses (in the case of SIT). This fitness effect could directly or indirectly (through reduced population sizes) lead to adverse outcomes in the biological control function they provide (#2 in Fig. 1).

3) In the case of *Wolbachia*-infected and GM organisms, both direct and indirect adverse effects could occur when natural enemies are feeding on herbivores that have hybridized with the released organisms (#3 in Fig. 1).

4) In the case of released biocontrol organisms, the native natural enemy populations

could be directly affected when hybridizing with the released species (#4 in Fig. 1) if the hybrids have a reduced fitness, altered behaviour etc., or indirectly when they are attacked or out-competed by the released species for example when sharing the same food source (#5 in Fig. 1).

5) In case of *Wolbachia*-infected and GM insects, the endosymbiont or the introduced gene could be transferred by horizontal gene transfer and then enter the food web with possible adverse outcomes for natural enemies feeding on those organisms (#6 in Fig. 1).

----- place Figure 1 approximately here -----

Only two of the technologies described in section 2 (i.e., *Wolbachia*-infected and GM insects) could also be deployed to achieve population alterations. In this case pathways #2, 3, and 6 caused by the reduced quality of prey/hosts of the altered organisms, by hybridization of the altered organisms with other species, and horizontal gene transfer would be equally relevant. There is, however, one obvious difference from the population suppression technologies: Pathway #1 is irrelevant (or less relevant) since the pest population size is not expected to change significantly (some reduction may occur if the released insects have a lower fitness than wildtype).

### 3. SYNTHETIC GENE DRIVES

#### 3.1. Synthetic Gene Drive Constructs

Over the last three decades, the development of synthetic genetic constructs has increased tremendously and our ability to engineer genomes offers novel potential for control of insect pests. In this section, we briefly review two broad classes of synthetic gene drives and their potential for insect pest control.

Toxin-antidote systems such as maternal-effect selfish genetic elements that were first described in flour beetles (*Tribolium castaneum*; Beeman et al., 1992) select for their own survival by inducing maternally driven lethality of all offspring not inheriting the specific selfish gene(s) from the maternal and/or paternal genome. These selfish elements have been developed in a number of insect systems (Buchman et al., 2018; Chen et al., 2007; Lorenzen et al., 2008). Synthetically engineered maternal factors are gene drives that rely on the combination of toxin-antidote system. One such

combination is a miRNA toxin (that disrupts embryonic development) expressed during egg formation in females that is linked to an antidote expressed during development. If the toxin is passed onto offspring from the miRNA-bearing mother, then development is disrupted. However, if the offspring also inherits the antidote (a miRNA resistant gene expressed only during development) normal development can be restored. Recently, it has been demonstrated that this sort of toxin-antidote system is potentially feasible for the control of *Drosophila suzukii* (Buchman et al., 2018).

Another broad class of synthetic gene drive system is based on nucleases. Nucleases are enzymes that break phosphodiester bonds and effect single- or double-stranded breaks in target molecules such as DNA. Nucleases need to associate with groups of (or single) nucleic acids and therefore carry specific site-recognition sequences (which might vary in base pair length). Specific engineered nucleases have been developed to bias sex ratios (through X-chromosome shredding), reduce fertility, alter refractoriness to pathogens or are linked to specific genes that induce lethality.

Nucleases can bias inheritance pattern through a process known as 'homing'. Alleles that carry a synthetic homing gene (say H+), an effector (the gene of action) that reduces fitness, will cut susceptible alleles (say H-) initiating repair of the damaged DNA. Using base sequence patterns on the undamaged (H+) chromosome (allele) that carries the nuclease this gene is copied into the cut site. Hence heterozygotes (H+/H-) are converted to homozygotes (H+/H+). This homing and cleaving of recognition sites with high efficiency and using cellular mechanisms of DNA repair (through recombination-driven repair - homology-directed repair) is applied by engineering gene drive constructs to target particular DNA sequences to achieve desired effects.

Synthetic nuclease constructs have been developed for insect disease vectors. For instance, homing endonuclease genes (HEGs) have been developed for *Anopheles* mosquitoes (e.g., Windbichler et al., 2007). One synthetic HEG, I-PpoI (which cuts DNA on the X-chromosome), has been shown to cause cytotoxicity, arresting cell proliferation in *Anopheles* cell lines. Targeting specific (highly conserved) ribosomal rDNA repeats located on the X-chromosome in *Anopheles*, when this HEG is expressed specifically during male meiosis, it can disrupt X-bearing sperm and lead to male biased sex ratios. Similar meiotic drive effects have been reported in other

insect systems (such as *Aedes* and *Culex* mosquitoes - see Wood and Newton, 1991).

The principles of synthetic gene drives have recently been extended to CRISPR-Cas systems. Like homing nucleases, these synthetic drives aim to cut DNA to disrupt or replace an original gene by converting heterozygote drive carriers into homozygote drive carriers. Guided by specific RNA (usually around 17-20 nucleotides in length) to loci on the DNA, the Cas nuclease enzyme induces a double strand break in the DNA. Homology-directed repair then copies the drive (and effector sequences carried by the CRISPR-Cas construct) into the adjacent break site. The advantage of CRISPR-Cas based systems is the ease by which gene editing can be developed. Major drawbacks of these CRISPR-Cas systems are potential off-target effects (guide RNAs misaligning) and the need to develop specific guide RNAs. Further, breaks in DNA can be repaired through an alternative mechanism (non-homologous end joining) which essentially glues the broken ends of the DNA together, mostly likely leading to the loss of the drive element. To date, synthetic CRISPR-Cas drive constructs have been developed as tools towards the control of several insect pests and vectors (Gantz et al., 2015; Hammond et al., 2016; Huang et al., 2016; Kyrou et al., 2018).

### **3.2. Application of synthetic gene drive constructs**

The application of synthetic gene drives constructs is wide. Low or no threshold drives (those that require low frequency of releases for establishment and spread) focus on the panmictic spread of constructs. In agricultural pest control, suppression drives - those aimed at developing genetic constructs that induce lethality, induce flightlessness or bias sex ratios so that populations decline - might be most applicable, although population alteration for vectors of plant diseases are also potential applications of synthetic drives.

Given that gene drives have the potential to be wide-spread, even panmictic, this technology might therefore lead to effects across large spatial and/or temporal scales. Developing gene drive systems that are spatially or temporally restricted, contained or confined in their ability to spread is the current focus of biotechnological development (e.g., Li et al., 2019; Noble et al., 2019; Oberhofer et al., 2019).

Furthermore, precaution can be added during the early phase of gene drive development by testing the organisms in “ecologically and/or physically contained



environments”, i.e. in regions/habitats where the organisms cannot establish in the case of escapes or on islands (NASEM, 2016; WHO, 2014).

One of the first things to bear in mind is that synthetic, loci-specific drives might have different population biologies (such as the rate of spread, fitness costs and replication frequency) than naturally occurring transposable elements that insert and spread rapidly through populations. Understanding the population genetic process of the synthetic construct spread in the relevant ecological context is essential to the application of these technologies.

Given the need for appropriate population biological understanding, we consider two broad aspects to the applicability of gene drive system for agricultural pest management. One of these might focus on the threshold allele frequency which must be exceeded to make the drive spread and the second might focus on designing contained drives. While clearly not mutually exclusive, we will explore the consequences of these approaches separately.

Synthetic nucleases, low threshold drives, require only a small seed (low numbers of released modified insects) for sufficient spread of the (costly) genetic element. Alternatively, developing high threshold drives might be more acceptable as only once the frequency of the releases is high enough will the element spread and if this frequency falls spread rates will fall. Accidental spill-over into a new population may be unlikely to spread if the threshold is fairly high. Designing drives with this sort of genetic structure through under-dominant genetic mechanisms (Davis et al., 2001; Khamis et al., 2018) might be one approach to developing acceptable synthetic drive systems for insect pest management.

Spatially and/or temporally contained drivers might be engineered in such a way that the drive element is dissociated from the effector (the gene of action). An effector gene that dynamically biases its own inheritance but is spread in a Mendelian fashion might be one such construct to limit spread. An alternative might be to consider a ‘daisy-chain’ drive system whereby each genetic element in the chain drives the next. In a CRISPR based drive system, dissociating the guide RNA from the Cas enzyme and from an effector might be one way to achieve this contained spread. As the effector is only spread in the presence of a cutting Cas, which is only spread in the presence of a guide RNA, fitness costs incurred over time of the effector would ensure that it is eventually lost through selection against genes with deleterious fitness consequences (Del Amo et al., 2019a; Esvelt and Gemmell, 2017). In this way

dissociated split gene drives are predicted to deliver physical or ecological containment while leading to local suppression of the pest insect. An alternative approach proposed is to activate the gene drive element by the application of a synthetic small molecule (Del Amo et al., 2019b).

### **3.3. Phased approach to developing gene drive organisms**

The development and application of synthetic gene drive constructs should address an impending ecological or economic problem caused by an agricultural pest or invasive species. Regulating gene drive products should then focus on aspects of efficacy and biosafety of the product through proportionate and appropriately formulated risk-based hypotheses. As for the other technologies described above, the differentiation between efficacy and risk is not very apparent and the evaluations are not clearly separated. It appears, though, that moving to the next developmental phase with a GD organism requires risk mitigations to be sufficiently effective.

#### **3.3.1. Risk Hypotheses: Efficacy and Biosafety Aspects**

Building on existing risk assessment paradigms, the efficacy and biosafety of synthetic gene drives for insect control has centred on a phased approach (EFSA 2013; Hayes et al., 2018; NASEM, 2016; WHO, 2014). While existing legislative frameworks might provide some guidance, the release of gene-drive products may present novel aspects related to biosafety. Guidance on a phased testing approach evaluates risks from the laboratory through to contained and confined small scale experiments then onto large scale field trials onwards to commercial release. At each phase, risk assessments and mitigation strategies evaluate whether, based on efficacy and/or biosafety criteria, a genetic modification can be moved on to the next tier of testing (Hayes et al., 2018; NASEM, 2016; WHO, 2014). However, for gene drive constructs this tiered phasing needs careful scrutiny since by their very nature these gene drives products are expected to spread, like classic biocontrol agents, beyond the point of release. This makes all development phases beyond the tight confines of the laboratory a continuum rather than a discrete set of tiers where risk evaluation is evaluated at each point of the tier. Before moving forward, the risk for synthetic gene drive products should be evaluated across the whole tiered phase testing scheme. To address this challenge, at an early stage of gene drive development, organism might be tested or released under “ecologically

and/or physically contained” environments as described above.

Risk hypotheses cover the adverse effects to wider biodiversity and/or human health. Yet for synthetic gene drive products, where a suppression drive is expected to reduce population abundance, that is a demonstrable expectation of the product. Impact on ecosystem processes and services may require different risk evaluations depending on the type of drive (low vs high threshold) and whether the aim is to suppress or replace the pest population.

### *3.3.2. Target product profiling for informing synthetic gene drive risk assessments*

Identifying specific uses or targets (e.g., agricultural pest control; plant disease reductions) for a particular product or regulated entity within an ERA is essential in defining plausible pathways to harm. Target Product Profiles (TPPs) used together with phased testing allow the relevant criteria (e.g., desired target, expected outcome; safety and efficacy characteristics) that profile products for development, release and/or commercialisation to be identified. This approach of developing product or application profiles is a valuable tool in early engagement with policy and decision makers (NASEM, 2016). Widely used in other product-driven disciplines such as pesticide development, they are now emerging tools in evaluating the efficacy of gene drive constructs for insect vector control (e.g., Carballar-Lejarazu and James, 2017; Killeen et al., 2011). TPPs align the needs of end-user application with the efficacy characteristics and biosafety criteria of the ERA together with the cost-efficiency targets that product developers should aim to meet.

These sorts of frameworks also provide evidence for informing an environmental risk assessment as they highlight the ideal outcomes and minimal acceptable outcomes from a product. These outcomes define boundaries for what can be deemed as ‘good enough’ for a product to pass the environmental risk assessment.

Identifying key measurable parameters provides information on efficacy criteria. For agricultural pests, these parameters might focus on pest life history (longevity, fecundity, dispersal, density dependence; population structure) or on the synthetic genetic construct (e.g., homing rate). Life history theory (e.g., Stearns, 1992) and evolutionary genetic theory (e.g., Charlesworth and Charlesworth, 2010) can help inform about the key aspects of product design, description and risk evaluation. For instance, life history parameters can be used to predict ecological rates of spread (e.g., through estimates on intrinsic rates of increase). Life history parameters might

feed into entomological endpoints (e.g., reduction of population size) or economic endpoints (e.g., crop yield criteria) on gene drive spread or expected duration of persistence.

Cost-effectiveness frameworks (Alphey et al., 2011; Hackett and Bonsall, 2016; Khamis et al., 2018) provide an appropriate way to connect how production, distribution and monitoring costs can be incorporated into efficacy, biosafety and product specification. For instance, considering how to monitor the impact and efficacy of a suppression gene drive requires appropriate cost comparators (e.g., conventional control intervention costs; national or local budgets for pest control). Furthermore, economic criteria such as discounting on the durability of the product and the emergence of resistance might also inform on efficacy or biosafety risk criteria associated with synthetic gene drive products for agricultural pest control. As noted, within the risk assessment paradigm it is important to consider whether it is the specific molecular construct or the pest strain (phenotype) that is the product under regulatory scrutiny as this will inform the product profiling, efficacy and safety criteria. Mathematical modelling of synthetic gene drives is essential and within this modelling parameter sensitivity is critical to understanding acceptable risks (and risk mitigation strategies) posed by the release of synthetic gene drive constructs (de Jong, 2017).

## **4. CASE STUDY *Drosophila suzukii***

### **4.1. Background**

The spotted wing *Drosophila*, *D. suzukii* is of Asian origin but has established widely in Europe, North- and South America in recent years (Asplen et al., 2015) and possesses the potential for further spread into Africa and Australia (dos Santos et al., 2017). *Drosophila suzukii* can oviposit into undamaged, ripening fruit with sufficiently soft skin and developing larvae render fruits unmarketable. Serious revenue losses for berry-, stone- and vine- fruit producers (Farnsworth et al., 2017; Mazzi et al., 2017) require efficient pest control measures. However, control of *D. suzukii* is challenging as developing larvae within the fruit are protected from the application of insecticides and numerous overlapping generations (Tochen et al., 2014) mean that newly emerging adults have to be targeted multiple times. Oviposition into almost ripe fruit and rapid development of larvae challenge farmers to meet the legally required pre-harvest intervals after pesticide application. Adults of *D. suzukii* can also re-infest

orchards after sheltering and reproducing in semi-natural habitats (Santoemma et al., 2018). Physical control measures such as intensive orchard sanitation (Leach et al. 2018), exclusion netting (Leach et al., 2016), or the application of kaolin, lime and rock dusts to alter the fruit surface (Strack et al., 2017) have shown to reduce fruit damage but are either extremely labour intensive, costly or not sufficiently effective to meet markets' zero-tolerance of *D. suzukii* infestation (Mazzi et al., 2017).

#### **4.2. Tools for area-wide *D. suzukii* management**

Area-wide management strategies have entered the focus of attention for the control of *D. suzukii*. Classical biological control using larval parasitoids of the families Braconidae and Figitidae (in particular: *Ganaspis* cf. *brasiliensis*) is currently being considered (Girod et al., 2018b; Wang et al., 2018). Another strategy aims to reduce offspring production in wild females by SIT using radiation-sterilized males (Lanouette et al., 2017). Also, IIT is a potential option given that *Wolbachia* are well-known from *Drosophila* and the rapid spread of an incompatibility factor within a natural population of *Drosophila simulans* has been observed in California (Turelli et al., 1991). Cattell et al. (2018) have infected *D. suzukii* with *Wolbachia* that induce CI. However, such a strategy for control of *D. suzukii* would require an efficient sexing technique or a sterilization of females with lower doses of radiation as a few accidentally released females carrying the *Wolbachia*-strain would compromise the efficacy of the IIT (Nikolouli et al., 2018). This, however, appears to be a challenge for *D. suzukii* (Cattell et al., 2018). In addition, horizontal gene transfer might be an issue that could lead to harm; for example in *D. ananassae*, an integrated whole genome of *Wolbachia* has been described (Choi et al., 2015).

Genetic modifications to control *D. suzukii* are also being considered and a dominant lethal genetic system (RIDL) is under development (<https://www.oxitec.com/>). The development of GM- based control strategies is not only fostered by the above-mentioned economic pressure but also by the extensive knowledge base that exists in similar systems. First, SIT (including genetically modified sterile insects) is well developed for various fruit flies (Tephritidae) that live in similar habitats and have a similar ecology to *D. suzukii*. Challenges such as developing efficient mass-rearing and to overcome reduced competitiveness of released males due to effects from domestication and storage (Gilchrist et al., 2012) can be addressed based on knowledge generated in these fruit fly systems. Second, *D. suzukii* is closely related

to *D. melanogaster*, the most common model arthropod species for genetic research. Knowledge generated for the latter species could be transferred to *D. sukukii*, for example the recently developed CRISPR-based system, which allows sterile males to be produced (Kandul et al., 2019).

#### **4.3. Developing Gene Drives to control *D. sukukii***

Embryonic lethal transgenes have been introduced into *D. melanogaster* (Horn and Wimmer, 2003) and could be adapted to *D. sukukii*. Cas9 and many of the promoters that have been developed for CRISPR/Cas9 systems in *D. melanogaster* (Port et al., 2014) also function in *D. sukukii* (Schetelig et al., 2018) making genome editing in this species feasible. For example, a CRISPR/Cas9 system in *D. sukukii* has been used to introduce site-specific mutations in a gene for eye colour to phenotypically mark individuals and in the master gene for female development *Sxl* to impair reproduction (Kalajdzic and Schetelig, 2017; Li and Scott, 2016). *D. melanogaster* females that are homozygous for the mutation in *Sxl* die early during development (Cline 1993) and embryos of *D. sukukii* injected with a plasmid containing the construct developed abnormal reproductive organs (Li and Scott, 2016). The construct has previously been used to engineer germline and somatic tissue in *D. melanogaster* (Kondo and Ueda, 2013; Port et al., 2014).

In a second system a *piggyback* transposon vector has been used to insert a female-specific lethality effector together with a fluorescent protein marker into *D. sukukii* (Schetelig and Handler, 2013) to eventually implement a conditional lethality system in this species. Whereas these systems could be used to remove females and to mark males for implementation of SIT, they have not yet been used for the construction of a gene drive in *D. sukukii*.

HEG-based gene drives are being investigated in the *D. melanogaster* model (Chan et al., 2013). As noted above, a *Medea* drive system, originally developed in *D. melanogaster* (Akbari et al., 2013; Chen et al., 2007), has recently been adapted to *D. sukukii* and patented in the United States (WO2017132207-A1). The system comprises a DNA sequence encoding a microRNA toxin under the control of a maternal germline-specific promoter and a second DNA sequence encoding an antidote under the control of an early embryo-specific promoter resulting in maternal-effect lethality. Crossing experiments demonstrated inheritance rates of up to 100%, depending on strain, while long term population cage experiments with a wild type

strain carrying pre-existing resistance and subsequent mathematical modelling revealed self-limiting dynamics of the system that confers a rather large fitness cost (Buchman et al., 2018).

#### **4.4. Pathways to harm for *D. suzukii* carrying a suppression drive**

Globally, the genus *Drosophila* contains about 2000 known species (O’Grady and DeSalle, 2018). While *Drosophila* spp. are best known for their role as decomposers of plant material, some species such as *D. suzukii* are considered important pests of fruit while others play a role as pollinators or predators (Markow and O’Grady, 2008; Skevington and Dang, 2002).

Focussing on the release of GM *D. suzukii* that contain a suppression drive with the aim to eradicate this pest in invaded areas, a number of potential harms to biodiversity have been identified that should be addressed in an ERA. These harms relate to important ecosystem services including regulating services (i.e., biological pest control), provisioning services (i.e., crop yield), supporting services (i.e., decomposition of organic matter, pollination). Figure 2 illustrates plausible pathways to harm (PTH) for those protection goals. In the following we will briefly summarize some of the evidence available to help address those PTHs.

##### **4.4.1. Impact on natural enemies**

In the invaded regions (USA and Europe), *D. suzukii* is attacked by generalist predators (Gabarra et al., 2015; Schmidt et al., 2019; Woltz and Lee, 2017; Wolf et al., 2018) and by pupal parasitoids of *Drosophila* spp. (Gabarra et al., 2015; Knoll et al., 2017; Mazzetto et al., 2016; Miller et al., 2015; Rossi-Stacconi et al., 2015). A similar natural enemy complex attacks the species in its region of origin (Daane et al., 2016; Guerrieri et al., 2016).

Indirect, food-web effects could occur as a consequence of the intended large-scale reduction in *D. suzukii* populations (Fig. 2, PTH #1). This is expected to be negligible in invaded areas where *D. suzukii* is not likely to become a key component in newly established prey/host-natural enemy associations (similar to *Ae. gambiae*; Collins et al., 2019). The situation might, however, be different in the area of origin. Asplen et al. (2015) highlight that this drosophilid can be common and cause damage to various fruits in East Asia indicating that it may be an important food-web component. In addition, apparently highly specialized parasitoids have been described (Nomano

et al., 2017; Girod et al., 2018a,b) and natural enemies that consume *D. sukuzii* could be adversely affected by toxic properties of the trait or protein encoded by the gene drive construct or due to the fact that the released insects have a reduced quality as prey or hosts (Fig. 2, PTH #2). Additional information might be required to assess the food-web effects of GD *D. sukuzii* and the population suppression in the area of origin in case that movement of the released GD *D. sukuzii* to those world areas cannot be excluded.

----- place Figure 2 approximately here -----

#### 4.4.2. Hybridization and horizontal gene transfer

One risk associated with the use of synthetic gene drives is that the drive element could be transferred to other distinct species. Gene transfer among *Drosophila* species could occur during hybridization of separate but closely related species (Fig. 2, PTH #3). The genus *Drosophila* contains a number of species groups. One of which is the *melanogaster* species group, an Old World clade with 193 species (Markow and O'Grady, 2005; O'Grady and DeSalle, 2018; Yang et al., 2012). The *melanogaster* species group is further separated into 12 species subgroups, one of which is *sukuzii* (Bock, 1980). Hybrid crosses resulting in viable offspring have been reported for closely related species (same species group) under laboratory conditions (Bock, 1984). A few instances of natural interspecific hybridization have also been reported; the phenomenon, however, appears to be a rare event (Bock, 1984; Garrigan et al., 2012). In the case of *D. sukuzii*, Fuyama (1983) reported hybridization with *D. pulchrella* under laboratory conditions. Studying *Wolbachia* infections, Conner et al. (2017) provide evidence for hybridization with *D. subpulchrella* from the *sukuzii* species subgroup. *D. subpulchrella* has been described from Japan and southern China (Takamori et al., 2006), uses a similar habitat and food sources and shows a similar seasonal pattern to *D. sukuzii* (Mitsui et al., 2010). Neither *D. subpulchrella* or *D. pulchrella* are yet present in the areas invaded by *D. sukuzii*. Consequently, hybridization of GD *D. sukuzii* with other *Drosophila* species is to be expected in the area of origin. Whether *D. sukuzii* is able to hybridize in other world areas, e.g., Europe, is unclear. To address this issue, the species that are present in the planned release area, their phylogenetic relatedness with *D. sukuzii* and potential to hybridize would have to be investigated.



Horizontal transfer of transposable elements has been reported for *Drosophila* spp. and may be more common than originally anticipated (Bartolomé et al., 2009; Herédia et al., 2004; Hill and Betancourt, 2018; Loreto et al., 2008). Introgression of the drive in non-target species due to hybridization or horizontal gene transfer does not automatically lead to a functional gene drive (Rüdelshheim and Smets, 2018). First, many factors determine the success of a drive. This includes the biology/ecology of the species, the driver's efficacy and fitness costs. Second, drives are multi-component systems and extremely sensitive to sequence variation in their recognition sequence. Despite the fact that the likelihood of a functional gene drive transfer between species is extremely low it should be evaluated before any environmental release (NASEM, 2016). Worst-case scenarios to be considered in the case of GD *D. sukuzii* would include hybridization experiments with *D. subpulchrella* or *D. pulchrella* to test whether the GD mechanism is still functional in the resulting progeny. Environmental harm could result when (a) the hybridized species would show an increased damage potential on fruit crops or (b) when the hybrids would have a reduced fitness leading to a reduction in important ecosystem services (Fig. 2).

## 5. DISCUSSION

To our knowledge problem formulation and pathways to harm (PTHs) to support the environmental risk assessment of organisms with gene drives are not yet well developed and have so far only been described for mosquitoes to eradicate malaria (Roberts et al., 2017; Teem et al., 2019). As yet, no PTHs are described for other applications of gene drives, such as for suppression drives in the control of agricultural insect pests. Above (and in Fig. 2), we discuss PTHs leading to the potential reduction of valued ecosystem services by introduction of *D. sukuzii* carrying a suppression drive. We focus mainly on PTHs resulting in loss of biological control provided by natural enemies (i.e., parasitoids and predators). While we describe PTHs for a suppression drive, we do not expect that they will differ for alteration drives. However, the importance of specific PTH under various drive scenarios may differ given the greater temporal and/or spatial exposure times. It appears that the PTHs for gene drive *D. sukuzii* leading to loss of biological control (Fig. 2) are the same as for the other agricultural insect pest control technologies described in this paper such as classical biocontrol, SIT, *Wolbachia* and use of GM

1106 insects (PTH #1-3 in Fig. 1).

1107 If genetic technology is used to introduce or create a gene drive, the resulting  
1108 organism will be a GMO and subject to regulation and the respective ERA. While  
1109 novel environmental harms associated with gene drive insects are unlikely, we  
1110 acknowledge that the environmental effects of gene drives could be more severe due  
1111 to a higher exposure (e.g., large scale, population wide) than with other technologies.  
1112 In addition, aspects / knowledge / experience from classical biocontrol and *Wolbachia*  
1113 application can be used to assess the environmental risks of gene drives.

1114 It appears that gene drive insects may have some specific peculiarities that need to  
1115 be addressed. These include: (i) the introduced trait spreads quickly even when it has  
1116 negative fitness consequences for its carrier; (ii) the genetic effect will spread and  
1117 influence populations or even entire species; (iii) the effect is potentially irreversible.  
1118 While this depends on whether we deal with a low or high threshold drive, we have  
1119 focused on the worst-case scenario (i.e., a low threshold drive). These specifics of  
1120 gene drives could make loss of biological control (Figs. 1, 2; PTHs #1-3) more likely  
1121 than for other insect control techniques.

1122 However, and most importantly, these peculiarities of gene drive organisms (i-iii  
1123 above) are not new. We have experience in assessing the environmental risks of  
1124 insects released to control target agricultural pests. These control strategies can  
1125 provide high levels of area-wide control and be irreversible. Potential risks that need  
1126 thorough evaluation are that area-wide and irreversible effects can also occur  
1127 through hybridisation of genetically modified insects with closely related species,  
1128 resulting in increased fitness or that are subject to selection pressure (comparable to  
1129 100 % gene flow). A non-drive trait will only spread if it has selective advantage or  
1130 positive selection pressure. However, gene drive traits could spread even with  
1131 negative fitness effects. An example from experience with GM crops is that of oil  
1132 seed rape, which can outcross with wild relatives. If the transferred trait gives the wild  
1133 relative a selective advantage in the specific receiving environment, this trait can also  
1134 spread though that population. However, it is the consequence of spread that informs  
1135 the risk assessment and decision making process.

1136 Given the potential of a low threshold gene drive to have potential population-wide  
1137 effects over larger geographical areas, an ERA for gene drive insects has to take into  
1138 account all receiving environments. Appropriate comparators for the effects of gene  
1139 drive insects must focus on the use of other methods for the control or eradication of

the agricultural pest. The use of a gene drive organism must thus be placed in the context of existing arthropod control/ replacement strategies (Roberts et al., 2017). Similar to classical biological control, studies conducted under confined conditions must ensure that environmental harm is unlikely to occur or similar to acceptable harm relative to expected benefits after release of the organisms into the environment since it is unlikely that they can be recovered. Appropriate interventions for controlling unwarranted spread or unwanted genetic outcomes must be considered. So, in contrast to most other technologies, a step-wise safety assessment from lab to glasshouse to restricted field release to open field release is not feasible in many cases. Further aspects of biosafety could be enhanced in the risk assessment by testing the functional trait and the drive mechanisms separately before combining them (Akbari et al., 2015).

As for other GM applications, risk management considerations are important to mitigate environmental risks. These include the following:

- Use of high threshold drives or contained drives (e.g., daisy drive)
- A gene drive insect with a low threshold should not be released without risk management measures; for example, a safeguard drive system could be developed that overwrites a previous one (Wu et al., 2016).

Risks can be linked to the accidental escape of gene drive organisms. Containment is thus very important. This includes laboratory containment, field cage containment, ecological containment (testing in regions where no natural population of the same insect can occur and interbreeding is not possible), geographical containment (e.g., releases on islands), and/or molecular containment (Akbari et al., 2015; Benedict et al., 2018; Di Carlo et al., 2015; James et al., 2018; NASEM, 2016; van der Vlugt et al., 2018).

Other aspects with specific relevance for use of gene drive insects, not directly related to environmental safety, include the question about the establishment and spread of the GMO. This focuses concerns on questions about the coexistence of different agricultural production systems (e.g., organic versus conventional) but also on transboundary movements (e.g., covered by the Cartagena Protocol (Marshall, 2010, 2011)). A number of international treaties are critically relevant to the global governance of gene drives (Brown, 2017; Redford et al., 2019). Besides the CBD, these include the International Plant Protection Convention (IPPC) which governs

phytosanitary standards that have been used to develop to international norms surrounding classical biocontrol (also see IPPC Standards for wood packaging, ISPM 15). The WHO has also developed guidelines for the governance of GM mosquitoes for malaria control (WHO, 2014) which is applicable to other gene drive organisms. Furthermore, the use of gene drive organisms has also already triggered debate about whether it is ethical, or seen as a risk, to attempt to eradicate an entire species (Simon et al., 2018; Thompson, 2018). It is important, however, to place all these questions into context and consider both risks and benefits. Species control might be achievable through a range of (more or less harmful) approaches and evaluating these requires appropriate benefits and risks of gene drives to be evaluated, as suggested by UK House of Lords (2015).

## **6. CONCLUSIONS**

Synthetic gene drive constructs have the potential to provide a suite of additional tools for the control of agricultural insect pests. Regulatory frameworks should be proportionate to risk-benefit considerations and consider the following facts:

- We have experience in assessing the environmental effects of insects released to control target pests. These can provide high levels of area-wide control and be irreversible.
- While we do not envisage any unforeseen risks with the use of gene drive organisms to control agricultural pests, effects might be more severe (e.g., large scale, population wide) (e.g., release of biocontrol agents)
- Existing risk assessment frameworks can be used to assess the potential adverse effects from insects carrying gene drives to control agricultural pests.
- Care has to be taken when requiring data for GD organisms to define whether data are required with the purpose of product characterization or to support ERA.
- The context (e.g., agricultural vs. conservation or human/animal health) in which the gene drive organisms are used has to be considered in the environmental risk assessment.
- Within the particular context it is important that the assessment also takes into account the benefits of using the technology. In particular, this relates to the environmental effects that are a direct consequence of the intended reduction of the target pest.

Gene drive technologies offer novel solutions to the control of insect pests and vectors. Drawing on existing practices, experiences and legislative frameworks provides a pragmatic and proportionate approach to evaluating the environmental risks of these technologies.

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## Conflict of interests

The authors declare no conflict of interests.

## REFERENCES

- Adelman, Z., Akbari, O., Bauer, J., Bier, E., Bloss, C., Carter, S.R., Callender, C., Costero-Saint Denis, A., Cowhey, P., Dass, B., Delborne, J., Devereaux, M., Ellsworth, P., Friedman, R.M., Gantz, V., Gibson, C., Hay, B.A., Hoddle, M., James, A.A., James, S., Jorgenson, L., Kalichman, M., Marshall, J., McGinnis, W., Newman, J., Pearson, A., Quemada, H., Rudenko, L., Shelton, A., Vinetz, J.M., Weisman, J., Wong, B., Wozniak, C., 2017. Rules of the road for insect gene drive research and testing. *Nat. Biotech.* 35, 716-718.
- Ahmed, M.Z., Li, S.J., Xue, X., Yin, X.J., Ren, S.X., Jiggins, F.M., Greeff, J.M., Qiu, B.L., 2015. The intracellular bacterium *Wolbachia* uses parasitoid wasps as phoretic vectors for efficient horizontal transmission. *PLoS Pathog.* 10, e1004672.
- Akbari, O.S., Buchmann, A., 2017. Use of medea elements for biocontrol of *D. suzukii* populations, US Patent Number WO2017132207-A1. <https://patents.google.com/patent/WO2017132207A1/en> (accessed 19 December 2019).
- Akbari, O.S., Matzen, K.D., Marshall, J.M., Huang, H., Ward, C.M., Hay, B.A., 2013. A synthetic gene drive system for local, reversible modification and suppression of insect populations. *Curr. Biol.* 23, 671-677.
- Akbari, O.S., Bellen, H.J., Bier, E., Bullock, S.L., Burt, A., Church, G.M., Cook, K.R., Duchek, P., Edwards, O.R., Esvelt, K.M., Gantz, V.M., Golic, K.G., Gratz, S.J., Harrison, M.M., Hayes, K.R., James, A.A., Kaufman, T.C., Knoblich, J., Malik, H.S., Matthews, K.A., O'Connor-Giles, K.M., Parks, A.L., Perrimon, N., Port, F.,

1242 Russell, S., Ueda, R., Wildonger, J., 2015. Safeguarding gene drive experiments  
1243 in the laboratory. *Science* 349, 927-929.

1244 Alphey, L., 2014. Genetic control of mosquitoes. *Annu. Rev. Entomol.* 59, 205-224.

1245 Alphey, L., Beech, C.J., 2012. Genetically engineered insects - regulatory progress  
1246 and challenges, in: Wozniak, C.A., McHughen, A. (Eds.), *Regulation of*  
1247 *Agricultural Biotechnology: The United States and Canada*. Springer, pp. 281-  
1248 299.

1249 Alphey, N., Bonsall, M.B., 2018. Genetics-based methods for agricultural insect pest  
1250 management. *Agric. Forest Entomol.* 20, 131-140.

1251 Alphey, N., Alphey, L., Bonsall, M.B. 2011. A model framework to estimate impact  
1252 and cost of genetics-based sterile insect methods for dengue vector control.  
1253 *PLoS ONE* 6, e25384.

1254 Asplen, M.K., Anfora, G., Biondi, A., Choi, D.S., Chu, D., Daane, K.M., Gibert, P.,  
1255 Gutierrez, A.P., Hoelmer, K.A., Hutchison, W.D., Isaacs, R., Jiang, Z.-L., Kárpáti,  
1256 Z., Kimura, M.T., Pascual, M., Philips, C.R., Plantamp, C., Ponti, L., Vétek, G.,  
1257 Vogt, H., Walton, V.M., Yu, Y., Zappalà, L., Desneux, N., 2015. Invasion biology  
1258 of spotted wing *Drosophila* (*Drosophila suzukii*): a global perspective and future  
1259 priorities. *J. Pest Sci.* 88, 469-494.

1260 Babendreier, D., Bigler, F., Kuhlmann, U., 2005. Methods used to assess non-target  
1261 effects of invertebrate biological control agents of arthropod pests. *BioControl* 50,  
1262 821-870.

1263 Bakri, A., Mehta, K., Lance, D.R., 2005. Sterilizing with ionizing radiation, in: Dyck,  
1264 V.A., Hendrichs, J., Robinson, A.S. (Eds.), *Sterile Insect Technique. Principles*  
1265 *and Practice in Area-Wide Integrated Pest Management*. IAEA, Springer, pp.  
1266 233-268.

1267 Barratt, B.I.P., Ehlers, C.A.C., 2017. Impacts of exotic biological control agents on  
1268 non-target species and biodiversity: Evidence, policy and implication, in: Coll, M.,  
1269 Wajnberg, E. (Eds.), *Environmental Pest Management: Challenges for*  
1270 *Agronomists, Ecologists, Economists and Policymakers*. John Wiley & Sons Ltd.,  
1271 Oxford, UK, pp. 325-346.

1272 Barratt, B.I.P., Howarth, F.G., Withers, T.M., Kean, J.M., Ridley, G.S., 2010.  
1273 Progress in risk assessment for classical biological control. *Biol. Contr.* 52, 245-  
1274 254.

1275 Bartolomé, C., Bello, X., Maside, X., 2009. Widespread evidence for horizontal

1276 transfer of transposable elements across *Drosophila* genomes. *Genome Biol.* 10,  
1277 R22. This article has been corrected. See *Genome Biol.* 2011, 12, 411.

1278 Bastide, H., Gérard, P.R., Ogereau, D., Cazemajor, M., Montchamp-Moreau, C.,  
1279 2013. Local dynamics of a fast-evolving sex-ratio system in *Drosophila simulans*.  
1280 *Mol. Ecol.* 22, 5352-5367.

1281 Beech, C.J., Miller, E., 2013. Regulation of genetically modified insects. *ISBR News*  
1282 20/08/2013. [http://isbr.info/news?news\\_id=24](http://isbr.info/news?news_id=24) (accessed 19 December 2019).

1283 Beech, C.J., Koukidou, M., Morrison, N.I., Alphey, L., 2012. Genetically modified  
1284 insects: Science, use, status and regulation. *Coll. Biosafety Rev.* 6, 66-124.

1285 Beech, C.J., Vasan, S.S., Quinlan, M.M., Capurro, M.L., Alphey, L., Bayard, V.,  
1286 Bouaré, M., McLeod, M.C., Kittayapong, P., Lavery, J.V., Lim, L.H., Marrelli,  
1287 M.T., Nagaraju, J., Ombongi, K., Othman, R.Y., Pillai, V., Ramsey, J., Reuben,  
1288 R., Rose, R.I., Tyagi, B.K., Mumford, J., 2009. Deployment of innovative genetic  
1289 vector control strategies: Progress on regulatory and biosafety aspects, capacity  
1290 building and development of best-practice guidance. *Asia-Pac. J. Mol. Biol.*  
1291 *Biotech.* 17, 75-85.

1292 Beeman, R.W., Friesen, K.S., Denell, R.E., 1992. Maternal-effect selfish genes in  
1293 flour beetles. *Science* 256, 89-92.

1294 Benedict, M., Burt, A., Capurro, M.L., De Barro, P., Handler, A.M., Hayes, K.R.,  
1295 Marshall, J.M., Tabachnick, W.J., Adelman, Z.N., 2018. Recommendations for  
1296 laboratory containment and management of gene drive systems in arthropods.  
1297 *Vector-Borne Zoonot. Dis.* 18, 2-13.

1298 Bian, G., Joshi, D., Dong, Y., Lu, P., Zhou, G., Pan, X., Xu, Y., Dimopoulos, G., Xi,  
1299 Z., 2013. *Wolbachia* invades *Anopheles stephensi* populations and induces  
1300 refractoriness to *Plasmodium* infection. *Science* 340, 748–751.

1301 Bigler, F., Bale, J.S., Cock, M.J.W., Dreyer, H., Greatrex, R., Kuhlmann, U.,  
1302 Loomans, A.J.M., van Lenteren, J.C., 2005. Guidelines on information  
1303 requirements for import and release of invertebrate biological control agents in  
1304 European countries. *Biocontr. News Info.* 26, 115N-123N.

1305 Bock, I.R., 1980. Current status of the *Drosophila melanogaster* species group  
1306 (Diptera). *Syst. Entomol.* 5, 341-356.

1307 Bock, I.R., 1984. Interspecific hybridization in the genus *Drosophila*, in: Hecht, M.K.,  
1308 Wallace, B., Prance, G.T. (Eds.), *Evolutionary Biolog.* Plenum Press, New York,  
1309 pp. 41-70.

1310 Bolton, M., Collins, H. L., Chapman, T., Morrison, N. I., Long, S. J., Linn, C. E, JR.,  
 1311 Shelton, A. M., 2019. Response to a synthetic pheromone source by OX4319L, a  
 1312 self-limiting diamondback moth (Lepidoptera: Plutellidae) strain, and field  
 1313 dispersal characteristics of its progenitor strain. J. Econ. Entomol. 112, 1546-  
 1314 1551.

1315 Borel, B., 2017. When the pesticides run out. Nature 543, 302-304.

1316 Bourtzis, K., Dobson, S.L., Xi, Z., Rasgon, J.L., Calvitti, M., Moreira, L.A., Bossi,  
 1317 H.C., Moretti, R., Baton, L.A., Hughes, G.L., Mavingui, P., Gilles, J.R.L., 2014.  
 1318 Harnessing mosquito-*Wolbachia* symbiosis for vector and disease control. Acta  
 1319 Trop. 132, S150-S163.

1320 Brelsfoard, C.L., Dobson, S.L., 2012. Population genetic structure of *Aedes*  
 1321 *polynesiensis* in the Society Islands of French Polynesia: implications for control  
 1322 using a *Wolbachia*-based autocidal strategy. Parasite Vector 5, 80.

1323 Brown, Z., 2017. Economic, regulatory and international implications of gene drives in  
 1324 agriculture. Choices 32, 1-8.

1325 Buchman, A., Marshall, J.M., Ostrovski, D., Yang, T., Akbari, O.S., 2018.  
 1326 Synthetically engineered *Medea* gene drive system in the worldwide crop pest  
 1327 *Drosophila suzukii*. Proc. Natl. Acad. Sci. USA 115, 4725-4730.

1328 Burt, A., 2003. Site-specific selfish genes as tools for the control and genetic  
 1329 engineering of natural populations. Proc. R. Soc. B. 270, 921-928.

1330 Burt, A., Trivers, R., 2006. Genes in Conflict. Harvard University Press.

1331 Carballar-Lejarazú, R., James, A.A., 2017. Population modification of Anopheline  
 1332 species to control malaria transmission. Pathog. Glob. Health 111, 424-435.

1333 Calkins, C.O., Parker, A.G., 2005. Sterile insect quality, in: Dyck, V.A., Hendrichs, J.,  
 1334 Robinson, A.S. (Eds.), Sterile Insect Technique. Principles and Practice in Area-  
 1335 Wide Integrated Pest Management. IAEA, Springer, pp. 269-296.

1336 Callaway, E., 2016. 'Gene drive' moratorium shot down at UN biodiversity meeting.  
 1337 Nat. Biotech., doi:10.1038/nature.2016.21216

1338 Callaway, E., 2018. Ban on 'gene drives' is back on the UN's agenda – worrying  
 1339 scientists. Nature 536, 454-455.

1340 Caltagirone, L.E., Doutt, R.L., 1989. The history of the vedalia beetle importation to  
 1341 California and its impact on the development of biological control. Annu. Rev.  
 1342 Entomol. 34, 1–16.

1343 Caplan, A.L., Parent, B., Shen, M., Plunkett, C., 2015. No time to waste-the ethical



1344 challenges created by CRISPR. EMBO Rep. 16, 1421-1426.  
 1345 Carballar-Lejarazú, R., James, A.A., 2017. Population modification of Anopheline  
 1346 species to control malaria transmission. Pathog. Glob Health, 111, 424-435.  
 1347 Carpenter, J.E., Bloem, S., Marec, F., 2005. Inherited sterility in insects, in: Dyck,  
 1348 V.A., Hendrichs, J., Robinson, A.S. (Eds.), Sterile Insect Technique. Principles  
 1349 and Practice in Area-Wide Integrated Pest Management. IAEA, Springer, pp.  
 1350 115-146.  
 1351 Carrière, Y., Eilers-Kirk, C., Sisterson, M., Antilla, L., Whitlow, M., Dennehy, T.J.,  
 1352 Tabashnik, B.E., 2003. Long-term regional suppression of pink bollworm by  
 1353 *Bacillus thuringiensis* cotton. Proc. Natl. Acad. Sci. USA 100, 1519-1523.  
 1354 Carvalho, D.O., McKemey, A.R., Garziera, L., Lacroix, R., Donnelly, C.A., Alphey, L.,  
 1355 Malavasi, A., Capurro, M.L., 2015. Suppression of a field population of *Aedes*  
 1356 *aegypti* in Brazil by sustained release of transgenic male mosquitoes. PLoS Negl.  
 1357 Trop. Dis. 9, e0003864.  
 1358 Cattel, J., Nikolouli, K., Andrieux, T., Martinez, J., Jiggins, F., Charlat, S., Vavre, F.,  
 1359 Lejon, D., Gibert, P., Mouton, L., 2018. Back and forth *Wolbachia* transfers reveal  
 1360 efficient strains to control spotted wing *Drosophila* populations. J. Appl. Ecol. 55,  
 1361 2408-2418.  
 1362 CBD (Convention on Biological Diversity), 2000. Cartagena Protocol on Biosafety to  
 1363 the Convention on Biological Diversity. <https://bch.cbd.int/protocol/> (accessed 19  
 1364 December 2019).  
 1365 CBD (Convention on Biological Diversity), 2016. Guidance on Risk Assessment of  
 1366 Living Modified Organisms and Monitoring in the Context of Risk Assessment.  
 1367 <https://www.cbd.int/doc/meetings/bs/mop-08/official/bs-mop-08-08-add1-en.pdf>  
 1368 (accessed 19 December 2019).  
 1369 Champer, J., Buchman, A., Akbari, O.S., 2016. Cheating evolution: engineering gene  
 1370 drives to manipulate the fate of wild populations. Nat. Rev. Genet. 17, 146-159.  
 1371 Chan, Y.-S., Huen, D.S., Glauert, R., Whiteway, E., Russell, S., 2013. Optimising  
 1372 homing endonuclease gene drive performance in a semi-refractory species: the  
 1373 *Drosophila melanogaster* experience. Plos One 8, e54130.  
 1374 Charlesworth, B., Charlesworth, D., 2010. Elements of Evolutionary Genetics.  
 1375 Roberts & Co Publishers, Greenwood Village, Colorado.  
 1376 Chen, C.-H., Huang, H., Ward, C.M., Su, J.T., Schaeffer, L.V., Guo, M., Hay, B.A.,  
 1377 2007. A synthetic maternal-effect selfish genetic element drives population

1378 replacement in *Drosophila*. Science 316, 597-600.

1379 Choi, J.Y., Bubnell, J.E., Aquadro, C.F., 2015. Population genomics of infectious and  
 1380 integrated *Wolbachia pipientis* genomes in *Drosophila ananassae*. Genome Biol.  
 1381 Evol. 7, 2362-2382.

1382 Cline, T.W., 1993. The *Drosophila* sex determination signal: how do flies count to  
 1383 two? Trends Genet. 9, 385-390.

1384 Cock, M.J.W., Murphy, S.T., Kairo, M.T.K., Thompson, E., Murphy, R.J., Francis,  
 1385 A.W., 2016. Trends in the classical biological control of insect pests by insects:  
 1386 an update of the BIOCAT database. BioControl 61, 349-263. An erratum to this  
 1387 article is available at <http://dx.doi.org/10.1007/s10526-017-9821-0>

1388 Collins, C.M., Bonds, J.A., Quinlan, M.M., Mumford, J.D., 2019. Effects of the  
 1389 removal or reduction in density of the malaria mosquito, *Anopheles gambiae* s.l.,  
 1390 on interacting predators and competitors in local ecosystems. Med. Vet. Entomol.  
 1391 33, 1-15.

1392 Conner, W.R., Blaxter, M.L., Anfora, G., Ometto, L., Rota-Stabelli, O., Turelli, M.,  
 1393 2017. Genome comparisons indicate recent transfer of wRi-like *Wolbachia*  
 1394 between sister species *Drosophila suzukii* and *D. subpulchrella*. Ecol. Evol. 7,  
 1395 9391-9404.

1396 CTNBio (Brazil National Biosafety Technical Commission), 2014. Technical opinion  
 1397 no. 3964/2014 (2014). Brasília: National Technical Biosafety Commission.  
 1398 Ministry of Science, Technology and Innovation.  
 1399 <http://bch.cbd.int/database/attachment/?id=14514> (accessed 19 December  
 1400 2019).

1401 Culliney, T.W., 2014. Crop losses to arthropods, in: Pimentel, D., Peshin, R. (Eds.),  
 1402 Integrated Pest Management. Springer, Dordrecht, pp. 201-225.

1403 Curtis, C.F., 1968. Possible use of translocations to fix desirable genes in insect pest  
 1404 populations. Nature 218, 368-369.

1405 Curtis, C.F., Sinkins, S.P., 1998. *Wolbachia* as a possible means of driving genes  
 1406 into populations. Parasitology 116, S111–S115.

1407 Daane, K.M., Wang, X.G., Biondi, A., Miller, B., Miller, J.C., Riedl, H., Shearer, P.W.,  
 1408 Guerrieri, E., Giorgini, M., Buffington, M., van Achterberg, K., Song, Y., Kang, T.,  
 1409 Yi, H., Jung, C., Lee, D.W., Chung, B.-K., Hoelmer, K.A., Walton, V.M., 2016.  
 1410 First exploration of parasitoids of *Drosophila suzukii* in South Korea as potential  
 1411 classical biological agents. J. Pest Sci. 89, 823–835.

1412 Daniels, S.B., Peterson, K.R., Strausbaugh, L.D., Kidwell, M.G., Chovnick, A., 1990.  
 1413 Evidence for horizontal transmission of the P transposable element between  
 1414 *Drosophila* species. *Genetics* 124, 339-355.  
 1415 Davis, S., Bax, N., Grewe, P., 2001. Engineered underdominance allows efficient and  
 1416 economical introgression of traits into pest populations. *J. Theor. Biol.* 212, 83-  
 1417 98.  
 1418 De Barro, P.J., Murphy, B., Jansen, C.C., Murray, J., 2011. The proposed release of  
 1419 the yellow fever mosquito, *Aedes aegypti* containing a naturally occurring strain  
 1420 of *Wolbachia pipientis*, a question of regulatory responsibility. *J. Consum. Prot.*  
 1421 *Food Saf.* 6 (Suppl. 1), S33-S40.  
 1422 De Clercq, P., Mason, P.G., Babendreier, D., 2011. Benefits and risks of exotic  
 1423 biological control agents. *BioControl* 56, 681-698.  
 1424 De Jong, T.J., 2017. Gene drives do not always increase in frequency: from genetic  
 1425 models to risk assessment. *J. Consum. Prot. Food Saf.* 12, 299-307.  
 1426 Del Amo, V.L., Bishop, A.L., Sánchez C., H.M., Bennett, J.B., Feng, X., Marshall,  
 1427 J.M., Bier, E., Gantz, V.M., 2019a. Split-gene drive system provides flexible  
 1428 application for safe laboratory investigation and potential field deployment.  
 1429 bioRxiv, doi: 10.1101/684597.  
 1430 Del Amo, V.L., Leger, B.S., Cox, K.J., Gill, S., Bishop, A.L., Scanlon, G.D., Walker,  
 1431 J.A., Gantz, V.M., Choudhary, A., 2019b. Small-molecule control of super-  
 1432 Mendelian inheritance in gene drives. bioRxiv, doi: 10.1101/6655620.  
 1433 Devos, Y., Romeis, J., Luttik, R., Maggiore, A., Perry, J.N., Schoonjans, R., Streissl,  
 1434 F., Tazazona, J.V., Brock, T.C.M., 2015. Optimising environmental risk  
 1435 assessments. Accounting for ecosystem services helps to translate broad policy  
 1436 protection goals into specific operational ones for environmental risk  
 1437 assessments. *EMBO Rep.* 16, 1060-1063.  
 1438 Devos, Y., Craig, W., Devlin, R.H., Ippolito, A., Leggatt, R.A., Romeis, J., Shaw, R.,  
 1439 Svendsen, C., Topping, C.J., 2019. Using problem formulation for fit-for-purpose  
 1440 pre-market environmental risk assessments of regulated stressors. *EFSA J.*  
 1441 17(S1), doi: 10.2903/j.efsa.2019.e170708.  
 1442 DiCarlo, J.E., Chavez, A., Dietz, S.L., Esvelt, K.M., Church, G.M., 2015.  
 1443 Safeguarding CRISPR-Cas9 gene drives in yeast. *Nat. Biotech.* 33, 1250-1255.  
 1444 Dively, G.P., Venugopala, P.D., Bean, D., Whalen, J., Holmstrom, K., Kuhar, T.P.,  
 1445 Doughty, H.B., Patton, T., Cissel, W., Hutchison, W.D., 2018. Regional pest

1446 suppression associated with widespread Bt maize adoption benefits vegetable  
 1447 growers. Proc. Natl. Acad. Sci. USA 115, 3320–3325.

1448 Dobson, S.L., Bordenstein, S.R., Rose, R.I., 2016. *Wolbachia* mosquito control:  
 1449 regulated. Nat. Biotech. 352, 526.

1450 dos Santos, L.A., Mendes, M.F., Krüger, A.P., Blauth, M.L., Gottschalk, M.S., Garcia,  
 1451 F.R., 2017. Global potential distribution of *Drosophila suzukii* (Diptera,  
 1452 Drosophilidae). Plos One 12, e0174318.

1453 Dyck, V.A., Hendrichs, J., Robinson, A.S. (Eds.), 2005. Sterile Insect Technique.  
 1454 Springer, Dordrecht, The Netherlands.

1455 EFSA (European Food Safety Authority), 2013. Guidance on the environmental risk  
 1456 assessment of genetically modified animals. EFSA J. 11, 3200.

1457 EPPO (European and Mediterranean Plant Protection Organization), 2014m PM 6/2  
 1458 (3) Import and release of non-indigenous biological control agents. EPPO Bull.  
 1459 44, 320-329.

1460 Esvelt, K.M., Gemmell, N.J., 2017. Conservation demands safe gene drives. PLoS  
 1461 Biol. 15, e2003850.

1462 Esvelt, K.M., Smidler, A.L., Catteruccia, F., Church, G.M., 2014. Concerning RNA-  
 1463 guided gene drives for the alteration of wild populations. eLife 3, e03401.

1464 European Commission (EU) (2018) Commission implementing decision (EU)  
 1465 2018/1623 of 29 October 2018 pursuant to Article 3(3) of Regulation (EU) No  
 1466 528/2012 of the European Parliament and of the Council on mosquitoes non-  
 1467 naturally infected with *Wolbachia* used for vector control purposes. Official  
 1468 Journal of the European Union L271, 30-31

1469 Evans, B.R., Kotsakiozi, P., Costa-da-Silva, A.L., Ioshino, R.S., Garziera, L.,  
 1470 Pedrosa, M.C., Malavasi, A., Virginio, J.F., Capurro, M.L., Powell, J.R., 2019.  
 1471 Transgenic *Aedes aegypti* mosquitoes transfer genes into a natural population.  
 1472 Sci. Rep. 9, 13047.

1473 Fang, J., 2010. A world without mosquitoes. Nature 466, 432-434.

1474 FAO (Food and Agriculture Organization of the United Nations), 2005. Guidelines for  
 1475 the export, shipment, import and release of biological control agents and other  
 1476 beneficial organisms. International Standard for Phytosanitary Measures 3.  
 1477 Secretariat of the International Plant Protection Convention (IPPC).  
 1478 <http://www.fao.org/3/a-j5365e.pdf> (accessed 19 December 2019).

1479 FAO/IAEA/USDA, 2003. Manual for Product Quality Control and Shipping

1480 Procedures for Sterile Mass-Reared Tephritid Fruit Flies, Version 5.0, Vienna,  
 1481 Austria. <http://www-naweb.iaea.org/nafa/ipc/public/ipc-mass-reared-tephritid.html>  
 1482 (accessed 19 December 2019).

1483 Farnsworth, D., Hamby, K., Bolda, M., Goodhue, R., Williams, J., Zalom, F., 2017.  
 1484 Economic analysis of revenue losses and control costs associated with the  
 1485 spotted wing drosophila (*Drosophila suzukii* (Matsumura)) in the California  
 1486 raspberry industry. *Pest Manag. Sci.* 73, 1083-1090.

1487 FDA (United States Food and Drug Administration), 2016. Environmental  
 1488 Assessment for Investigational Use of *Aedes aegypti* OX513A.  
 1489 [https://www.fda.gov/files/animal%20&%20veterinary/published/Oxitec-Mosquito--](https://www.fda.gov/files/animal%20&%20veterinary/published/Oxitec-Mosquito--Final-Environmental-Assessment.pdf)  
 1490 [-Final-Environmental-Assessment.pdf](https://www.fda.gov/files/animal%20&%20veterinary/published/Oxitec-Mosquito--Final-Environmental-Assessment.pdf) (accessed 19 December 2019).

1491 Fuyama, Y., 1983. Species-specificity of paragonial substances as an isolating  
 1492 mechanism in *Drosophila*. *Experientia* 39, 190-192.

1493 Gabarra, R., Riudavets, J., Rodríguez, G.A., Pujade-Villar, J., Arnó, J., 2015.  
 1494 Prospects for the biological control of *Drosophila suzukii*. *BioControl* 60, 331–  
 1495 339.

1496 Gantz, V.M., Bier, E., 2015. The mutagenic chain reaction: a method for converting  
 1497 heterozygous to homozygous mutations. *Science* 348, 442-444.

1498 Gantz, V.M., Jasinskiene, N., Tatarenkova, O., Fazekas, A., Macias, V.M., Bier, E.,  
 1499 James, A.A., 2015. Highly efficient Cas9-mediated gene drive for population  
 1500 modification of the malaria vector mosquito *Anopheles stephensi*. *Proc. Natl.*  
 1501 *Acad. Sci. USA* 112, E6736-E6743.

1502 Garrigan, D., Kingan, S.B., Geneva, A.J., Andolfatto, P., Clark, A.G., Thornton, K.R.,  
 1503 Presgraves, D.C., 2012. Genome sequencing reveals complex speciation in the  
 1504 *Drosophila simulans* clade. *Genome Res.* 22, 1499-1511.

1505 Gilchrist, A., Cameron, E., Sved, J., Meats, A., 2012. Genetic consequences of  
 1506 domestication and mass rearing of pest fruit fly *Bactrocera tryoni* (Diptera:  
 1507 Tephritidae). *J. Econ. Entomol.* 105, 1051-1056.

1508 Girod, P., Borowiec, N., Buffington, M., Chen, G., Fang, Y., Kimura, M.T., Peris-  
 1509 Felipo, F.J., Ris, N., Wu, H., Xiao, C., Zhang, J., Aebi, A., Haye, T., Kenis, M.,  
 1510 2018a. The parasitoid complex of *D. suzukii* and other fruit feeding *Drosophila*  
 1511 species in Asia. *Sci. Rep.* 8, 11839.

1512 Girod, P., Liehmann, O., Urvois, T., Turlings, T.C., Kenis, M., Haye, T., 2018b. Host  
 1513 specificity of Asian parasitoids for potential classical biological control of

1514 *Drosophila suzukii*. J. Pest Sci. 91, 1241-1250.

1515 Glandorf, D.C.M., 2017. Technical evaluation of a potential release of OX513A *Aedes*  
 1516 *aegypti* mosquitoes on the island of Saba. RIVM Letter report 2017-0087.  
 1517 [https://www.rivm.nl/publicaties/technical-evaluation-of-a-potential-release-of-](https://www.rivm.nl/publicaties/technical-evaluation-of-a-potential-release-of-ox513a-aedes-aegypti-mosquitoes-on)  
 1518 [ox513a-aedes-aegypti-mosquitoes-on](https://www.rivm.nl/publicaties/technical-evaluation-of-a-potential-release-of-ox513a-aedes-aegypti-mosquitoes-on) (accessed 19 December 2019).

1519 Glandorf, D.C.M., Breyer, D., 2016. Field trials with GM trees: A step-by-step  
 1520 approach, in: Vettori, C., Gallardo, F., Häggman, H., Kazana, V., Migliacci, F.,  
 1521 Pilate, G., Fladung, M. (Eds.), Biosafety of Forest Transgenic Trees, Springer,  
 1522 pp. 141-154.

1523 Gorman, K., Young, J., Pineda, L., Márquez, R., Sosa, N., Bernal, D., Torres, R.,  
 1524 Soto, Y., Lacroix, R., Naish, N., Kaiser, P., Tepedino, K., Philips, G., Kosmanna,  
 1525 C., Cáceres, L., 2015. Short-term suppression of *Aedes aegypti* using genetic  
 1526 control does not facilitate *Aedes albopictus*. Pest Manag. Sci. 72, 618-628.

1527 Gray, A.J., 2012. Problem formulation in environmental risk assessment for  
 1528 genetically modified crops: A practitioner's approach. Coll. Biosafety Rev. 6, 10–  
 1529 65.

1530 Greathead, D.J., Greathead, A.H., 1992. Biological control of insect pests by insect  
 1531 parasitoids and predators: the BIOCAT database. Biocontr. News Info. 13, 61N–  
 1532 68N.

1533 Guerrieri, E., Giorgini, M., Cascone, P., Carpenito, S., van Achterberg, C., 2016.  
 1534 Species diversity in the parasitoid genus *Asobara* (Hymenoptera: Braconidae)  
 1535 from the native area of the fruit fly pest *Drosophila suzukii* (Diptera:  
 1536 Drosophilidae). PLoS One 11, e0147382.

1537 Hackett, S.C., Bonsall, M.B., 2016. Type of fitness cost influences the rate of  
 1538 evolution of resistance to transgenic Bt crops. J. Appl. Ecol. 53, 1391-1401.

1539 Hamilton, W.D. 1967. Extraordinary sex ratios. Science 156, 477-488.

1540 Hammond, A., Galizi, R., Kyrou, K., Simoni, A., Siniscalchi, C., Katsanos, D., Gribble,  
 1541 M., Baker, D., Marois, E., Russell, S., Burt, A., Windbichler, N., Crisanti, A.,  
 1542 Nolan, T., 2016. A CRISPR-Cas9 gene drive system targeting female  
 1543 reproduction in the malaria mosquito vector *Anopheles gambiae*. Nat. Biotech.  
 1544 34, 78-83.

1545 Harris, A., Nimmo, D., McKemey, A., Kelly, N., Scaife, S., Donnelly, C.A., Beech, C.,  
 1546 Petrie, W., Alphey, L., 2011. Field performance of engineered male mosquitoes.  
 1547 Nat. Biotech. 29, 1034-1037.

1548 Harris AF, McKemey AR, Nimmo D, Curtis Z, Black I, Morgan SA, Oviedo, M.N.,  
 1549 Lacroix, R., Naish, N., Morrison, N.I., Collado, A., Stevenson, J., Scaife, S.,  
 1550 Dafa'alla, T., Fu, G., Phillips, C., Miles, A., Raduan, N., Kelly, N., Beech, C.,  
 1551 Donnelly, C.A., Petrie, W.D., Alphey, L., 2012. Successful suppression of a field  
 1552 mosquito population by sustained release of engineered male mosquitoes. *Nat.*  
 1553 *Biotech.* 30, 828–830.

1554 Harvey-Samuel, T., Morrison, N.I., Walker, A.S., Marubbi, T., Yao, J., Collins, H.L.,  
 1555 Gorman, K., Davies, T.G.E., Alphey, N., Warner, S., Shelton, A.M., Alphey, L.,  
 1556 2015. Pest control and resistance management through release of insects  
 1557 carrying a male-selecting transgene. *BMC Biol.* 13, 49.

1558 Hastings, I.M., 1994. Selfish DNA as a method of pest control. *Philos. Trans. R. Soc.*  
 1559 *London B* 344, 313-324.

1560 Hayes, K.R., Hosack, G.R., Dana, G.V., Foster, S.D., Ford, J.H., Thresher, R.,  
 1561 Ickowicz, A., Peel, D., Tizard, M., De Barro, P., Strive, T., Dambacher, J.M.,  
 1562 2018. Identifying and detecting potentially adverse ecological outcomes  
 1563 associated with the release of gene-drive modified organisms. *J. Resp. Inno.*  
 1564 5(No. S1), S139-S158.

1565 HCSP Haut Conseil de la Santé Publique), 2018. Avis relatif à l'élaboration de  
 1566 recommandations pour autoriser le lâcher de moustiques stériles à des fins de  
 1567 lutte anti-vectorielle. Haut Conseil de la Santé Publique, France. 28 juin 2018, 35  
 1568 pp.

1569 Heath, B.D., Butcher, R.D.J., Whitfield, W.G.F., Hubbard, S.F., 1999. Horizontal  
 1570 transfer of *Wolbachia* between phylogenetically distant insect species by a  
 1571 naturally occurring mechanism. *Curr. Biol.* 9, 313–316.

1572 Heimpel, G.E., Mills, N.J., 2017. *Biological Control. Ecology and Applications.*  
 1573 Cambridge University Press, Cambridge, UK.

1574 Hendrichs, J., Vreysen, M.J.B., Enkerlin, W.R., Cayol, J.P. 2005. Strategic options in  
 1575 using sterile insects for area-wide integrated pest management, in: Dyck, V.A.,  
 1576 Hendrichs, J., Robinson, A.S. (Eds.), *Sterile Insect Technique. Principles and*  
 1577 *Practice in Area-Wide Integrated Pest Management.* IAEA, Springer, pp. 563-  
 1578 600.

1579 Herédia, F., Loreto, E.L.S., Valente, V.L.S., 2004. Complex evolution of gypsy in  
 1580 drosophilid species. *Mol. Biol. Evol.* 21, 1831-1842.

1581 Hickey, W.A., Craig, G.B. Jr., 1966a. Genetic distortion of sex ratio in a mosquito,

1582 *Aedes aegypti*. Genetics 53, 1177-1196.

1583 Hickey, W.A., Craig, G.B. Jr., 1966b. Distortion of sex ratio in populations of *Aedes*  
 1584 *aegypti*. Can. J. Genet. Cytol. 8, 260-278.

1585 Hilgenboecker, K., Hammerstein, P., Schlattmann, P., Telschow, A., Werren, J.H.,  
 1586 2008. How many species are infected with *Wolbachia*? – a statistical analysis of  
 1587 current data. FEMS Microbiol. Lett. 281, 215–220.

1588 Hill, T., Betancourt, A.J., 2018. Extensive exchange of transposable elements in the  
 1589 *Drosophila pseudoobscura* group. Mobile DNA 9, 20.

1590 Hoddle, M.S., 2006. Historical review of control programs for *Levuana iridescens*  
 1591 (Lepidoptera: Zygaenidae) in Fiji and examination of possible extinction of this  
 1592 moth by *Bessa remota* (Diptera: Tachinidae). Pac. Sci. 60, 439-453.

1593 Hoffmann, A.A., Montgomery, B.L., Popovici, J., Iturbe-Ormaetxe, I., Johnson, P.H.,  
 1594 Muzzi, F., Greenfield, M., Durkan, M., Leong, Y.S., Dong, Y., Cook, H., Axford,  
 1595 J., Callahan, A.G., Kenny, N., Omodei, C., McGraw, E.A., Ryan, P.A., Ritchie,  
 1596 S.A., Turelli, M., O'Neill, S.L., 2011. Successful establishment of *Wolbachia* in  
 1597 *Aedes* populations to suppress dengue transmission. Nature 476, 454-457.

1598 Hoffmann, A.A., Iturbe-Ormaetxe, I., Callahan, A.G., Phillips, B., Billington, K.,  
 1599 Axford, J.K., Montgomery, B., Turley, A.P., O'Neill, S.L., 2014. Stability of the  
 1600 *wMel* *Wolbachia* infection following invasion into *Aedes aegypti* populations.  
 1601 PLoS Negl. Trop. Dis. 8, e3115.

1602 Hoffmann, A.A., Ross, P.A., Rašić, G., 2015. *Wolbachia* strains for disease control:  
 1603 ecological and evolutionary considerations. Evol. Appl. 8, 751-768.

1604 Horn, C., Wimmer, E.A., 2003. A transgene-based, embryo-specific lethality system  
 1605 for insect pest management. Nat. Biotech. 21, 64.

1606 Horner, R.M., Walker, J.T.S., Rogers, D.J., Lo, P.L., Suckling, D.M., 2016. Use of  
 1607 sterile insect technique in New Zealand: benefits and constraints. N. Z. Plant  
 1608 Protect. 69, 296-304.

1609 House of Lords, Science and Technology Committee, 2015. Genetically modified  
 1610 insects. HL Paper 68.  
 1611 [www.publications.parliament.uk/pa/ld201516/ldselect/ldsctech/68/6802.htm](http://www.publications.parliament.uk/pa/ld201516/ldselect/ldsctech/68/6802.htm)  
 1612 (accessed 5 July 2019).

1613 Houses of Parliament, 2014. GM Insects and Disease Control. Postnote 483,  
 1614 Parliamentary Office of Science and Technology, UK.  
 1615 <https://researchbriefings.files.parliament.uk/documents/POST-PN-483/POST-PN->



483.pdf (accessed 19 December 2019).

Howarth, F.G., 1983. Classical biocontrol: panacea or Pandora's box? *Proc. Hawaii. Entomol. Soc.* 24, 239-244.

Howarth, F.G., 1991. Environmental impacts of classical biological control. *Annu. Rev. Entomol.* 36, 485-509.

HSNO Act (1996) Hazardous Substances and New Organisms Act, Reprinted 1 December 2017. Ministry for the Environment, New Zealand Government, Wellington, New Zealand.

<http://legislation.govt.nz/act/public/1996/0030/99.0/DLM381222.html> (accessed 19 December 2019)

Huang, Y., Chen, Y., Zeng, B., Wang, Y., James, A.A., Gurr, G.M., Yang, G., Lin, X., Huang, Y., You, M., 2016. CRISPR/Cas9 mediated knockout of the abdominal-A homeotic gene in the global pest, diamondback moth (*Plutella xylostella*). *Insect Biochem. Molec. Biol.* 75, 98-106.

Hughes, G.L., Rasgon, J.L., 2014. Transinfection: A method to investigate *Wolbachia*-host interactions and control arthropod-borne disease. *Insect Molec. Biol.* 23, 141–151.

Hulme, P.E., 2009. Trade, transport and trouble: managing invasive species pathways in an era of globalization. *J. Appl. Ecol.* 46, 10-18.

Hulme, P.E., 2017. Climate change and biological invasions: evidence, expectations, and response options. *Biol. Rev.* 92, 1297-1313.

Hunt, E.J., Kuhlmann, U., Sheppard, A., Qin, T.-K., Barratt, B.I.P., Harrison, L., Mason, P.G., Parker, D., Flanders, R.V., Goolsby, J., 2008. Review of invertebrate biological control agent regulation in Australia, New Zealand, Canada and the USA: recommendations for a harmonized European system. *J. Appl. Entomol.* 132, 89-123.

Hunter-Fujita, F.R., Entwistle, P.E., Evans, H.F., Crook, N.E. (Eds.), 1998. *Insect Viruses and Pest Management*. Wiley, New York.

Hurst, T.P., Pittman, G., O'Neill, S.L., Ryan, P.A., Nguyen, H.L., Kay, B.H., 2012. Impacts of *Wolbachia* infection on predator prey relationships: evaluating survival and horizontal transfer between *wMelPop* infected *Aedes aegypti* and its predators. *J. Med. Entomol.* 49, 624-630.

Hutchison, W.D., Burkness, E.C., Mitchell, P.D., Moon, R.D., Leslie, T.W., Fleischer, S.J., Abrahamson, M., Hamilton, K.L., Steffey, K.L., Gray, M.E., Hellmich, R.L.,

1650 Kaster, L.V., Hunt, T.E., Wright, R.J., Pecinovsky, K., Rabaey, T.L., Flood, B.R.,  
 1651 Raun, E.S., 2010. Areawide suppression of European Corn Borer with Bt maize  
 1652 reaps savings to non-Bt maize growers. *Science* 330, 222-225.  
 1653 Iturbe-Ormaeche, I., Walker, T., O'Neill, S.L., 2011. *Wolbachia* and the biological  
 1654 control of mosquito-borne disease. *EMBO Rep.* 12, 508–518.  
 1655 James, S., Collins, F.H., Welkhoff, P.A., Emerson, C., Godfray, C.J., Gottlieb, M.,  
 1656 Greenwood, B., Lindsay, S.W., Mbogo, C.M., Okumu, F.O., Quemada, H.,  
 1657 Savadogo, M., Singh, J.A., Tountas, K.H., Touré, Y.T., 2018. Pathway to  
 1658 deployment of gene drive mosquitoes as a potential biocontrol tool for elimination  
 1659 of malaria in Sub-Saharan Africa: Recommendations of a scientific working  
 1660 group. *Am. J. Trop. Med. Hyg.* 98 (Suppl. 6), 1-49.  
 1661 Jenner, W.H., Kuhlmann, U., Miall, J.H., Cappuccino, N., Mason, P.G., 2014. Does  
 1662 parasitoid state affect host range expression? *Biol. Contr.* 78, 15-22.  
 1663 Kakazu, H., 2002. Economic evaluation of the melon fly eradication project in  
 1664 Okinawa, Japan, in: *Cooperation on fruit fly control research and technology in*  
 1665 *the Asia-Pacific region*. Research Institute for Subtropics, Okinawa, Japan, pp.  
 1666 31–54.  
 1667 Kalajdzic, P., Schetelig, M.F., 2017. CRISPR/Cas-mediated gene editing using  
 1668 purified protein in *Drosophila suzukii*. *Entomol. Exp. Appl.* 164, 350-362.  
 1669 Kandul, N.P., Liu, J., Sanchez, C.H.M., Wu, S.L., Marshall, J.M., Akbari, O.S., 2019.  
 1670 Transforming insect population control with precision guided sterile males with  
 1671 demonstration in flies. *Nature Commun.* 10, 84.  
 1672 Kean, J.M., Suckling, D.M., Sullivan, N.J., Tobin, P.C., Stringer, L.D., Smith, G.R.,  
 1673 Kimber, B., Lee, D.C., Flores Vargas, R., Fletcher, J., Macbeth, F., McCullough,  
 1674 D.G., Herms, D.A., et al., 2019. Global eradication and response database.  
 1675 <http://b3.net.nz/gerda> (accessed 19 December 2019).  
 1676 Kenis, M., Auger-Rozenberg, M.-A., Roques, A., Timms, L., Péré, C., Cock, M.J.W.,  
 1677 Settele, J., Augustin, S., Lopez-Vaamonde, C., 2009. Ecological effects of  
 1678 invasive alien insects. *Biol. Invasions* 11, 21-45.  
 1679 Khamis, D., El Mouden, C., Kura, K., Bonsall, M.B., 2018. Ecological effects on  
 1680 underdominance threshold drives for vector control. *J. Theor. Biol.* 456, 1-15.  
 1681 Killeen, G.F., Fillinger, U., Kiche, I., Gouagna, L.C., Knols, B.G., 2002. Eradication of  
 1682 *Anopheles gambiae* from Brazil: lessons for malaria control in Africa? *Lancet*  
 1683 *Infect. Dis.* 2, 618-627.

1684 Killeen, G.F., Chitnis, N., Moore, S.J., Okumu, F.O., 2011. Target product profile  
 1685 choices for intra-domiciliary malaria vector control pesticide products: repel or  
 1686 kill? *Malaria J.* 10, 207.

1687 Klassen, W., 2005. Area-wide integrated pest management and the sterile insect  
 1688 technique, in: Dyck, V.A., Hendrichs, J., Robinson, A.S. (Eds.) *Sterile Insect  
 1689 Technique. Principles and Practice in Area-Wide Integrated Pest Management.*  
 1690 IAEA, Springer, pp. 39-68.

1691 Klassen, W., Curtis, C.F., 2005. History of the sterile insect technique, in: Dyck, V.A.,  
 1692 Hendrichs, J., Robinson, A.S. (Eds.) *Sterile Insect Technique. Principles and  
 1693 Practice in Area-Wide Integrated Pest Management.* IAEA, Springer, pp. 3-36.

1694 Klasson, L., Kambris, Z., Cook, P.E., Walker, T., Sinkins, S.P., 2009. Horizontal gene  
 1695 transfer between *Wolbachia* and the mosquito *Aedes aegypti*. *BMC Genom.* 10,  
 1696 33.

1697 Knoll, V., Ellenbroek, T., Romeis, J., Collatz, J., 2017. Seasonal and regional  
 1698 presence of hymenopteran parasitoids of *Drosophila* in Switzerland and their  
 1699 ability to parasitize the invasive *Drosophila suzukii*. *Sci. Rep.* 7, 40697.

1700 Kofler, R., Hill, T., Nolte, V., Betancourt, A.J., Schlötterer, C., 2015. The recent  
 1701 invasion of natural *Drosophila simulans* populations by the P-element. *Proc. Natl.*  
 1702 *Acad. Sci USA* 112, 6659-6663.

1703 Kofler, N., Collins, J.P., Kuzma, J., Marris, E., Esvelt, K., Nelson, M.P., Newhouse,  
 1704 A., Rothschild, L.J., Vigliotti, V.S., Semenov, M., Jacobsen, R., Dahlman, J.E.,  
 1705 Prince, S., Caccone, A., Brown, T., Schmitz, O.J., 2018. Editing nature: Local  
 1706 roots of global governance. *Science* 362, 527-529.

1707 Kondo, S., Ueda, R., 2013. Highly improved gene targeting by germline-specific Cas9  
 1708 expression in *Drosophila*. *Genetics* 195, 715-721.

1709 Krafur, E.S. 1998. Sterile insect technique for suppressing and eradicating insect  
 1710 population: 55 years and counting. *J. Agric. Entomol.* 15, 303-317.

1711 Krafur, E.S., Townson, H., Davidson, G., Curtis, C.F., 1986. Screwworm eradication  
 1712 is what it seems. *Nature* 323, 495-496.

1713 Kriesner, P., Hoffmann, A.A., Lee, S.F., Turelli, M., Weeks, A.R., 2013. Rapid  
 1714 sequential spread of two *Wolbachia* variants in *Drosophila simulans*. *PLoS*  
 1715 *Pathog.* 9, e1003607.

1716 Kuhlmann, U., Schaffner, U., Mason, P.G., 2006. Selection of non-target species for  
 1717 host specificity testing, in: Bigler, F., Babendreier, D., Kuhlmann, U. (Eds.),

1718 Environmental Impact of Invertebrates for Biological Control of Arthropods –  
 1719 Methods and Risk Assessment. CABI Publishing, Wallingford, UK, pp. 15–37.

1720 Kyrou, K., Hammond, A.M., Galizi, R., Kranjc, N., Burt, A., Beaghton, A.K., Nolan, T.,  
 1721 Crisanti, A., 2018. A CRISPR-Cas9 gene drive targeting doublesex causes  
 1722 complete population suppression in caged *Anopheles gambiae* mosquitoes. Nat.  
 1723 Biotech. 36, 1062-1066.

1724 Lacroix, R., McKemey, A.R., Norzahira, R., Lim, K.W., Wong, H.M., Teoh, G.N., Siti  
 1725 Rahidah, A.A., Salman, S., Subramaniam, S., Nordin, O., Norhaida Hanum, A.T.,  
 1726 Angamuthu, C., Mansor, S.M., Lees, R.S., Naish, N., Scaife, S., Gray, P., Labbé,  
 1727 G., Beech, C., Nimmo, D., Alphey, L., Vasan, S.S., Lim, L.H., Nazni Wasi, A.,  
 1728 Murad, S., 2012. Open field release of genetically engineered sterile male *Aedes*  
 1729 *aegypti* in Malaysia. PLoS One 7, e42771.

1730 Lanouette, G., Brodeur, J., Fournier, F., Martel, V., Vreysen, M., Cáceres, C., Firlej,  
 1731 A., 2017. The sterile insect technique for the management of the spotted wing  
 1732 drosophila, *Drosophila suzukii*: Establishing the optimum irradiation dose. Plos  
 1733 One 12, e0180821.

1734 Laven, H., 1967. Eradication of *Culex pipiens fatigans* through cytoplasmic  
 1735 incompatibility. Nature 216, 383-384.

1736 Leach, H., Van Timmeren, S., Isaacs, R., 2016. Exclusion netting delays and reduces  
 1737 *Drosophila suzukii* (Diptera: Drosophilidae) infestation in raspberries. J. Econ.  
 1738 Entomol. 109, 2151-2158.

1739 Leach, H., Moses, J., Hanson, E., Fanning, P., Isaacs, R., 2018. Rapid harvest  
 1740 schedules and fruit removal as non-chemical approaches for managing spotted  
 1741 wing Drosophila. J. Pest Sci. 91, 219-226.

1742 Ledford, H., 2015. Caution urged over DNA editing in wild. Nature 524, 16.

1743 Lee, H.L., Vasan, S., Ahmad, N.W., Idris, I., Hanum, N., Selvi, S., Alphey, L., Murad,  
 1744 S., 2013. Mating compatibility and competitiveness of transgenic and wild type  
 1745 *Aedes aegypti* (L.) under contained semi-field conditions. Transgenic Res. 22,  
 1746 47-57.

1747 LePage, D.P., Metcalf, J.A., Bordenstein, S.R., On, J., Perlmutter, J.I., Shropshire,  
 1748 J.D., Layton, E.M., Funkhouser-Jones, L.J., Beckmann, J.F., Bordenstein, S.R.,  
 1749 2017. Prophage WO genes recapitulate and enhance Wolbachia-induced  
 1750 cytoplasmic incompatibility. Nature 543, 243.

1751 Li, F., Scott, M.J., 2016. CRISPR/Cas9-mediated mutagenesis of the *white* and *Sex*

1752 *lethal* loci in the invasive pest, *Drosophila suzukii*. Biochem. Biophys. Res.  
1753 Commun. 469, 911-916.

1754 Li, M., Yang, T., Kandul, N.P., Bui, M., Gamez, S., Raban, R., Bennett, J., Sanchez  
1755 C., H.M., Lanzaro, G.C, Schmidt, H., Lee, Y., Marshall, J.M., Akbari, O.S., 2019.  
1756 Development of a confinable gene-drive system in the human disease vector,  
1757 *Aedes aegypti*. bioRxiv, doi: /10.1101/645440.

1758 Lindholm, A.K., Dyer, K.A., Firman, R.C., Fishman, L., Forstmeier, W., Holman, L.,  
1759 Johannesson, H., Knief, U., Kokko, H., Larracuenta, A.M., Manser, A.,  
1760 Montchamp-Moreau, C., Petrosyan, V.G., Pomiankowski, A., Presgraves, D.C.,  
1761 Safronova, L.D., Sutter, A., Unckless, R.L., Verspoor, R.L., Wedell, N., Wilkinson,  
1762 G.S., Price, T.A.R., 2016. The ecology and evolutionary dynamics of meiotic  
1763 drives. Trends Ecol. Evol. 31, 315-326.

1764 Lindsey, A.R.I., Rice, D.W., Bordenstein, S.R., Brooks, A.W., Bordenstein, S.R.,  
1765 Newton, I.L.G., 2018. Evolutionary genetics of cytoplasmic incompatibility genes  
1766 *cifA* and *cifB* in prophage WO of *Wolbachia*. Genome Biol. Evol. 10, 434-451.

1767 Lorenzen, M.D., Gnirke, A., Margolis, J., Garnes, J., Campbell, M., Stuart, J.J.,  
1768 Aggarwal, R., Richards, S., Park, Y., Beeman, R.W., 2008. The maternal-effect,  
1769 selfish genetic element *Medea* is associated with a composite Tc1 transposon.  
1770 Proc. Natl. Acad. Sci. USA 105, 10085-10089.

1771 Loreto, E.L.S., Carareto, C.M.A., Capy, P., 2008. Revisiting horizontal transfer of  
1772 transposable elements in *Drosophila*. Heredity 100, 545-554.

1773 Lorimer, N. 1981. Long-term survival of introduced genes in a natural population of  
1774 *Aedes aegypti* (L.) (Diptera: Culicidae). Bull. Ent. Res. 71, 129-132.

1775 Lorimer, N., Lounibos, L.P., Petersen, J.L., 1976. Field trials with a translocation  
1776 homozygote in *Aedes aegypti* for population replacement. J. Econ. Entomol. 69,  
1777 405-409.

1778 Louda, S.M., Pemberton, R.W., Johnson, M.T., Follett, P.A., 2003. Nontarget  
1779 effects—the Achilles' heel of biological control? Retrospective analyses to reduce  
1780 risk associated with biocontrol introductions. Annu. Rev. Entomol. 48, 365-396.

1781 Lounibos, L.P., 2002. Invasions by insect vectors of human diseases. Annu. Rev.  
1782 Entomol. 47, 233-266.

1783 McDonald, P.T., Häusermann, W., Lorimer, N., 1977. Sterility introduced by release  
1784 of genetically altered males to a domestic population of *Aedes aegypti* at the  
1785 Kenya coast. Am. J. Trop. Med. Hyg. 26, 553-561.

1786 Mangan, R.L., 2005. Population suppression in support of the sterile insect  
 1787 technique. in: Dyck, V.A., Hendrichs, J., Robinson, A.S. (Eds.) Sterile Insect  
 1788 Technique. Principles and Practice in Area-Wide Integrated Pest Management.  
 1789 IAEA, Springer, pp. 407-425.

1790 Markow, T.A., O'Grady, P., 2005. Chapter I: Phylogenetic relationships of  
 1791 Drosophilidae, in: *Drosophila: A guide to Species Identification and Use*.  
 1792 Academic Press, London, pp. 3-64.

1793 Markow, T.A., O'Grady, P., 2008. Reproductive ecology of *Drosophila*. *Funct. Ecol.*  
 1794 22, 747-759.

1795 Marshall, J.M., 2009. The effect of gene drive on containment of transgenic  
 1796 mosquitoes. *J. Theor. Biol.* 258, 250-265.

1797 Marshall, J.M., 2010. The Cartagena protocol and genetically modified mosquitoes.  
 1798 *Nat. Biotech.* 28, 896-897.

1799 Marshall, J.M., 2011. The Cartagena protocol in the context of recent releases of  
 1800 transgenic and *Wolbachia*-infected mosquitoes. *Asia Pac. J. Mol. Biol.*  
 1801 *Biotechnol.* 19, 93-100.

1802 Mason, P.G., Everatt, M.J., Loomans, A.J.M., Collatz, J., 2017. Harmonizing the  
 1803 regulation of invertebrate biological control agents in the EPPO region: using the  
 1804 NAPPO region as a model. *EPPO Bull.* 47, 79-90.

1805 Mazzetto, F., Marchetti, E., Amiresmaeili, N., Sacco, D., Francati, S., Jucker, C.,  
 1806 Dindo, M.L., Lupi, D., Tavella, L., 2016. *Drosophila* parasitoids in northern Italy  
 1807 and their potential to attack the exotic pest *Drosophila suzukii*. *J. Pest Sci.* 89,  
 1808 837–850.

1809 Mazzi, D., Bravin, E., Meraner, M., Finger, R., Kuske, S., 2017. Economic impact of  
 1810 the introduction and establishment of *Drosophila suzukii* on sweet cherry  
 1811 production in Switzerland. *Insects* 8, 18.

1812 MEA (Millennium Ecosystem Assessment), 2005. *Ecosystems and Human Well-*  
 1813 *Being: Synthesis*. Washington, DC.  
 1814 <http://www.millenniumassessment.org/documents/document.356.aspx.pdf>  
 1815 (accessed 19 December 2019).

1816 Miller, B., Anfora, G., Buffington, M., Daane, K.M., Dalton, D.T., Hoelmer, K.M., Rossi  
 1817 Staccioni, M.V., Grassi, A., Ioratti, C., Loni, A., Miller, J.C., Ouantar, M., Wang,  
 1818 X., Wiman, N.G., Walton, V.M., 2015. Seasonal occurrence of resident  
 1819 parasitoids associated with *Drosophila suzukii* in two small fruit production

regions of Italy and the USA. Bull. Insectol. 68, 255–263.

Mitsui, H., Beppu, K., Kimura, M.T., 2010. Seasonal life cycles and resource uses of flower- and fruit-feeding drosophilid flies (Diptera: Drosophilidae) in central Japan. Entomol. Sci. 13, 60-67.

Mora, C., Tittensor, D.P., Adl, S., Simpson, A.G.B., Worm, B. 2011. How many species are there on earth and in the ocean? PLoS Biol. 9, e1001127.

Mumford, J.D., 2012. Science, regulation and precedent for genetically modified insects. PLoS One 6, e51504.

Murphy, B.D., Jansen, C., Murray, J., De Barro, P., 2010. Risk analysis on the Australian release of *Aedes aegypti* (L.) (Diptera: Culicidae) containing *Wolbachia*. CSIRO Report.  
[http://www.eliminatedengue.com/library/publication/document/csiro\\_report\\_australia\\_2010.pdf](http://www.eliminatedengue.com/library/publication/document/csiro_report_australia_2010.pdf) (accessed 19 December 2019)

Murray, J.V., Jansen, C.C., De Barro, P., 2016. Risk associated with the release of *Wolbachia*-infected *Aedes aegypti* mosquitoes into the environment in an effort to control dengue. Front. Public Health 4, 43.

Myers, J.H., Savoie, A., van Randen, E., 1998. Eradication and pest management. Annu. Rev. Entomol. 43, 471-491.

Nagel, P., Peveling, R., 2005. Environment and the sterile insect technique, in: Dyck, V.A., Hendrichs, J., Robinson, A.S. (Eds.) Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management. IAEA, Springer, pp. 499-524.

NAPPO (North American Plant Protection Organisation), 2007. Regional Standards for Phytosanitary Measures RSPM 27: Guidelines for Importation and Confined Field Release of Transgenic Arthropods in NAPPO Member Countries. 2007, North American Plant Protection Organisation (NAPPO).

NAPPO (North American Plant Protection Organisation), 2015. Guidelines for Petition for First Release of Non-indigenous Phytophagous or Phytopathogenic Biological Control Agents. Regional Standards for Phytosanitary Measures No. 7.  
[http://www.nappo.org/files/1814/4065/1908/RSPM\\_7\\_30-07-2015\\_-e.pdf](http://www.nappo.org/files/1814/4065/1908/RSPM_7_30-07-2015_-e.pdf) (accessed 19 December 2019).

Naranjo, S.E., Ellsworth, P.C., 2011. Fourteen years of Bt cotton advances IPM in Arizona. Southw. Entomol. 35, 437-444.

NASEM (National Academies of Sciences, Engineering, and Medicine), 2016. Gene

1854 Drives on the Horizon. The National Academies Press, Washington DC, doi:  
1855 10.17226/23405

1856 Neuenschwander, P., 2001. Biological control of the cassava mealybug in Africa: a  
1857 review. Biol. Contr. 21, 214-229.

1858 Nikolouli, K., Colinet, H., Renault, D., Enriquez, T., Mouton, L., Gibert, P., Sassu, F.,  
1859 Cáceres, C., Stauffer, C., Pereira, R., Bourtzis, K., 2018. Sterile insect technique  
1860 and *Wolbachia* symbiosis as potential tools for the control of the invasive species  
1861 *Drosophila suzukii*. J. Pest Sci. 91, 489-503.

1862 Noble, C., Min, J., Olejarz, Buchthal, J., Chavez, A., Smidler, A.L., DeBenedicts,  
1863 E.A., Church, G.M., Nowak, N.A., Esvelt, K.M., 2019. Daisy-gene drives for the  
1864 alteration of local populations, Proc. Natl. Acad. Sci. USA 116, 8275-8282.

1865 Nomano, F.Y., Kasuya, N., Matsuura, A., Suwito, A., Mitsui, H., Buffington, M.L.,  
1866 Kimura, M.T., 2017. Genetic differentiation of *Ganaspis brasiliensis*  
1867 (Hymenoptera: Figitidae) from East and Southeast Asia. Appl. Entomol. Zool. 52,  
1868 429–437.

1869 Oberhofer, G., Ivy, T., Hay, B.A., 2019. Cleave and rescue, a novel selfish genetic  
1870 element and general strategy for gene drive. Proc. Natl. Acad. Sci. USA 116,  
1871 6250-6259.

1872 O'Connor, L., Plichart, C., Sang, A.C., Brelsfoard, C.L., Bossin, H.C., Dobson, S.L.,  
1873 2012. Open release of male mosquitoes infected with a *Wolbachia* biopesticide:  
1874 field performance and infection containment. PLoS Negl. Trop. Dis. 6, e1797.

1875 OECD (Organisation for Economic Co-operation and Development), 2004. Guidance  
1876 for Information Requirements for Regulation of Invertebrates as Biological  
1877 Control Agents (IBCA). Series on Pesticides Number 21, OECD, Environment  
1878 Directorate, 22 pp.

1879 Oerke, E.-C., 2006. Crop losses to pests. J. Agric. Sci. 144, 31-43.

1880 O'Grady, P.M., DeSalle, R., 2018. Phylogeny of the genus *Drosophila*. Genetics 209,  
1881 1-25.

1882 O'Neill, S.L., Hoffmann, A.A., Werren, J.H., 1997. Influential Passengers. Oxford  
1883 University Press Inc., New York, USA.

1884 Oye, K.A., Esvelt, K., Appleton, E., Catteruccia, F., Church, G., Kuiken, T., Lightfoot,  
1885 S.B.-Y., McNamara, J., Smidler, A., Collins, J.P., 2014. Regulating gene drives.  
1886 Science 345, 626-628.

1887 Parker, A.G., 2005. Mass-rearing for sterile insect release, in: Dyck, V.A., Hendrichs,



1888 J., Robinson, A.S. (Eds.) Sterile Insect Technique. Principles and Practice in  
1889 Area-Wide Integrated Pest Management. IAEA, Springer, pp. 209-232.

1890 Pattabhiramaiah, M., Brückner, D., Reddy, M.S., 2011. Horizontal transmission of  
1891 *Wolbachia* in the honeybee subspecies *Apis mellifera carnica* and its  
1892 ectoparasite *Varroa destructor*. Int. J. Environ. Sci. 2, 526-535.

1893 Phuc, H.K., Andreasen, M.H., Burton, R.S., Vass, C., Epton, M.J., Pape, G., Fu, G.,  
1894 Condon, K.C., Scaife, S., Donnelly, C.A., Coleman, P.G., White-Cooper, H.,  
1895 Alphey, L., 2007. Late-acting dominant lethal genetic systems and mosquito  
1896 control. BMC Biol. 5, 11.

1897 Popovici, J., Moreira, L.A., Poinsignon, A., Iturbe-Ormaetxe, I., McNaughton, D.,  
1898 O'Neill, S.L., 2010. Assessing key safety concerns of a *Wolbachia*-based  
1899 strategy to control dengue transmission by *Aedes* mosquitoes. Mem. Inst.  
1900 Oswaldo Cruz 105, 957-964.

1901 Port, F., Chen, H.-M., Lee, T., Bullock, S.L., 2014. Optimized CRISPR/Cas tools for  
1902 efficient germline and somatic genome engineering in *Drosophila*. Proc. Natl.  
1903 Acad. Sci. USA 111, E2967-E2976.

1904 Quesneville, H., Anxolabéhère, D., 1997. A simulation of P element horizontal  
1905 transfer in *Drosophila*. Genetica 100, 295-307.

1906 Raybould, A., 2006. Problem formulation and hypothesis testing for environmental  
1907 risk assessments of genetically modified crops. Environ. Biosaf. Res. 5, 119–125.

1908 Redford, K.H., Brooks, T.M., Macfarlane, N.B.W., Adams, J.S., Eds., 2019. Genetic  
1909 frontiers for conservation: An assessment of synthetic biology and biodiversity  
1910 conservation. Technical assessment. Gland, Switzerland: IUCN. xiv + 166pp.

1911 Roberts, A., de Andrade, P.P., Okumu, F., Quemada, H., Savadogo, M., Singh, J.A.,  
1912 James, S., 2017. Results from the Workshop “Problem Formulation for the Use of  
1913 Gene Drive in Mosquitoes”. Am. J. Trop. Med. Hyg. 96, 530-533.

1914 Robinson, A.S., 2005. Genetic basis of the sterile insect technique in: Dyck, V.A.,  
1915 Hendrichs, J., Robinson, A.S. (Eds.) Sterile Insect Technique. Principles and  
1916 Practice in Area-Wide Integrated Pest Management. IAEA, Springer, pp. 95-114.

1917 Robinson, A.S., Hendrichs, J., 2005. Prospects for the future development and  
1918 application of the sterile insect technique, in: Dyck, V.A., Hendrichs, J., Robinson,  
1919 A.S. (Eds.) Sterile Insect Technique. Principles and Practice in Area-Wide  
1920 Integrated Pest Management. IAEA, Springer pp. 727-760.

1921 Romeis, J., Bartsch, D., Bigler, F., Candolfi, M.P., Gielkens, M.M.C., Hartley, S.E.,

1922 Hellmich, R.L., Huesing, J.E., Jepson, P.C., Layton, R., Quemada, H., Raybould,  
 1923 A., Rose, R.I., Schiemann, J., Sears, M.K., Shelton, A.M., Sweet, J., Vaituzis, Z.,  
 1924 Wolt, J.D., 2008. Assessment of risk of insect-resistant transgenic crops to  
 1925 nontarget arthropods. *Nat. Biotech.* 26, 203-208.  
 1926 Rossi-Stacconi, M.V., Buffington, M., Daane, K.M., Dalton, D.T., Grassi, A., Kaçar, G.,  
 1927 Miller, B., Miller, J.C., Baser, N., Ioriatti, C., Walton, V.M., Wiman, N.G., Wang,  
 1928 X., Anfora, G., 2015. Host stage preference, efficacy and fecundity of parasitoids  
 1929 attacking *Drosophila suzukii* in newly invaded areas. *Biol. Contr.* 84, 28–35.  
 1930 Rüdelsheim, P.L.J., Smets, G., 2018. Gene Drives. Experience with gene drive  
 1931 systems that may inform an environmental risk assessment. Perseus Report.  
 1932 COGEM Report CGM 2018-03.  
 1933 Santoiemma, G., Mori, N., Tonina, L., Marini, L., 2018. Semi-natural habitats boost  
 1934 *Drosophila suzukii* populations and crop damage in sweet cherry. *Agric. Ecosyst.*  
 1935 *Environ.* 257, 152-158.  
 1936 Sanvido, O., Romeis, J., Gathmann, A., Gielkens, M., Raybould, A., Bigler, F., 2012.  
 1937 Evaluating environmental risks of genetically modified crops – ecological harm  
 1938 criteria for regulatory decision-making. *Environ. Sci. Policy* 15, 82-91.  
 1939 Savary, S., Willocquet, L., Pethybridge, S.J., Esker, P., McRoberts, N., Nelson, A.,  
 1940 2019. The global burden of pathogens and pests on major food crops. *Nat. Ecol.*  
 1941 *Evol.* 3, 430-439.  
 1942 Schetelig, M.F., Lee, K.-Z., Otto, S., Talmann, L., Stökl, J., Degenkolb, T., Vilcinskas,  
 1943 A., Halitschke, R., 2018. Environmentally sustainable pest control options for  
 1944 *Drosophila suzukii*. *J. Appl. Entomol.* 142, 3-17.  
 1945 Schetelig, M.F., Handler, A.M., 2013. Germline transformation of the spotted wing  
 1946 drosophilid, *Drosophila suzukii*, with a piggyBac transposon vector. *Genetica*  
 1947 141, 189-193.  
 1948 Schmidt, T.L., Barton, N.H., Rasic, G., Turley, A.P., Montgomery, B.L., Iturbe-  
 1949 Ormaetxe, I., Cook, P.E., Ryan, P.A., Ritchie, S.A., Hoffmann, A.A., O'Neill, S. L.,  
 1950 Turelli, M., 2017. Local introduction and heterogeneous spatial spread of dengue-  
 1951 suppressing *Wolbachia* through an urban population of *Aedes aegypti*. *PLoS*  
 1952 *Biol.* 15, e2001894.  
 1953 Schmidt, J.M., Whitehouse, T.S., Green, K., Krehenwinkel, H., Schmidt-Jeffris, R.,  
 1954 Sial, A.A., 2019. Local and landscape-scale heterogeneity shape spotted wing  
 1955 drosophila (*Drosophila suzukii*) activity and natural enemy abundance:

1956 implications for trophic interactions. *Agric. Ecosyst. Environ.* 272, 86-94.

1957 Scott, M.J.C., Concha, C., Welch, J.B., Phillips, P.L., Skoda, S.R., 2017. Research  
1958 advances in the screwworm eradication program over the past 25 years.  
1959 *Entomol. Exp. Appl.* 164, 226-236.

1960 Scott, M.J., Gould, F., Lorenzen, M., Grubbs, N., Edwards, O., O'Brochta, D., 2018.  
1961 Agricultural production: assessment of the potential use of Cas9-mediated gene  
1962 drive systems for agricultural pest control. *J. Resp. Inno.* 5(No. S1), S98-S120.

1963 Serebrovsky, A.S., 1940. On the possibility of a new method for the control of insect  
1964 pests. *Zool. Zh.* 19, 618-30.

1965 Simberloff, D., Stiling, P., 1996. How risky is biological control? *Ecology* 77, 1965–  
1966 1974.

1967 Simmons, G., Alphey, L., Vasquez, T., Morrison, N., Epton, M., Miller, E., Miller, T.,  
1968 Staten, R., 2007. Potential use of a conditional lethal transgenic pink bollworm  
1969 *Pectinophora gossypiella* in area-wide eradication or suppression programmes,  
1970 in: Vreysen, M.B., Robinson, A.S., Hendrichs, J. (Eds.), *Area-Wide Control of*  
1971 *Insect Pests*. Springer, Dordrecht, pp. 119-123.

1972 Simmons, G.S., McKemey, A.R., Morrison, N.I., O'Connell, S., Tabashnik, B.E.,  
1973 Claus, J., Fu, G., Tang, G., Sledge, M., Walker, A.S., Phillips, C.E., Miller, E.D.,  
1974 Rose, R.I., Staten, R.T., Donnelly, C.A., Alphey, L., 2011. Field performance of a  
1975 genetically engineered strain of pink bollworm. *PLoS One* 6, e24110.

1976 Simon, S., Otto, M., Engelhard, M. 2018. Synthetic gene drive: between continuity  
1977 and novelty. *EMBO Rep.* 19, e45760.

1978 Sinkins, S.P., Gould, F., 2006. Gene drive systems for insect disease vectors. *Nat.*  
1979 *Rev. Genet.* 7, 427-435.

1980 Skevington, J.H., Dang, P.T. (eds.), 2002. Exploring the diversity of life. *Biodiversity*  
1981 3, 3-27.

1982 Slade, G., Morrison, N., 2014. Developing GM insects for sustainable pest control in  
1983 agriculture and human health. *BMC Proc.* 8(Suppl 4), O43.

1984 Stearns, S.C., 1992. *The Evolution of Life Histories*. Oxford University Press, London.

1985 Sternberg, S.H., Doudna, J.A., 2015. Expanding the biologist's toolkit with CRISPS-  
1986 Cas9. *Mol. Cell* 58, 568-574.

1987 Stouthamer, R., Breeuwer, J.A., Hurst, G.D., 1999. *Wolbachia pipientis*: microbial  
1988 manipulator of arthropod reproduction. *Annu. Rev. Microbiol.* 53, 71-102.

1989 Strack, T., Cahenzli, F., Daniel, C., 2017. Kaolin, lime and rock dusts to control

1990 *Drosophila suzukii*, in: Wolfrum, S., Heuwinkel, H., Wiesinger, K., Reents, H.J.,  
1991 Hülsbergen, K.-J. (Eds.), Ökologischen Landbau weiterdenken: Verantwortung  
1992 übernehmen, Vertrauen stärken. Verlag Dr. Köster, Berlin, pp. 262-263.

1993 Suckling, D.M., 2003. Applying the sterile insect technique for biosecurity: benefits  
1994 and constraints. N. Z. Plant Protect. 56, 21-26.

1995 Takamori, H., Watanabe, H.-A., Fuyama, Y., Zhang, Y.-P., Aotsuka, T., 2006.  
1996 *Drosophila subpulchrella*, a new species of the *Drosophila suzukii* species  
1997 subgroup from Japan and China (Diptera: Drosophilidae). Entomol. Sci. 9, 121-  
1998 128.

1999 Teem, J.L., Ambali, A., Glover, B., Ouedraogo, J., Makinde, D., Roberts, A., 2019.  
2000 Problem formulation for gene drive mosquitoes designed to reduce malaria  
2001 transmission in Africa: results from four regional consultations 2016–2018.  
2002 Malaria J. 18, 347.

2003 Thompson, P.B., 2018. The roles of ethics in gene drive research and governance. J.  
2004 Resp. Inno. 5(No. S1), S159-S179.

2005 Tochen, S., Dalton, D.T., Wiman, N., Hamm, C., Shearer, P.W., Walton, V.M., 2014.  
2006 Temperature-related development and population parameters for *Drosophila*  
2007 *suzukii* (Diptera: Drosophilidae) on cherry and blueberry. Environ. Entomol. 43,  
2008 501-510.

2009 Turelli, M., Hoffmann, A.A., 1991. Rapid spread of an inherited incompatibility factor  
2010 in California *Drosophila*. Nature 353, 440.

2011 USDA (United States Department of Agriculture), 2001. Fruit fly cooperative control  
2012 program — final environmental impact statement. United States Department of  
2013 Agriculture, Marketing and Regulatory Programs, Animal and Plant Health  
2014 Inspection Service, Riverdale, MD, USA.

2015 US EPA (United States Environmental protection Agency), 2017. Pesticide Product  
2016 with a New Active Ingredient (*Wolbachia pipientis*, ZAP Strain in male *Aedes*  
2017 *albopictus* (Asian tiger mosquitoes) – FIFRA). Docket ID: EPA-HQ-OPP-2016-  
2018 0205, <https://www.regulations.gov/docket?D=EPA-HQ-OPP-2016-0205>  
2019 (accessed 19 December 2019).

2020 van der Vlugt, C.J.B., Brown, D.D., Lehmann, K., Leunda, A., Willemarck, N., 2018. A  
2021 framework for the risk assessment and management of gene drive technology in  
2022 contained use. Appl. Biosaf. 23, 25-31.

2023 van Lenteren, J.C., Babendreier, D., Bigler, F., Burgio, G., Hokkanen, H.M.T., Kuske,

S., Loomans, A.J.M., Menzler-Hokkanen, I., van Rijn, P.C.J., Thomas, M.B., Tommasini, M.G., Zeng, Q.-Q., 2003. Environmental risk assessment of exotic natural enemies used in inundative biological control. *BioControl* 48, 3-38.

Vietnam Eliminate Dengue Project, 2011. Risk Assessment of the Pilot Release of *Aedes aegypti* mosquitoes containing *Wolbachia*. [http://www.eliminatedengue.com/library/publication/document/july\\_2011\\_ra\\_report\\_eng.pdf](http://www.eliminatedengue.com/library/publication/document/july_2011_ra_report_eng.pdf) (accessed 19 December 2019).

Walker, T., Johnson, P.H., Moreira, L.A., Iturbe-Ormaetxe, I., Frentiu, F.D., McMeniman, C.J., Leong, Y.S., Dong, Y., Axford, J., Kriesner, P., Lloyd, A.L., Ritchie, S.A., O'Neill, S.L., Hoffmann, A.A., 2011. The wMel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. *Nature* 476, 450-453.

Waltz, E., 2017. US government approves 'killer' mosquitoes to fight disease. *Nature*, doi:10.1038/nature.2017.22959

Wan, P., Huang, Y., Tabashnik, B.E., Huang, M., Wu, K., 2012. The halo effect: Suppression of pink bollworm on non-Bt cotton by Bt cotton in China. *PLoS ONE* 7, e42004.

Wang, X.G., Nance, A.H., Jones, J.M., Hoelmer, K.A., Daane, K.M., 2018. Aspects of the biology and reproductive strategy of two Asian larval parasitoids evaluated for classical biological control of *Drosophila suzukii*. *Biol. Contr.*, 121, 58-65.

Webber, B.L., Raghu, S., Edwards, O.R., 2015. Opinion: Is CRISPR-based gene drive a biocontrol silver bullet or global conservation threat? *Proc. Natl. Acad. Sci. USA* 112, 10565-10567.

Weeks, A.R., Turelli, M., Harcombe, W.R., Reynolds, K.T., Hoffmann, A.A., 2007. From parasite to mutualist: rapid evolution of *Wolbachia* in natural populations of *Drosophila*. *PLoS Biol.* 5, e114.

Weinert, L.A., Araujo-Jnr, E.V., Ahmed, M.Z., Welch, J.J., 2015. The incidence of bacterial endosymbionts in terrestrial arthropods. *Proc. R. Soc. B* 282, 20150249.

Wentworth, J., 2014. GM Insects and Disease control. Postnote 483, Houses of Parliament. Parliamentary Office of Science and Technology, UK.

Werren, J.H., Zhang, W., Guo, L.R., 1995. Evolution and phylogeny of *Wolbachia*: reproductive parasites of arthropods. *Proc. R. Soc. B* 261, 55-71.

Werren, J.H., Baldo, L., Clark, M.E., 2008. *Wolbachia*: master manipulators of invertebrate biology. *Nat. Rev. Microbiol.* 6, 741-751.

WHO (World Health Organisation), 2014. Guidance Framework for Testing Genetically Modified Mosquitoes. Available from: [http://www.who.int/tdr/news/2012/guidance\\_framework/en/](http://www.who.int/tdr/news/2012/guidance_framework/en/) (accessed 19 December 2019).

Windbichler, N., Papathanos, P.A., Catteruccia, F., Ranson, H., Burt, A., Crisanti, A., 2007. Homing endonuclease mediated gene targeting in *Anopheles gambiae* cells and embryos. *Nucleic Acids Res.* 35, 5922-5933.

Wolf, S., Zeisler, C., Sint, S., Romeis, J., Traugott, M., Collatz, J., 2018. A simple and cost-effective molecular way to track predation on *Drosophila suzukii* in the field. *J. Pest Sci.* 91, 927-935.

Wolt, J.D., Keese, P., Raybould, A., Fitzpatrick, J.W., Burachik, M., Gray, A., Olin, S.S., Schiemann, J., Sears, M., Wu, F., 2010. Problem formulation in the environmental risk assessment for genetically modified plants. *Transgenic Res.* 19, 425-436.

Woltz, J.M., Lee, J.C., 2017. Pupation behavior and larval and pupal biocontrol of *Drosophila suzukii* in the field. *Biol. Contr.* 110, 62-69.

Wood, R.J., Newton, M.E., 1991. Sex-ratio distortion caused by meiotic drive in mosquitoes. *Am. Nat.* 137, 379-391.

Wu, B., Luo, L., Gao, X.J., 2016. Cas9-triggered chain ablation of Cas9 as a gene drive brake. *Nat. Biotech.* 34, 137-138.

Wu, K.-M., Lu, Y.-H., Feng, H.-Q., Jiang, Y.-Y., Zhao, J.-Z., 2008. Suppression of cotton bollworm in multiple crops in China in areas with Bt toxin-containing cotton. *Science* 321, 1676-1678.

Xi, Z., Khoo, C.C., Dobson, S.L., 2005. *Wolbachia* establishment and invasion in an *Aedes aegypti* laboratory population. *Science* 310, 326-328.

Yang, Y., Hou, Y.-C., Qian, Y.-H., Kang, H., Zeng, Q.-T., 2012. Increasing the data size to accurately reconstruct the phylogenetic relationships between nine subgroups of *Drosophila melanogaster* species group (*Drosophilidae*, *Diptera*). *Mol. Phylogenet. Evol.* 62, 214-223.

Zabalou, S., Apostolaki, A., Livadaras, I., Franz, G., Robinson, A.S., Savakis, C., Bourtzis, K., 2009. Incompatible insect technique: incompatible males from a *Ceratitis capitata* genetic sexing strain. *Entomol. Exp. Appl.* 132, 232-240.

Zabalou, S., Riegler, M., Theodorakopoulou, M., Stauffer, C., Savakis, C., Bourtzis, K., 2004. *Wolbachia*-induced cytoplasmic incompatibility as a means for insect

pest population control. Proc. Natl. Acad. Sci. USA 101, 15042-15045.

Zheng, X., Zhang, D., Li, Y., Yang, C., Wu, Y., Liang, X., Liang, Y., Pan, X., Hu, L., Sun, Q., Wang, X., Wei, Y., Zhu, J., Qian, W., Yan, Z., Parker, A.G., Gilles, J.R.L., Bourtzis, K., Bouyer, J., Tang, M., Zheng, B., Yu, J., Liu, J., Zhuang, J., Hu, Z., Zhang, M., Gong, J.-T., Hong, X.-Y., Zhang, Z., Lin, L., Liu, Q., Hu, Z., Wu, Z., Baton, L.A., Hoffmann, A.A., Xi, Z., 2019. Incompatible and sterile insect techniques combined eliminate mosquitoes. Nature 572, 56-61.

Zug, R., Hammerstein, P., 2012. Still a host of hosts for *Wolbachia*: analysis of recent data suggests that 40% of terrestrial arthropod species are infected. PLoS ONE 7, e38544.

## Figure captions

**Fig. 1.** Plausible pathways on how control interventions that require the release of living insects to suppress populations of target pest(s) might harm the valued ecosystem service of biological control. Note that steps for which data are requested as part of an environmental risk assessment are coloured according to the particular technology.

**Fig. 2.** Plausible pathways on how the release of *Drosophila suzukii* carrying a suppression drive could lead to harm to valued ecosystem services involving biological control.