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INDUSTRY-DRIVEN AREA-WIDE MANAGEMENT OF QUEENSLAND FRUIT FLY IN QUEENSLAND AND NEW SOUTH WALES, AUSTRALIA: CAN IT WORK?

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SUMMARY

Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Tephritidae), is one of Australia's most problematic and costly horticultural pests. As key insecticides traditionally used to manage the pest have recently been restricted, area-wide management (AWM) of Queensland fruit fly is becoming a key recommended practice. The increased push for AWM coincides with several state governments reducing direct on-ground support for pest management. It is increasingly up to local industries to take the reins of implementing AWM programmes. This study explored the social and institutional aspects of industry-driven AWM to understand how these programmes can best be supported. The findings are based on AWM case studies in Queensland and New South Wales in Australia, as well as interviews with people who operate in Australia's broader fruit fly management innovation system. The findings reported here complement the prevailing techno-centric emphasis relating to Queensland fruit fly management. They are summarised in five principles: (i) the local social profile influences the prospects of successful AWM; (ii) AWM needs to be based on adaptive co-management; (iii) local industries need help to help themselves; (iv) AWM programmes in Queensland need strong two-way connectivity with the broader Queensland fruit fly management innovation system; and (v) industry-driven AWM programmes need institutional adjustment to share public roles and responsibilities. These principles are discussed, as well as their policy implications. The study concludes that industry-driven AWM is only possible in certain circumstances.

Key Words: Bactrocera tryoni, Tephritidae, community support, enabling environment, institutional design, fruit flies, social factors, fruit industry, horticulture, stakeholder involvement, adaptive co-management, international trade

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1. INTRODUCTION

In various countries there is an increasing push for local agricultural industries to be less dependent on direct government support and take on more responsibility for pest management (Higgins et al. 2016). One such pest in Australia is the Queensland fruit fly (*Bactrocera tryoni* (Froggatt)), which is established throughout parts of eastern Australia (Clarke et al. 2011). Queensland fruit fly is a particularly problematic pest as most fruit and vegetables are susceptible to infestation to varying degrees. Queensland fruit fly numbers can quickly soar under favourable conditions and the pest has the ability to cause considerable damage to crops (Dominiak and Ekman 2013). The pest can therefore have a huge economic impact, especially as several of Australia's horticulture international trading partners place costly requirements or restrictions on produce from Queensland fruit fly-infested areas (PHA 2008). In addition, the application of two key chemical insecticides that were traditionally used to manage the pest, fenthion and dimethoate, have been restricted (Dominiak and Ekman 2013).

Area-wide management (AWM) is seen as a key alternative Queensland fruit fly management strategy (NFFC 2016; PHA 2008). It promises a reduced need for insecticides and is acknowledged in international trade regulations as an acceptable phytosanitary approach for fruit fly (FAO 2016). However, many regions face reduced direct on-ground government support for Queensland fruit fly management resulting from cuts in pest monitoring and treatment activities, and extension services. Local industries now need to drive collaboration between various stakeholder groups and risk contributors (such as landholders with Queensland fruit fly hosts on their land) in order to initiate and maintain AWM programmes.

Responsibility for the management of Queensland fruit fly is addressed across a federated system involving delegated people in various organizations and roles. These organizations include the Australian Government, which oversees international trade matters that are affected by Queensland fruit fly. State/territory governments are responsible for providing support for Queensland fruit fly suppression and domestic market access impacted by Queensland fruit fly. Various public and private organizations conduct Queensland fruit fly-related research. Several recent initiatives were introduced to address the Queensland fruit fly problem, including the National Fruit Fly Council, the National Fruit Fly Research, Development and Extension Plan (PBCRC 2015), and the establishment of a Sterile Insect Technique consortium (*SITplus*). As these initiatives occurred after the initial field work of this research, their impact on the results presented here was limited.

This paper contains a summary of the key findings of a PhD project that investigated the social and institutional aspects of industry-driven Queensland fruit fly AWM in Australian horticulture industries, with special focus on three case studies in the states of Queensland and New South Wales. Traditionally, pest management has predominantly been approached as a technical issue in need of technical solutions (Schut et al. 2014). This chapter complements this techno-centric thinking.

2. METHODS

The study involved mixed methods research and included qualitative interviews and focus groups (facilitated small group discussions), and a quantitative grower survey. In phase 1 (2013-2014) an assessment was made of how the success of industry-driven AWM can be bolstered at the local on-ground level. It comprised three case studies (Table 1), together with a review of scholarly literature about socio-ecological systems (e.g. Ostrom 2005; Armitage et al. 2008) and community-based natural resource management (such as Berkes 2010; Klerkx et al. 2012; Curtis et al. 2014).

The online grower survey, involving the same three case study areas, was carried out between phases 1 and 2 towards the end of 2015. For a more detailed summary of the phase 1 methods see Kruger (2016a, 2016b).

In phase 2 (2015-2016), ways were identified to create an enabling environment for industry-driven AWM, i.e. the conditions needed that will support local industries to take the lead in AWM programmes. This was done both in terms of Queensland fruit fly suppression and market access. It involved 33 interviews with people operating in the broader fruit fly management innovation system (Table 2).

Table 2. An overview of the people interviewed in phase 2 about how to create an enabling environment for industry-driven fruit fly AWM

Organizational background	Number of interviewees[c]
State government[a]	
- Queensland fruit fly researcher	7
- Policy	6
- Industry support	4
- Operational management	1
Australian Government - Policy	3
University	2
Industry body	7
Local industry	2
Local government[b]	1
Consultant	3

[a] *New South Wales and Queensland only*
[b] *Five others were interviewed during phase 1 (see Table 1)*
[c] *Some interviews involved more than one interviewee*

These findings, together with the grower survey results, were analysed by applying agriculture innovation systems thinking (Klerkx et al. 2012; Schut et al. 2014). Such thinking sees innovation as a co-evolutionary process involving not only technical, but also social and institutional change that results in on-ground progress (Klerkx et al. 2012). For a more detailed summary of the phase 2 methods see Kruger (2017). For a detailed explanation of the full PhD study's methods see Kruger (2018).

Table 1. Overview of the three AWM (area-wide management) case studies investigated, including for each case study information about the areas they cover, the number of growers, kinds of crops produced, local support for AWM, indicators of success, factors that enable or hinder AWM, and the data collected from these regions (adjusted from Kruger 2016a)

	Central Burnett (Queensland)	**Riverina** (New South Wales)	**Young-Harden** (New South Wales)
Local Government Areas [a]	Along the Burnett River within the North Burnett Region Local Government Area	Local Government Areas of Carrathool, Griffith, Leeton, Murrumbidgee, Narrandera	Local Government Areas of Young and Harden
No. of growers	40 (2016 - mainly citrus) [b]	Approx. 420 [b] citrus; 372 [b] grape; 55 [b] prune, and many other growers of horticultural crops (2014)	Approx. 35 [b] cherry, 20 [b] grape (2015)
Crops produced and areas involved	2,266 ha [c] citrus (mainly mandarins); 370 ha table grapes (2010)	8,133 ha [c] citrus; 7,200 ha vegetables; 15,000 ha grapes; 1,365 ha walnuts; 885 ha prunes; 300 ha cherries; and smaller plantings of other hosts (approx. 30,000 ha of horticulture)	Estimated 650-700 ha [b] cherries; and up to 1,000 ha [b] of wine grapes
Support for AWM	▪ All growers implement rigorous Queensland fruit fly baiting ▪ Growers voluntary fund town treatments, but contributions are dwindling	▪ The local citrus industry pushes for broad-scale Queensland fruit fly management to support market access ▪ Other horticultural industries show little interest as Queensland fruit fly does not affect them economically	▪ Cherry industry pushes for AWM to support market access ▪ Grape growers are less engaged as unaffected economically by fruit fly
Indicators of AWM success	▪ Successful Queensland fruit fly suppression in season ▪ Queensland fruit fly management practices led to protocol ICA-28, increasing domestic market access	▪ Too early to tell	▪ Too early to tell
Factors enabling AWM	▪ Trust relationships between growers and consultants ▪ Small industry ▪ Growers have similar on-farm objectives ▪ Small, horticulture-dependent towns	▪ Cold winters ▪ Some packing houses insist that their grower suppliers provide proof of Queensland fruit fly management through spray records and receipts of fruit fly management inputs	▪ Cold winters ▪ Strong local government support ▪ Small industry
Factors hindering AWM	▪ Lack of enforcement mechanism to sustain grower contributions for town treatments ▪ Queensland fruit fly is endemic ▪ Disappointment with inaccessibility to overseas markets without cold treatment contributes to dwindling grower contributions for town treatments	▪ Many horticulture growers with multiple on-farm objectives ▪ Lack of representative leadership ▪ Many part-time, low-input growers ▪ Large/medium-sized towns dependent on multiple industries ▪ No local power to enforce cooperation ▪ Lack of communication channels to all horticulture growers	▪ Medium-sized towns ▪ Little local power to enforce cooperation ▪ Limited local communication and influence across all horticulture growers
Qualitative data	▪ Thirteen interviews [d] ▪ One focus group ▪ October 2013	▪ Twenty interviews [d] ▪ One focus group ▪ March 2014	▪ Nine interviews [d] ▪ One focus group ▪ September 2013
Grower survey response rate	70% (28/40) [e]	51% (50/98) [e]	63% (20/32) [e]

[a] *Local Government Areas are political entities that form the third tier of government in Australia*

[b] *Numbers provided by local industries*

[c] *Personal communication with Nathan Hancock, Citrus Australia Ltd (12 Feb 2016)*

[d] *Included local programme coordinators, growers, crop pest consultants and representatives of AWM programme management groups, packing houses, local councils and industry associations*

[e] *Number of respondents / total accessible grower population*

3. FINDINGS AND DISCUSSION

Fig. 1 represents the consolidation of the key findings of the PhD research, and shows that AWM programmes can be conceptualised as comprising three key components that stretch across various levels of activity:

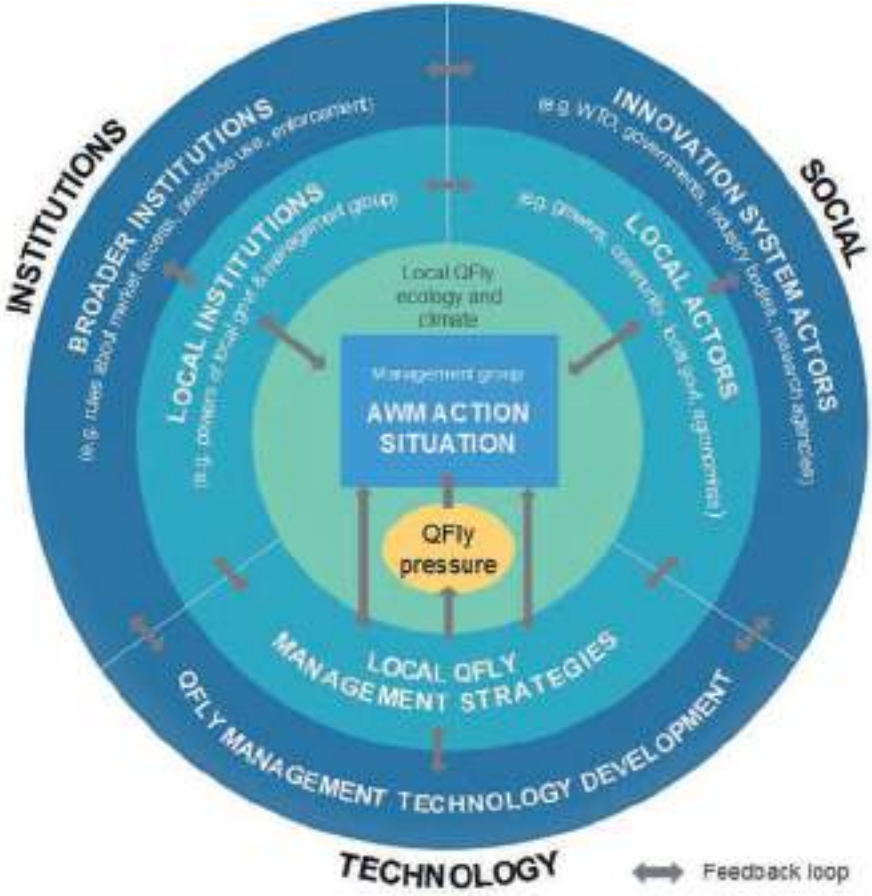

Figure 1. Conceptualised fruit fly management innovation system that will enable industry-driven AWM.

- *Social* – people and groups at local, state, national and international levels whose actions, interactions and decisions (or lack thereof) can either facilitate or hinder AWM.
- *Institutions* – formal (laws, regulations, standards) and informal (norms and accepted behaviour) that apply at local, state, national and international levels that influence the design and implementation of AWM.

- *Technology* – Queensland fruit fly-related technologies and information that are available at the local level and flow predominantly from the broader fruit fly management innovation system that stretches across state, national and international levels.

Following agriculture innovation systems thinking, each AWM programme is therefore embedded in a broader innovation system at higher levels comprising social, institutional and technological components that influence the feasibility of industry-driven AWM programmes.

The two-way arrows in Fig. 1 emphasise the importance of multi-directional information flow across levels and between the three key components. This is necessary to ensure that the different components are responsive to each other.

The key findings can be summarised in the following five principles that are also reflected in Fig. 1. A detailed explanation of the study's findings is contained in Kruger (2018).

3.1. *Principle 1. The Local Social Profile Influences the Prospects of Successful AWM*

With the international drive for harmonised phytosanitary measures, the impact due to the uniqueness of different local regional communities on their ability to achieve AWM can be easily underestimated. This research found four social variables that determine the degree of difficulty to achieve AWM, i.e. the transaction cost to develop local institutions (formal and informal rules) that support AWM that are widely supported by risk contributors (Kruger 2016b).

First variable: High heterogeneity of the contributors to Queensland fruit fly risk complicates setting local rules, such as identifying who needs to implement fruit fly management, what is required from them and how to win their commitment. Different on-farm objectives, pest impacts and market requirements for Queensland fruit fly management make it harder to identify a common AWM objective. For example, in the Riverina area in New South Wales, a small proportion of citrus growers is interested in exporting to premium Queensland fruit fly-sensitive markets. However, a large proportion is comprised of part-time growers supplying the domestic juice market. As their horticultural operations are not their primary income source, it lessens their incentive to participate in AWM, whereas exporters would like to see fruit fly management implemented to a high standard. Moreover, a heterogeneous mix of grower sectors means that the local AWM management group needs to establish trust and communication channels with more diverse stakeholders. This can challenge the management group's legitimacy and credibility across the region.

When stakeholder investment is not proportionate to the distribution of eventual benefits, they might view any or some rules as unfair, which discourages cooperation (Ostrom 2005). Identifying who will gain most, and therefore who ought to contribute most, is not always initially evident. For example, AWM is seen as a good candidate to be included in systems approaches for trade, e.g. applying at least two independent risk management measures that cumulatively achieve acceptable levels of pest risk (Dominiak and Ekman 2013; FAO 2018). However, in practice many markets remain wary of accepting systems approaches and achieving AWM may not necessarily render post-harvest treatments, such as cold sterilisation, obsolete, as is the case in Central Burnett in Queensland.

Second variable: High levels of social capital (i.e. personal trust-based amicable relationships) between key participants may facilitate the establishment of AWM, including established trust and communication networks, champions and leadership, such as in Central Burnett (see Box 1). However, it is important that social capital is not located in just some participating groups, as cliques may hinder progress (Pretty 2003).

Third variable: Existing social mechanisms that provide opportunities for monitoring on-farm Queensland fruit fly management, e.g. to keep a check on potential 'free-riders', can facilitate collective action and lower transaction costs. For example, in Central Burnett crop pest consultants making routine field visits enable low-cost monitoring of whether growers are managing Queensland fruit fly. In Riverina, some packing houses insist that their grower suppliers provide proof of Queensland fruit fly management. However, this could be thwarted in times of produce shortage when supply chains ease their grower requirements in order to secure supply.

Fourth variable: The ratio between the number of growers that are keen to pursue AWM and risk contributors who have little incentive to manage Queensland fruit fly (such as several town residents and part-time growers) affects the cost and effort needed to establish AWM. In Central Burnett growers were able to fund town treatments as towns are small relative to the production area. In the Riverina, with the large urban centre of Griffith, this would be less feasible, rendering AWM supporters reliant on awareness-raising to urge town residents to manage Queensland fruit fly on their land.

Policy Implications: The varying profiles of the three case studies illustrate that finding 'one-size-fits-all' local institutions ideal for all AWM programmes is unlikely. The rules relating to how the programme is run, what it involves and what it sets out to achieve are best negotiated locally and tailored to local circumstances. The AWM action situation (Fig. 1) needs to allow for Enticott's (2008) 'spaces of negotiation' to find ways to achieve wide stakeholder support.

3.2. Principle 2. AWM Needs to be Based on Adaptive Co-management

The formal requirements for fruit fly AWM (e.g. International Standards for Phytosanitary Measures (ISPMs) and the Queensland fruit fly Code of Practice (Jessup et al. 2007)), could suggest that AWM constitutes standardised 'spaces of prescription' (Enticott 2008), where AWM relies on introducing standard Queensland fruit fly management measures. Local industries' attempts to achieve AWM often involve social, institutional and technical complexity and uncertainty.

Aspects of AWM that involve complexity and uncertainty include its collaborative nature, various on-farm objectives and that not all stakeholders and risk contributors may have incentives to continually manage Queensland fruit fly, as was especially the case in the Riverina. Every local community is different. External expertise should not be privileged at the expense of local knowledge and learning processes rooted in local socio-economic, cultural and political contexts (Kruger 2016a).

Designing AWM programmes requires a good understanding of the regional Queensland fruit fly situation, including Queensland fruit fly behaviour in and amongst crops, and other local hosts, and this might vary across years with different weather conditions (Clarke et al. 2011).

In the system shown in Fig. 1, an AWM management group needs to continually monitor Queensland fruit fly pressure and adjust local Queensland fruit fly management strategies accordingly. Moreover, achieving market access is complex and uncertain. This was illustrated by the situation in Central Burnett where, despite a successful AWM programme, anticipated access to lucrative markets sensitive to Queensland fruit fly did not occur.

Global experience with a wide range of natural resource management situations has shown that complexity and uncertainty are best surmounted through adaptive co-management, i.e. a flexible process of 'learning by doing' that draws on different knowledge systems (Armitage et al. 2008). Agricultural innovation systems literature refers to the need for co-producing integrated knowledge between different stakeholder groups to provide a holistic understanding of how to best improve plant protection systems (e.g. Schut et al. 2014). For Queensland fruit fly this includes knowledge on the biology and behaviour of the pest, and its host distribution and phenology. It also requires knowledge on the effectiveness of cultural and other phytosanitary measures and their integration, trade requirements and politics, institutions, risk contributors' motivations and attitudes, and community engagement. Taking these factors into consideration will maximise the chances of designing a successful AWM programme tailored to local conditions.

In the case studies analysed, co-production of knowledge typically occurred within the local management group. Here, participants engaged in social learning, learning from the activities implemented and each other, to continually refine Queensland fruit fly-related management activities. From an adaptive co-management perspective, outcomes need to be closely monitored when changes are introduced to ensure that the system does not loose functionality. Social learning requires sound communication processes that are well-facilitated, both horizontally between local players and vertically across levels as illustrated in Fig. 1. Adaptive co-management was evident in the successful Central Burnett AWM programme (see Box 1).

Adaptive co-management does not offer a 'quick fix', and the investment (transaction cost) to engage with different players and learning processes can be considerable, especially in the early stages of an AWM programme. However, it does offer several important benefits over time (Kruger 2016a):

- Local knowledge of Queensland fruit fly behaviour in the local environment increases
- Management activities are suited to the local region and continually improve
- Local adaptive capacity strengthens, as lessons are learnt about what works and does not work in the local region and under different circumstances
- A common narrative develops between key stakeholders, which is fundamental to developing the much-needed shared vision for AWM
- A more sustainable, fit-for-context and locally-owned initiative is developed.

Box 1. Adaptive Co-management of Queensland fruit fly in Central Burnett

The successful Central Burnett AWM programme represents many aspects of adaptive co-management. Local crop consultants, state government researchers and citrus growers worked closely together on various regional projects from the 1990s, resulting in trust-based amicable relationships. Jointly they decided on research priorities and Queensland fruit fly-related activities for the region, and discussed findings, which subsequently shaped in-field activities. Research was carried out in the region, including in growers' orchards. Growers participated in some research activities, such as when the male annihilation technique was introduced. When AWM was launched in 2003, the local government assisted with community engagement to minimise Queensland fruit fly pressure originating from towns. Other local horticultural industries were engaged in the AWM effort. State government researchers were in close contact with their colleagues responsible for negotiating domestic trade protocols. This assisted in fine-tuning a domestic market access protocol to be accepted by some jurisdictions and be practical for growers to implement. Most growers employed a local crop consultant, with whom they regularly communicated, implying that they had the ear of key decision-makers, as all crop consultants were management group members. When changes were introduced, such as less intense town treatments, the results were closely monitored to allow for rapid response in case of unfavourable results.

Policy Implications: Adaptive co-management requires a mind-shift from focusing exclusively on implementing Queensland fruit fly management measures – such as when the now-restricted chemicals were still available – to implementing measures with the intention of continually learning and adjusting. This is illustrated in Fig. 1 by the two-way arrows between the AWM action situation, the local Queensland fruit fly management strategies and Queensland fruit fly pressure.

Adaptive co-management implies that grower groups need to actively build networks with others, including other growers (or their representatives), experts in Queensland fruit fly behaviour and market access, and community representatives, such as local government. Several of these may involve representatives of organizations that are active in the broader fruit fly management innovation system (Fig. 1).

Several aspects of market access represent 'top-down' elements where growers have little control, such as some trade protocol requirements that are negotiated bilaterally at the federal level. While it is important for those designing AWM programmes to take the technical requirements for market access into consideration as early as possible, it is best for AWM programmes to first focus on achieving Queensland fruit fly suppression. Unmet trade requirements relating to Queensland fruit fly can then be seen as 'bolt-on' components. Government and industry bodies can facilitate access to relevant experts. Other ways in which local industries can be supported are discussed below.

3.3. Principle 3. Local Industries Need Help to Help Themselves

An 'ideal type' of knowledge and capabilities that local industries need to access in order to achieve and maintain AWM, was developed during this research. It demonstrates the importance of dedicated staff to oversee and coordinate an AWM programme. In summary, these capabilities are:

- *Programme administration and management* – Effective programme implementation, including planning, implementation and monitoring; securing funding, sound financial management, understanding broader institutional requirements, organising and facilitating meetings, and record-keeping.
- *Stakeholder interaction* – Including achieving a shared local vision, maximising uptake across risk contributors, networking, advocating the programme to key stakeholders, conflict management and supporting growers with recommended practice implementation.
- *Understanding Queensland fruit fly behaviour and management* – Including general Queensland fruit fly biology and behaviour within the target region, on-ground management options, and consistently implementing regional Queensland fruit fly management strategies.
- *Understanding market access requirements:*
    - Phytosanitary measure options – including effectiveness and limitations
    - Formal market access standards – e.g. relevant ISPMs and Queensland fruit fly Code of Practice; and concepts such as Probit 9 levels, appropriate level of protection and risk management
    - Informal aspects and requirements – e.g. market expectations and politics
    - Market access application and approval process, including data gathering, and
    - Consistent implementation, e.g. monitoring and corrective actions, where needed.

Phase 1 respondents talked about the difficulty of establishing the needed networks to access the knowledge and capabilities required. Some phase 2 interviewees believed that stakeholders at higher levels may easily assume that growers have certain levels of knowledge or capabilities, but in reality, this varies. Interviewees across phases 1 and 2 spoke about issues that added cost and effort to achieve AWM that could be made easier. For example, a high level of government staff turnover contributes to growers struggling to maintain relationships built on a mutual understanding of their local Queensland fruit fly situation, and possible ways forward. Moving forward sometimes depends on joint decision-making between stakeholders from different agencies and ‘getting them in one room’ is difficult for industry.

Policy Implications: Given the considerable decline in public extension support in Australia, training could be offered to those who are likely to fill at least part of this gap, such as private crop consultants, key growers and other interested local people. This can strengthen local knowledge and capacity on issues such as trade; Queensland fruit fly biology, behaviour and management; and community engagement. However, training without strengthened intermediation between local level actors and other Queensland fruit fly management innovation system actors will do little to encourage ‘upward’ information flow (see *Principle 4*). Training can also be a tool to support collaborations across levels by more quickly facilitating in-depth conversations between growers and other stakeholders. Feedback from interviewees also stressed the importance of effective stakeholder coordination between states/territories and between government and industry bodies; minimising staff changes and fostering a client-oriented ethos in government departments. Innovative policy-making can contribute to overcoming local reliance on voluntary approaches (see *Principle 5*).

3.4. Principle 4. AWM Programmes Need Strong Two-way Connectivity with the Broader Queensland fruit fly Management Innovation System

This research found that local industries easily become disconnected from the broader system. Phase 1 interviewees spoke about the difficulty of establishing networks and finding information and guidance to instigate AWM. Some phase 2 interviewees spoke about inaccessible research findings, as much research is not publicly available, and many growers will not read scientific articles. The disconnect is intensified by the loss of public extension services. The 'grower voice' was considered as under-represented in national Queensland fruit fly management dialogues. Higher level governance bodies may underestimate local complexities and overestimate the knowledge and abilities of local stakeholders. While peak industry bodies make much contribution to filling this gap, not all growers may see their peak industry body as representing their concerns. Limited resources prevent industry bodies from being involved in all AWM attempts to develop an in-depth understanding of the local issues.

Innovation studies show that successful innovation that results in positive on-ground change tends to result from a co-evolutionary process involving concurrent technological, social, organizational, and institutional change. As such, growers tend to be partners and entrepreneurs in the innovation system. The prevailing innovation approaches that focus primarily on technology production are increasingly criticized for not achieving intended outcomes often due to a lack of adoption (Klerkx et al. 2012). Instead, a well-functioning innovation system, in this case one that promotes industry-driven Queensland fruit fly AWM, needs to deliver the required institutional, social, and technological change that will maximise the chances for it to flourish (Hekkert et al. 2007). This requires strong feedback loops between local AWM attempts and the broader Queensland fruit fly management innovation system (see Fig. 1).

Policy Implications: Australia's National Fruit Fly Council has made considerable effort to make information on Queensland fruit fly management more accessible since the research was conducted, including online. However, establishing knowledge brokering to enable feedback loops across levels (Fig. 1) can support co-producing integrated knowledge (Kruger 2017). This requires fulfilling key functions to support information flow and collaboration between stakeholder groups (Klerkx et al. 2012), which are easily overlooked as they are often invisible and hard to measure (Klerkx and Leeuwis 2009; Meyer 2010). These key functions are:

- Demand articulation – including assisting local industries with finding a shared vision to identify their technology, knowledge, funding, and policy needs (Klerkx and Leeuwis 2009; Meyer 2010).
- Network establishment – including local horizontal networks and vertical networks with policy-makers and researchers (Klerkx et al. 2012; Meyer 2010).
- Information translation – to connect 'external' information with the local context and growers' existing knowledge in summarized form and language that growers find useful (Klerkx et al. 2012); and local issues are 'translated' to other innovation system players to inform their decision-making (Klerkx and Leeuwis 2009; Meyer 2010).

- Innovation process management – working towards better arrangements in the multi-actor network, including facilitating cooperation and learning (Klerkx and Leeuwis 2009).

As in *Principle 2*, collaborations between heterogeneous stakeholders to learn from each other are needed to produce integrated knowledge. Such collaborations represent innovation platforms. Interconnected innovation platforms across levels can be created, e.g. by connecting local AWM groups with multi-stakeholder groups at state and national levels. It enables representation of a stronger 'grower voice' at higher level deliberations and decision-making. The design of such intervention is best negotiated amongst key stakeholders to meet their needs and expectations, ensure maximum buy-in, and to enable a good fit with existing structures. Queensland fruit fly *management coordinators* at regional, state and national levels can facilitate interconnected innovation platforms and can potentially be co-funded between government and industry.

3.5. Principle 5. Industry-driven AWM Programmes Need Institutional Adjustment to Share Public Responsibilities and Roles

A major challenge for industry-driven AWM is dealing with Queensland fruit fly pressure from host plants in town backyards, and on peri-urban and public land. Therefore, public support is vital for AWM success (Dyck et al. 2021). All case studies were reliant on voluntary approaches to address Queensland fruit fly pressure from towns, such as the awareness-raising activities in Young-Harden and Riverina. Currently, legislative power rests predominantly with state governments, but there may be reluctance to introduce enforceable measures that favour industry over other rural groups (NSW 2014). Many local governments have very limited powers to put in place enforceable rules and in the case of study areas they did not have powers to enter backyards without resident permission.

The case studies revealed barriers to behaviour change other than a lack of awareness, e.g. community apathy or recommended Queensland fruit fly management practices not making economic sense. For example, routine sanitation in orchards to remove fallen fruit is another essential AWM component that many growers are reluctant to fund. Other challenges included absentee landholders and derelict orchards.

At least 89 per cent of growers surveyed across all case studies agreed that Queensland fruit fly infestation in towns increases on-farm Queensland fruit fly pressure. However, only 42 per cent of respondents in the Riverina and 40 per cent in Young-Harden had a strong or some belief that regular educational activities would ensure that town residents would adequately manage Queensland fruit fly on their properties. This suggests a limited potential for growers voluntarily contributing to a reliance on awareness-raising activities in towns.

Another major concern is maintaining commitment and AWM programme funding over the longer term. Establishing an income stream often depends on voluntary contributions from growers. However, Central Burnett demonstrates that this is thwarted by 'free-riding' when some growers refuse to contribute while still benefiting from reduced Queensland fruit fly pressure from towns. This causes others to also ‘opt out’ of voluntary contributions. For example, 59 per cent of Central Burnet survey respondents said that they would contribute to town treatments only if others contributed too.

Studies about resource governance, involving resource-users taking the lead in setting the rules around resource usage, do not exclude complementary state intervention to back-up the collective action driven by resource users. In various contexts it is seen as important to sustain the trust among resource-users that others will also cooperate, and that a lack of cooperation will not jeopardise individual efforts (Ostrom 2005).

Policy Implications: A recommendation from this work is to apply ‘smart regulation’, i.e. using complementary policy instruments and behavioural interventions to assist in overcoming the weaknesses of individual instruments, while still capitalising on their strengths. For example, by combining approaches that draw on people’s intrinsic motivation to ‘do the right thing’ with legal instruments that can be enforced and that provide legitimacy to local industry AWM efforts.

Several policy instruments were explored as part of the PhD research project that were drawn from the case studies, as well as Australia’s Landcare Programme for natural resource management, and effective overseas AWM programmes. These instruments could be used in combination with others to ensure that the overall approach is locally tailored:

- *Community education and awareness-raising* – if well implemented, this approach can influence people who have an intrinsic motivation to manage the pest, such as households valuing their backyard produce. The investment needed to sustain effective awareness campaigns are easily under-estimated. However, such campaigns will do little to overcome behavioural change barriers beyond a lack of awareness (Curtis et al. 2014).
- *Broad-scale state regulation enforcing Queensland fruit fly management on all land* – while this can be applied consistently across risk contributors, it is very costly to monitor and enforce. There are also moral challenges, such as when landholders are physically or financially unable to manage Queensland fruit fly. Magistrate courts easily misunderstand the level of Queensland fruit fly control required and have rejected cases brought for prosecution (personal communication, state government interviewee, 24 September 2013).
- *Co-opting local stakeholders to support better implementation of government powers* – including authority to enter private property and/or prosecute non-compliant landowners. This lessens the monitoring and enforcement burden on state governments. It is applied in the US states of Oregon and Washington, for example under the 2011 Washington Code (Washington State Legislature 2011). However, some authorities may be reluctant to favour industry needs over those of other community groups.

- *Devolved power to enable industry, in partnership with local communities, to devise rules appropriate for the local context* – including possibly allowing enforcement as a ‘back-up’ mechanism. This aligns most closely with the underlying principles contained in much of the community engagement literature that values community involvement in the decision-making of issues affecting them. However, results elsewhere have been mixed in natural resource management (Berkes 2010). Potential adversarial effects include conflict and 'power grabs' by some groups (Berkes 2010). Dealing with uninformed people trying to influence the programme is difficult (Dyck et al. 2021). Such approaches tend to require considerable investment and skill.
- *Legislated cost-recovery structures* – A legislated income-stream can facilitate industry and other appropriate local stakeholders to implement on-going and reliable pest treatments in Queensland fruit fly risk areas in combination with community awareness activities. It can come from mandatory contribution from growers, the state and possibly town residents, such as in the successful OKSIR programme for codling moth control in British Columbia, Canada (Dyck et al. 2021; Nelson et al., this volume). However, such schemes could encounter resistance from those expected to contribute unless the contributions are adjusted to be proportionate to the expected benefits.

4. CONCLUSION

The research reported here explored whether industry-driven fruit fly area-wide management is feasible, with special focus on Queensland fruit fly AWM programmes in New South Wales and Queensland, Australia. This manuscript contains a summary of a social science PhD project that involved an investigation of three case studies of AWM programmes (or attempts thereof), a grower survey and interviews with people operating in the broader Queensland Fruit Fly management innovation system.

It found that the feasibility of industry-driven AWM depends on social and institutional factors at the local level and within the broader Queensland fruit fly management innovation system. Advantageous local factors include a favourable social profile, such as growers with relatively homogeneous on-farm goals and high levels of social capital, as well as the application of adaptive co-management. AWM programmes need to be adjusted to the local context, with market access requirements seen as ‘bolt-on’ components.

An enabling environment for industry-driven AWM requires a broader innovation system that is responsive to the needs of local industries. This requires strong two-way information flow between local programmes and other players in the innovation system, such as policy-makers and technology developers, which can be supported through knowledge-brokers and vertically interconnected innovation platforms. ‘Smart regulation’ can assist local industries to overcome the limitations of voluntary approaches (such as depending on awareness-raising and education alone), by influencing people through a combination of policy instruments tailored to local conditions.

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A SUCCESSFUL COMMUNITY-BASED PILOT PROGRAMME TO CONTROL INSECT VECTORS OF CHAGAS DISEASE IN RURAL GUATEMALA

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SUMMARY

The adoption of novel integrated vector management (IVM) strategies requires proof-of-concept demonstrations. To implement a community-based intervention, for the control of vectors of Chagas disease in Guatemala, we engaged all relevant stakeholder groups. Based on this and previous experiences of the authors on engaged research and community-based interventions, several key factors can help facilitate effective integration of stakeholders in support of area-wide integrated vector management (AW-IVM) programmes. First and foremost, the diversity of stakeholders needs to be engaged early-on in the participatory action research and implementation processes, to provide ownership and contribute ideas on how to design and implement an intervention. Another important component, situational analysis regarding current pest control policies, practices and relevant stakeholders, is generated through interviews with key informants, at both national and local levels (governmental and non-governmental organizations); it can facilitate the joint identification of strengths, weaknesses, opportunities and threats regarding current pest control strategies and proposing solutions through an AW-IVM approach. In addition, successful AW-IVM can result from identifying locally relevant strategies to implement the proof-of-concept demonstrative project. Further, it is critical to maintain constant communication with the local and national leaders, involving them throughout the implementation and evaluation processes. Flexibility should also be built into the project to allow for community-driven changes in the strategy, through a cyclical joint reflective process. Periodic feedback of project development needs to be scheduled with key stakeholders to maintain rapport. Finally, the results of the evaluation should be shared and discussed with stakeholders to ensure long-term sustainability of the programme, intervention, or project. Here we present the citizen engagement procedures used to integrate community members, health officials, and non-governmental organization staff for Chagas disease control in a region of Guatemala. We demonstrate how these methods can be applied to support AW-IVM programmes, so that communities and authorities are actively involved in the development and implementation of a jointly agreed intervention. In 2012, we developed the IVM intervention in an area of Guatemala with persistent *Triatoma dimidiata* (Latreille) infestation that is associated with the presence of infected rodents (rats and mice), that act as reservoirs of the *Trypanosoma cruzi* Chagas parasites inside the households. Nine control communities received only the Ministry of Health insecticide application against the vector and nine intervention communities participated in the

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AW-IVM intervention. The intervention included a programme for rodent control by the community members, together with education about the risk factors for vector infestation, and insecticide application by the Ministry of Health. Entomological evaluations in 2014 and 2015 showed that vector infestation remained significantly lower in both intervention and control communities. In 2015, we found that there was a higher acceptance of vector surveillance activities in the intervention communities compared to control communities, suggesting that participatory activities increase programme sustainability. Finally, we found that there was a significant increase over time in the number of households with infected vectors in the control group, whereas there was no significant increase in the communities that participated in the programme. Thus, an AW-IVM programme including simultaneous rodent and vector control could reduce the risk of Chagas infection in communities with persistent vector infestation.

Key Words: Central America, Ministry of Health, Jutiapa, community-driven changes, citizen engagement, Reduviidae, *Trypanosoma cruzi*, *Rhodnius prolixus*, *Triatoma dimidiata*, *Triatoma infestans*, stakeholders, participatory action research, vector surveillance, area-wide integrated vector management (AW-IVM), peridomestic environments, insecticide application, rodent control

1. BACKGROUND ON CHAGAS DISEASE

1.1. Chagas Disease in Latin America

Chagas disease is widespread in the Americas, affecting 6-7 million people (WHO 2017). It is considered one of the most neglected tropical diseases with serious public health implications, causing the loss of more than 600 000 disability-adjusted life years in Latin America (Mathers et al. 2007). The causing agent, *Trypanosoma cruzi* Chagas, is transmitted primarily by a few species of blood-feeding triatomine insects of the Reduviidae family (Dias et al. 2002). Strategies to control the vector species associated with domestic environments have been successful in several Latin American regions.

There are three area-wide regional initiatives in the Americas for Chagas disease vector control: the Southern Cone, the Andean Pact, and the Central American Initiative (Dias et al. 2002). All three initiatives aim to reduce the incidence of Chagas disease through vertically coordinated multi-country vector control programmes, blood supply screening, and health education (Dias et al. 2002). The two area-wide South American initiatives targeted *Triatoma infestans* Klug for vector control, as it is the main vector for the transmission of the disease in these regions (Massad 2008). The control activities for *T. infestans* resulted in the interruption of vector-borne transmission in Brazil, Chile and Uruguay (Dias 2007). Also, it reduced the incidence of infection in children and young adults in its member countries (Moncayo and Silveira 2009).

The Central American Initiative focused on the coordinated use of indoor residual spraying (IRS) to eliminate *Rhodnius prolixus* (Stähl) and reduce *Triatoma dimidiata* (Latreille) domestic populations (PAHO 2011). This area-wide programme included an attack and a surveillance phase that was coordinated by the Ministers of Health of Central America and several cooperation agencies, including local universities and the Japanese International Cooperation Agency (JICA 2014).

The success of the IRS interventions resulted in the interruption of Chagas transmission by *R. prolixus* in Guatemala, Honduras and Nicaragua, and the elimination of the vector in Costa Rica, El Salvador and Mexico (Hashimoto and Schofield 2012). These initiatives were successful and led to a significant decrease in the incidence and prevalence of Chagas disease. However, as with many area-wide pest/vector control programmes (Vreysen et al. 2007), remaining foci with persistent infestations hinder regional success. To succeed in these areas will require approaches that integrate novel ecological, biological and social factors.

Since early 2000s, housing improvement was proposed for sustainable control in regions with persistent *T. dimidiata* infestation (Lucero et al. 2013). In 2009, the World Health Organization (WHO) promoted the development of novel interventions for the control of Chagas and dengue in Latin America (Sommerfeld and Kroeger 2015). The interventions included multi-sectoral and -disciplinary ecosystem management strategies. Our study was part of an initiative by several countries to develop novel approaches for the control of *T. dimidiata* and *T. infestans* (Gürtler and Yadon 2015). We aimed to develop a community-based strategy for sustainable control of an area with persistent *T. dimidiata* in south-eastern Guatemala (De Urioste-Stone et al. 2015).

1.2. Vector Control Programme in Jutiapa, Guatemala

Guatemala started a major vector control programme in 2000 as part of the National Strategic Plan for Chagas Control (Hashimoto and Schofield 2012). This included several rounds of IRS application in the endemic area that covered over 45 000 km^2 in the initial and second programme phase (2000-2005) (Hashimoto et al. 2012). In 2009, Guatemala was certified to have interrupted transmission of the disease by *R. prolixus,* and *T. dimidiata* infestation was reduced nine-fold in the endemic region (Hashimoto and Schofield 2012; Manne et al. 2012). Vector control activities during the 2000-2010 period were estimated to have reduced the number of seropositive school-age children from 5.3% (1998) to 1.3% (2005-2006) (Hashimoto et al. 2012). Thus, successful Chagas disease control was achieved in most of the endemic area.

The effectiveness of IRS was evaluated by Hashimoto et al. (2006) across the department of Jutiapa, located in the south-eastern region of Guatemala. In areas where the baseline infestation rates were originally 20%, one spraying cycle reduced infestation to a mean of 1.4% within 3-21 months (Fig. 1). However, the infestation levels increased to an average of 8.1% during a second screening 20-45 months after spraying. In villages with an initial 40% infestation rate, the first spraying cycle reduced it to an average of 12.2%. Given that the control programme aims to reduce infestation to below 5%, a second spraying cycle was carried out in these villages. This effort reduced infestation to 4.8% in 40 of 52 villages 3-10 months after spraying. However, 12 of the 52 villages showed higher than 5% infestation after two spraying cycles, necessitating a third cycle that reduced the infestation from 10.9% to 4.1% 3-5 months after spraying.

With uneven results of prevention and control efforts, emphasis shifted to exploring long-term sustainability of surveillance and control interventions (Schofield et al. 2006). Despite a growing recognition of the role of social, cultural, economic and political conditions as risk factors linked to the disease, research and control efforts focusing on these factors have remained scarce (Ventura-García et al. 2013). Vulnerable groups such as indigenous populations and groups living in poverty continue to be at a high risk for disease transmission due to cultural, social, political, and health system barriers (Dell'Arciprete et al. 2014).

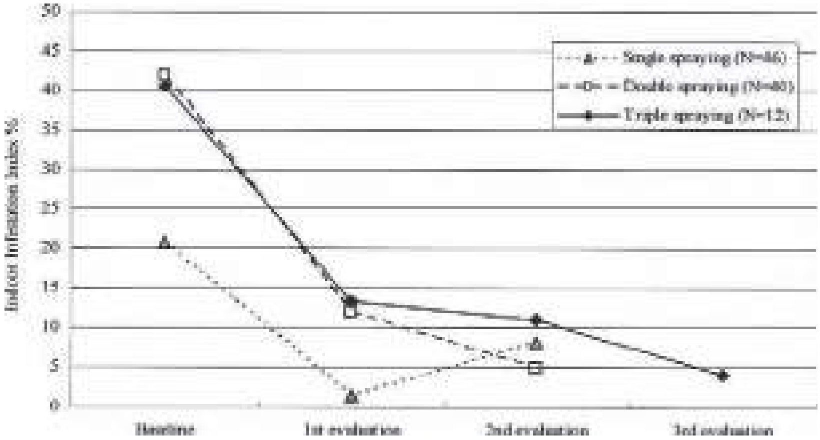

Figure 1. Changes of indoor infestation index for the single, double, and triple spraying areas (from Hashimoto et al. 2006).

2. DEVELOPMENT OF PROCEDURES AND SURVEYS

2.1. Setting the Foundations

2.1.1. Area of Interest and Initial Field Visit

The municipality of Comapa in the department of Jutiapa has been recognized as an endemic focus for Chagas disease, with persistent *T. dimidiata* infestation (Hashimoto et al. 2006) and high prevalence of *T. cruzi* infection (Rizzo et al. 2003). After multiple cycles of IRS and house improvement interventions (Bustamante et al. 2014), transmission continues at low levels in school-age children (Juárez et al 2018). A multidisciplinary team was formed that was composed of social and biological scientists with the aim to develop a project for improved Chagas disease control in this region. Our aim was to create a process that allowed stakeholders to contribute ideas, and implement an intervention that considered local conditions, resources, and concerns.

2.1.2. PRECEDE-PROCEED Framework

The PRECEDE-PROCEED model (PRECEDE: *Predisposing, Reinforcing, and Enabling Causes in Educational Diagnosis and Evaluation*; PROCEED: *Policy, Regulatory and Organizational Constructs in Educational and Environmental Development*) was used as a framework to guide the identification of risk factors through an analysis of the situation, and to develop and implement the intervention programme considering the risk conditions. This framework has been widely used for planning, implementing and evaluating health promotion programmes (Edberg 2007). It uses a multidisciplinary approach that includes disciplines such as sociology, psychology, epidemiology, business, and education (Blank 2006).

The steps required by the process before the intervention – PRECEDE – are based on a situational analysis: social, environmental, entomological, epidemiological, psychological, educational, and institutional assessments. A key component is the identification of risk factors (predisposing and reinforcing), as well as an informed development of the intervention proposal, with input from participants during group meetings. The intervention is based on the PRECEDE findings and implemented through the PROCEED steps in the formative and final evaluation of the intervention. As suggested by Edberg (2007), for the evaluation component, attention needs to be placed on assessing 1) the process of implementation, 2) the impact of the intervention (i.e. changes in knowledge, changes in practices and policies, changes in awareness), and 3) a limited number of outcomes due to the short timeframe of the project.

2.1.3. Defining the Study Design

In the PRECEDE stage of the project, a mixed methodology design was used to gain in-depth understanding of social, economic and environmental factors associated with persistent triatomine infestation (Bustamante et al. 2014). The approach allowed for a situational analysis of the community context, as well as for generalization of results and the credibility of the conclusions due to triangulation across research methods (Mertens 2014; Patton 2002).

Each method was selected based on its usefulness to the intervention, framed within the PRECEDE component of the framework. The methodology consisted of five stages: a) building rapport and gaining entry, b) mapping of households and sampling design, c) conducting baseline entomological and household surveys on knowledge, attitudes and practices regarding Chagas disease and possible risk factors, d) facilitating group meetings, and e) analysing documented evidence regarding local Chagas disease control activities and the socio-economic context.

Quantitative and qualitative data analyses allowed jointly developing a situational analysis report and an intervention proposal through a participatory process of individual and group learning and reflection (Bustamante et al. 2014; De Urioste-Stone et al. 2015). The PROCEED component of the study included a pre-test and post-test control group study design (De Urioste-Stone et al. 2015). Once again, a mixed methodology was used to generate both quantitative and qualitative measures during the intervention (Fig. 2).

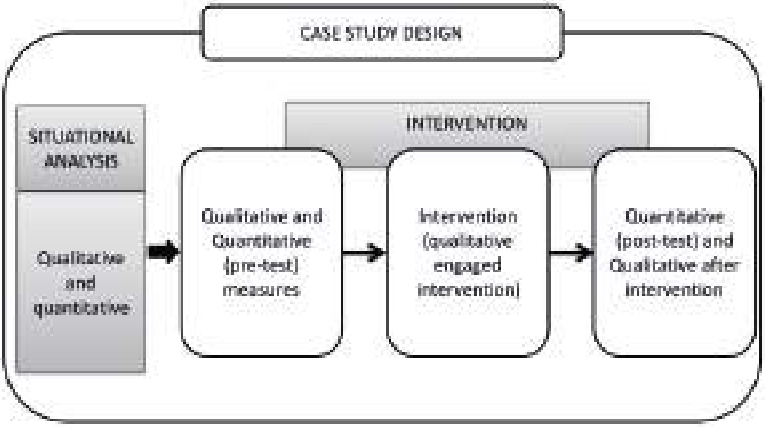

Figure 2. Case study diagram embedded mixed methods and design of the qualitative-engaged intervention (modified from Creswell and Plano Clark 2007).

One-year after completion of the intervention, an interim evaluation was performed in 2014 with household surveys and semi-structured interviews, and two-years after completion, in 2015, a final entomological survey was performed by the Ministry of Health (MoH) vector control programme. Given that change in behaviour requires time, this stage included pre- and post-surveys as indicators of change in knowledge and attitudes related to identified risk factors and to the disease. This allowed the study to determine the impact of participatory activities on these indicators. Small behavioural changes were measured qualitatively via participatory activities such as giving each participant a calendar to keep track of their activities (e.g. household cleaning and rodent trapping) throughout the month. Entomological indicators at baseline and follow-up were used as a proxy of reduced disease transmission.

2.1.4. Quality Assurance in Case Study Research

Trustworthiness strategies (Mertens 2014; Patton 2015) must be applied for quality assurance. Triangulation in case study research is key to enhance credibility (Creswell 1998). We used triangulation across stakeholders (Erlandson et al. 1993; Flick 1998; Mertens 2014; Patton 2015), and by generating information through different research techniques (Erlandson et al. 1993; Flick 1998; Patton 2002; De Urioste-Stone et al. 2015). We validated, through collective and iterative dialogue with the participants, our understanding and interpretation of the main concepts and ideas generated during participatory activities (Creswell 1998; Erlandson et al. 1993; Flick 1998; Mertens 2014). This was done immediately after generating data to enhance credibility.

2.1.5. Ethical Considerations of a Multidisciplinary Approach

A variety of informed consent forms were used; confidentiality was assured by creating coding systems with IDs (questionnaires, interviews, and group meetings), and careful and secure data management. Written consent forms were read and signed by the participants; in case the person could not sign the form, a fingerprint was requested, as well as a signature of a witness. For group activities, a written consent form was used; the consent form was read and signed by a representative of the group and a member of the research team (and included a list of the participants). Participants were requested to sign a consent form for photographs and videos during participatory group meetings. Potential benefits of the study were described and agreed upon prior to data collection.

The consent protocols for the cognitive study were collectively developed with the communities during the ethnographic phase, taking as model the consent forms used for interviews and signing a letter of conformity with the communities according to their own terms. We also were flexible in changing procedures and techniques to respond to contextual particularities in the communities of study. For example, the informed consent originally proposed was a verbal consent, but the communities requested to sign them during the first pilot study, so change to a written consent form was approved by the ethics committee.

After facilitation of each meeting, the research team went through a reflective process and preliminary analysis of results before planning the next group session. Hence, formative data analysis informed data collection.

2.2. Gaining Entry and Building Rapport

Gaining entry and building rapport are essential when conducting qualitative research (Ely et al. 1991). We considered this element of the research process essential for carrying out any type of intervention, and to ensure active collaboration of participants. Early on, a meeting was called by the leader of the Community Development Councils (COCODEs) for the entire community to share the study objectives, methods and expected participation at the community and individual level. For participatory activities, the entire community was invited.

For surveys and intervention phases, communities were randomly selected, and a set number of households was randomly selected to be included in the activities. The selection process was explained at these meetings to prevent any misunderstanding.

The following activities were carried out to enhance the success in gaining access and building trust with stakeholders in the study area:

- Periodic communication with gatekeepers from organizations and communities.
- Meetings in Comapa to present the intervention strategies and results from each stage of the project to local leaders, communities, participants and other stakeholders. In every meeting there was a space for dialogue to obtain feedback about concerns and ideas.
- Rapport with the national authorities was also enhanced, and the interim and final results were presented annually at the National Chagas Vector Control Programme evaluation meetings.

- Presentation of results from each stage of the study to communities and stakeholders.
- Flexibility to change procedures and techniques to respond to the situations, getting Institutional Review Board approval for protocol modifications as the project progressed.
- Respect and value for the time provided by co-participants according to their cultural norms and conception of time.
- Recognition, adaptation and respect for organizational and ethnic cultures and ways.
- Collaborative definition of the location, dates, and times to conduct meetings, interviews and workshops.
- Ongoing reflection on the processes of gaining entry and building trust.

Community meetings were conducted to share general results of the Knowledge, Attitude and Practices (KAP) questionnaires and entomological surveys after the PRECEDE and PROCEED stages. Interim and final results were presented annually at the National Chagas Vector Control Programme evaluation meetings.

2.2.1. Engaging National and Local Health Authorities

Before preparing the proposal, the idea was first presented to the head of the National Chagas Vector Control Programme. After approval at the national level, the idea was presented to the Jutiapa Health Area epidemiologist. During this meeting, a visit to the field site provided context and shaped the proposal for local relevance. This visit was critical to gain support from the local health authorities, who provided valuable input for the final proposal. Brainstorming sessions looked at potential stakeholders to approach, and potential collaboration activities and resources. Meetings were also conducted with leaders from the Municipality of Comapa. The main objective of these meetings was to gain stakeholder permission to conduct the research, to explain its different components and to discuss and gain feedback on how to implement them. Local authorities provided us with baseline data from the communities, maps, and an up-dated list of community leaders.

2.2.2. Engagement of Community Leaders

In 2002, the COCODEs were created in Guatemala to serve as the local organization that identifies and brings together community leaders. The aim of the COCODEs is to serve as a channel to facilitate the participation of the population, to plan and implement development efforts using a democratic approach (Congreso de la República de Guatemala 2002). COCODEs have usually facilitated introduction of projects (or any external initiative) to their communities, serving as the first contact to approach the community and gain access and project approval.

For our project, a list of communities and their leaders was generated at the municipality level. A meeting was organized with 74 members of the COCODEs from Comapa, where the leaders were informed about the duration and objectives of the project. The methodology was explained in detail, as well as the expected participation of the community members. Lists of problems related to Chagas and organizations working in the area were generated and the overall scope of knowledge and experiences with Chagas at the community level was detailed. The meeting with the COCODEs leaders was essential to gaining entry and ensure proper communication about the project.

However, the COCODEs are also an institution that can influence decision-making, which may lead to politicization of initiatives. New projects with no former experience in the area should pay attention to these political dynamics, which might be very challenging. In our case, some communities had one COCODE appointed by the municipality and another appointed by the community members. At the end two meetings were done for each community. We found throughout the study that identifying leaders who were recognized by the community allowed us to gain entry and build a relationship of trust with each community, without politicizing the project. In the absence of organizations such as COCODE, one option is to determine which other institutions collaborate with local leaders and which organizations (governmental and non-governmental) are working in the area. It is very likely that relevant structures are already in place, i.e. a network of leaders, youth teams, volunteers or women groups, that could be invited to collaborate, rather than starting interactions from scratch.

2.2.3. Community Engagement

Based on our experience, securing collaboration from the communities is essential to avoid a paternalistic approach. It was important to acknowledge that we did not have all the answers and that we could not propose all potential solutions using solely ideas generated in the office or by conducting experiments in the laboratory. We recognized the need to listen to the local community members, and value their opinions to make the intervention successful and sustainable.

After evaluating the eco-bio-social baseline information, we conducted participatory activities at three different communities, inviting all members of each community to each meeting through the local leader. The communities were selected based on different social organization characteristics and vector infestation levels. In these meetings, we actively listened to the ideas and concerns of local participants regarding their role in risk factor mitigation.

Based on this approach, we proposed potential strategies that were discussed with all stakeholders and could be modified later if required. Actively listening to ideas, experiences and respecting the knowledge of local populations was key to engaging and empowering participants. Through this engagement, the project moved from being an endeavour from a traditional research team, to becoming a collective undertaking with the active participation of local populations – this is essential for the activities to continue after the project has come to an end.

2.3. Stakeholders Analysis

2.3.1. Stakeholder Context

We developed a list of stakeholders at the national, regional and local levels that stipulated their roles in Chagas vector control. The different roles and areas of emphasis for stakeholders are shown in Fig. 3.

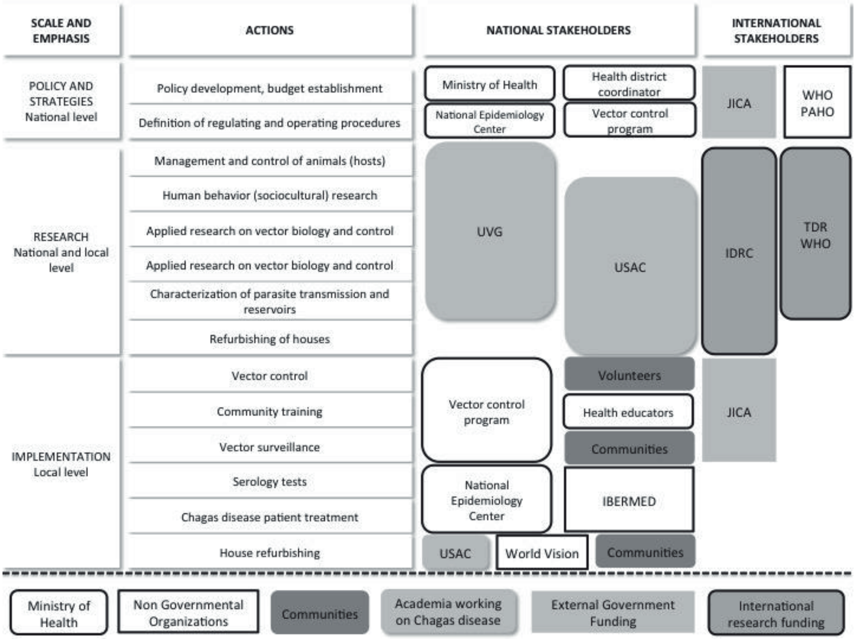

Figure 3. Stakeholder mapping of the National System for Chagas Control at the local and national levels (with permission from De Urioste-Stone et al. 2015). (Universidad del Valle de Guatemala: UVG; Universidad de San Carlos de Guatemala: USAC; International Development Research Centre: IDRC; Japan International Cooperation Agency (JICA); Tropical Disease Research-WHO: TDR-WHO; Pan American Health Organization: PAHO; Médicos con Iberoamérica: IBERMED).

The relationships we observed between the different stakeholders are portrayed in Fig. 4. This type of analysis is useful to understand and identify the persons who can positively contribute to the project and those who can potentially be obstructive. During the study, we continuously discussed newly developing power relationships between the different stakeholders to predict potential conflicts, but also opportunities for collaboration and to leverage resources.

We strived to understand the different roles and interactions among stakeholders to build the relationships for a participatory process. We acknowledged and considered knowledge about Chagas disease and its control, including interests, positions, alliances and relevance in Chagas disease control of those involved (Schmeer 1999).

2.3.2. Training of Personnel/Collaborators

Several training sessions were undertaken with local field staff of the MoH. The first efforts focused on standard operating procedures, use of maps and global positioning system (GPS) equipment. Field staff received training on how to apply the household/KAP questionnaire, specifically on how to approach interviewees, how to ask each of the questions, how to facilitate consent request and other ethical concerns.

Training sessions on the biosafety and handling of vector specimens were undertaken by all members of the vector control MoH team. These activities were crucial to generate understanding and empowerment related to the proposed activities for the intervention; all staff and collaborators need to fully understand the procedures to generate high quality data. This approach also allowed for collaboration across disciplines and forming a strong interdisciplinary team.

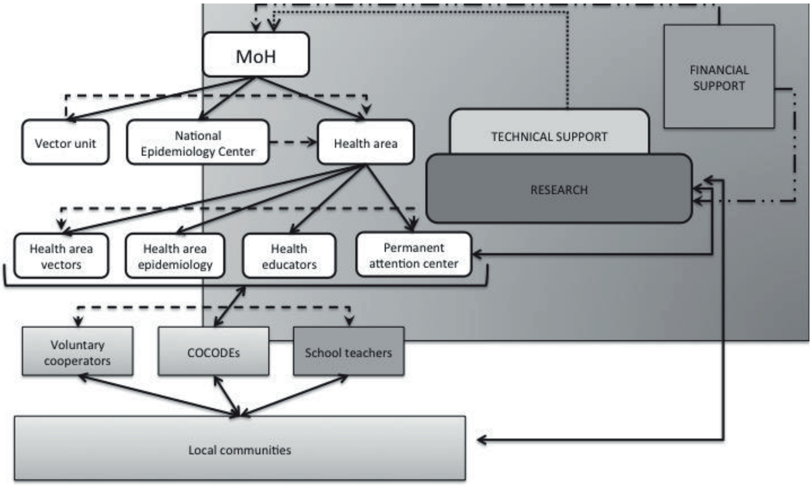

Figure 4. Mapping of stakeholder relationships. Arrows denote direction of relationships between stakeholders: unidirectional denoting supervision relationships, bidirectional being collaborative interactions. The dotted lines indicate secondary linkages through cooperation relationships, the solid lines indicate primary linkages through collaborative relationships.

2.3.3. Pre-testing of Survey Procedures and Questionnaire

We validated all survey procedures, questionnaires and the consent forms before starting data collection, in two communities from the same municipality that were not part of our study. These communities were selected from the sample of communities found over 850 meters above sea level that were not selected for the survey. Two validation rounds were conducted in two different communities with four households randomly selected in each. We obtained feedback to make the required modifications.

Each household was visited to assess the household/KAP and entomological forms, with the goal of reducing measurement error (Dillman et al. 2008; Krosnick et al. 2014). The reactions of interviewed community members to wording and ordering of questions was observed and analysed, and participants were asked to suggest better phrasing and more appropriate wording when questions were not clear. As a result, the accuracy of the questions was improved, several questions were added, and the order of questions revised. The order of the procedures was also modified to improve the interviewing process by performing the KAP survey instrument before the entomological survey.

The instruments were modified, and a third round took place to pilot test the effectiveness of the selected protocols, with four more households randomly selected. After the feedback from the pilot test was included, the household/KAP questionnaires were further reviewed and revised by staff of the MoH.

3. THE INTERVENTION

3.1. Developing the Intervention

Before the intervention, three communities of the Municipality of Comapa, selected based on their vector infestation levels (two with the highest infestation and one without persistent infestation), were invited to share information regarding animal management in relation to Chagas disease risk factors and to identify their problems and potential solutions (Bustamante et al. 2014). All community members were invited to these activities. We used a participatory model to gain in-depth understanding regarding local practices related to risk factors. Through a series of community reflection exercises, it became evident that community members wanted to better understand the disease, and to identify actions to change the current conditions.

An anthropological study was undertaken to better understand the economic production practices, further adding to the knowledge generated previously. We observed very distinct activities based on gender, with agricultural activities mainly carried out by men, whereas women carried out household chores, raised children, cared for peridomestic animals (e.g. chickens), and traded goods with other community members.

The first evaluation of the region showed that, with respect to Chagas disease, chicken management practices and the presence of dogs and rodents posed an important risk for the household (Bustamante et al. 2014). The findings indicated the importance of developing sustainable animal and environmental management practices that would modify behaviours of community members that posed risk factors in relation to triatomine infestation, as part of a gender-oriented education programme.

3.2. The Intervention Framework

The intervention framework included a close collaboration with the communities to generate an integral animal and environmental management programme. It also aimed to implement relevant components of another educational programme in the area called “Clean House, Clean Patio” developed by the MoH and JICA (De Urioste-Stone et al. 2015). The general objective was to provide a strategy to improve Chagas disease prevention in different ecological and social settings. This was achieved by better understanding the ecological, vector-biological and social ("eco-bio-social") determinants of peridomestic animal management in relation to vector infestation. As a result, we developed and evaluated a community-centred intervention to reduce habitats for rodents and chickens inside the household, both of which were found to be important blood sources and risk factors for triatomines.

Eighteen communities with baseline infestation levels above 15% were selected from the 30 communities surveyed in the situational analysis (De Urioste-Stone et al. 2015). Nine communities were randomly assigned to the intervention (AW-IVM) and the other nine communities to the control (only the MoH insecticide application) groups (Fig. 5).

3.3. *Participation and Community Engagement during Intervention*

We used some elements of Participatory Action Research (PAR) to guide changes in perception and behaviour at multiple levels, through "direct involvement, intervention or insertion in processes of social action" (Fals-Borda 2001). The model facilitated active listening to the concerns and ideas of participants, and it promoted a reflexive process about the implications of the interventions amongst researchers and participants. We relied on an iterative process of reflection and action (Freire 1970).

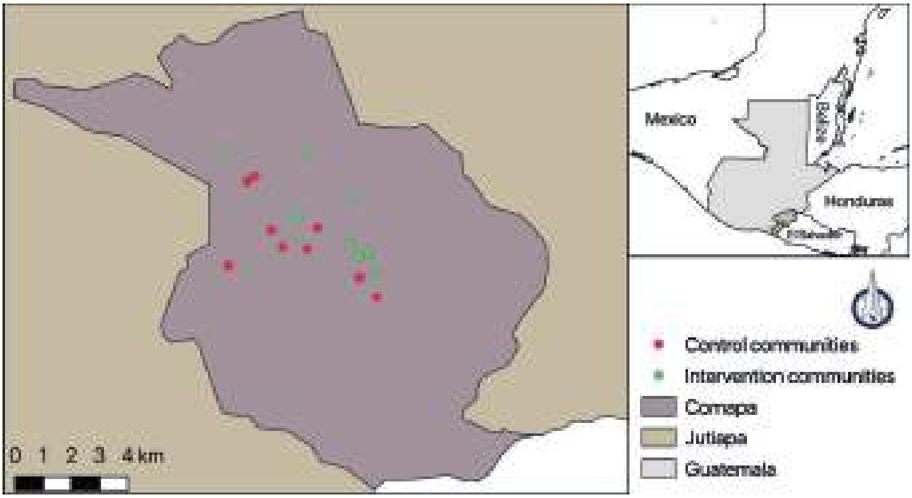

Figure 5. Distribution of communities assigned to intervention and control groups for the intervention phase, Comapa, Jutiapa, Guatemala, 2012.

We - participants, researchers and collaborating institutions (non-governmental organizations, MoH) - engaged in a cycle of sharing knowledge, following up changes in practices, and reflecting about the research process during the intervention (De Urioste et al. 2015). We believe that through this knowledge-reflection-action iterative method, we facilitated a process best described by Kemmis and McTaggart (2003) as:

> *"...systematic inquiry, process; participation and collaboration; bridging local and scientific knowledge; empowerment; and action".*

Participatory techniques (FAO 1990; Chambers 2002) were used as a vehicle to stimulate dialogue and reflection among participants about Chagas disease. Educational material about Chagas and topics related to chicken, dog, rodent/grain management were discussed and analysed with participants. Seven monthly participatory meetings were held within each of the intervention communities:

- *Meeting 1:* A full description of the intervention methodology and timeline was presented to the selected members of the intervention communities. Consent of all the participants was obtained. Methods were discussed and feedback of the participants was incorporated into the research tools to be developed in the following meetings. An informative brochure that contained results obtained in the baseline was developed and shared with the participants.
- *Meeting 2:* A community level Strengths, Weaknesses, Opportunities, Threats (SWOT) analysis was generated, to have a better understanding of the situation and to identify specific issues to be addressed in the following meetings. The analysis included topics such as (1) current practices to manage and control the triatomines, (2) current knowledge about *T. dimidiata* behaviour and presence in the houses, and (3) other institutions working directly or indirectly with the disease and the vector.
- *Meeting 3:* Group narrative related to knowledge and experience with Chagas disease. The research team presented information on the disease and its vector, forms of transmission, symptoms, effects and treatment. A calendar was presented and validated as a personal matrix to document household activities.
- *Meeting 4:* Group narrative related to knowledge regarding rodents and grain storage as risk factors for vector presence in the house. The research team presented information on the biology and ecology of rodents, the danger they pose in relation to Chagas disease, and proposals for control strategies. The calendars were delivered to the meeting participants for use as personal matrices to record the activities and practices related to rodent and grain storage, to be implemented during that month. During this meeting, rodent traps were delivered, and a practical session demonstrated the use of the traps, protocols to kill the rodents easily and ethically, and to manage and bury the carcasses.
- *Meeting 5:* Group evaluation with respect to the dynamics of the PAR process, i.e. assessment of the knowledge gained on the vector, the disease and the rodents, the activities proposed for rodent control, and about the use of the matrix (a calendar) to document rodent and grain storage practices. Discussion of needed changes in design and new personal commitments acquired to continue advancing.
- *Meeting 6:* Group discussion on the use of the matrix for rodent control activities and any related changes made. Sharing of knowledge and experiences related to chickens, vegetation and waste management as risk factors for vector presence. Presentation by the research team on the importance of chicken management, and proposal of a strategy for integral waste management, through compost production, a chicken coop and food production. Validation and discussion about the matrices to have a record of the activities and practices related to environmental management.

- *Meeting 7:* Group discussion regarding the use of the matrix for grain storage, and rodent, chicken, dog and environmental management practices and any changes made, and all steps of the PAR process. Closing presentation with findings, achievements and identification of possible knowledge gaps. Opinion survey among participants and local leaders regarding the PAR process.

4. RESULTS AND CONCLUDING REMARKS

Comparison of KAP survey results with Student´s t test, in a pre-test in 2012 and post-test in 2014, showed that the intervention with participatory activities produced a significant change in protective practices against risk factors for persistent *T. dimidiata* infestation, including rodent control using mechanical traps and environmental management, as well as chicken management (Student´s t test, $p<0.001$) (De Urioste-Stone 2015). An odds ratio comparison of entomological indices in 2012 and 2014 showed higher early instar reinfestations in the communities that received no treatment (control), compared to the intervention communities (OR 8.3, 95% CI 2.4-28.4). In addition, there was a significant reduction in rodent infestations in the intervention group over time (OR 1.9, 95% CI 1.09-3.45). The overall infestation levels were maintained below 10% in both intervention and control groups during the first evaluation in 2014 (Fig. 6).

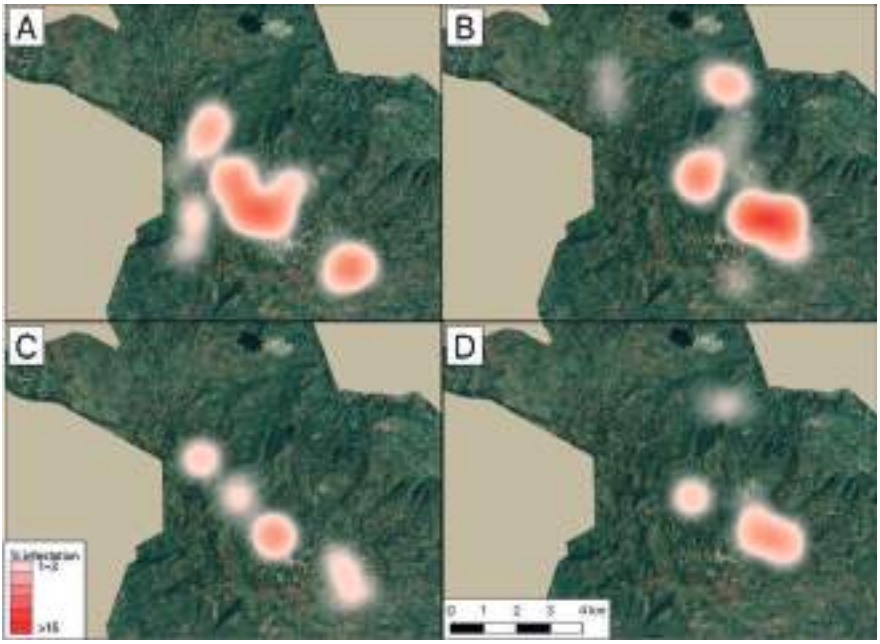

Figure 6. Reduction of Triatoma dimidiata *infestation in the Municipality of Comapa, Department of Jutiapa, Guatemala in the control and intervention groups pre- (2012) and post-intervention (2014). A) Control group 2012. B) Intervention group 2012. C) Control group 2014. D) Intervention group 2014.*

In 2015, an entomological survey was performed as part of the MoH surveillance activities. The survey was performed simultaneously with a serological survey of household inhabitants (Juarez et al 2018). During this 2015 survey, we observed that communities where Participatory Action Research was conducted had a higher participant retention, when comparing treatments (Pearson Chi^2= 3.298, p= 0.046, one-tailed test). A trend was also observed, at a 90% CI, for lower drop-out rates for the intervention communities with an Odds Ratio of 1.67 (90% CI= 1.05, 2.65) (Table 1). This suggests that the participatory process increases long-term community acceptance of MoH surveillance activities.

Table 1. Community recruitment and continued participation between control and intervention treatments in numbers (%) for the municipality of Comapa, Department of Jutiapa, Guatemala

	2012	2014	2014	2015	2015
Treatment	Recruitment	Drop-out	Remained	Drop-out	Remained
Control	215 (49.9)	15 (3.5)	200 (46.4)	37 (8.6)	178 (41.3)*
Intervention	216 (50.1)	23 (5.3)	193 (44.8)	24 (5.6)	192 (44.5)*
Total	431 (100)	38 (8.8)	393 (91.2)	61 (14.2)	370 (85.8)

* *Significant difference, p<0.05. We were unable to observe any statistical difference for domestic infestation levels between the control (21%; 8.5% and 15%) and intervention (20%; 8.3% and 12%) by year (2012-2014-2015). We did observe a trend that may suggest that intervention practices may prevent long-term reinfestation of the houses (n=192) that were originally infested (OR 2.5, 95% CI 0.93-6.99). On the other hand, the control group (n=178) showed a higher probability of finding infestation if the household was previously infested (OR 3.16, 95% CI 1.1-9.00)*

We also evaluated the effects of rodent infestation on vector infection by *T. cruzi*, for the triatomines collected in 2012 and 2015. Triatomine infections were confirmed using PCR for the parasite *T. cruzi* (Maddren 2018). We found that the proportion of infested houses with infected triatomines significantly increased over time in the control group (Table 2). However, in the intervention group the proportion of infested houses with infected triatomines did not increase over time. This suggests that rodent control may reduce the risk of infection in the households. It appears that the participatory process gave the household inhabitants the tools to reduce risk factors for infection. Through this community-based programme, we learned that complex health problems such as vector-borne zoonotic diseases require multidisciplinary and community-based approaches to develop innovative solutions that target ecological, socio-economic, cultural, institutional, and biophysical factors of risk.

To develop relevant solutions, an in-depth understanding of the dynamics of communities and the role/interactions among stakeholders are first needed to be able to understand the context that frames the public health issue. Once biophysical and social science data are collected and triangulated, and the problems are identified based on this data integration, solutions should include the input from those that will be involved and affected by the intervention.

Table 2. Percentage of sampled houses in the Municipality of Comapa with Triatoma dimidiata *infected with* Trypanosoma cruzi

Treatment	Year	Infested Houses	Percent of houses with *T. cruzi* infected *T. dimidiata* (95% CI)
Control	2012	30	65.1 (50.3-79.8)*
	2015	18	90.4 (78.2-100.0)*
Intervention	2012	17	68.1 (44.2-91.9)
	2015	20	77.2 (59.9-94.4)

* *Significant difference, $Chi^2 = 5.8$, $p<0.05$*

A participatory-reflexive process can help improve the effectiveness of strategies aimed at achieving changes in human social and behavioural contexts. These activities empower the community to engage in practices that are truly relevant and feasible to them. Introduction of AW-IVM innovations should engage citizens throughout the process to ensure public awareness and involvement in the projects.

In our case, given that these vectors can colonize peridomestic environments, it will be necessary to implement additional strategies in the future to reduce the availability of these habitats and prevent future domestic infestations. In addition, all members of the communities should be engaged in the process, to ensure that peridomestic environments do not become a source of infestation for neighbouring households and communities.

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CITIZEN SCIENCE AND ASIAN TIGER MOSQUITO: A PILOT STUDY ON PROCIDA ISLAND, A POSSIBLE MEDITERRANEAN SITE FOR MOSQUITO INTEGRATED VECTOR MANAGEMENT TRIALS

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SUMMARY

During the past twenty years, the number of research projects involving people not trained as scientists, the so-called citizen science, has increased consistently, including mosquito monitoring and control projects. The involvement of citizens in mosquito monitoring programmes not only helps scientists during the data collection phase, but also raises public awareness on mosquito-transmitted diseases and educates citizens about virtuous behaviours that can help in reducing mosquito populations and their spread. The Asian tiger mosquito *Aedes albopictus* (Skuse) is an invasive species that became established in Europe starting in 1979, with Italy representing currently one of the most infested countries. Procida, a small Mediterranean island in the Naples gulf (Campania region, southern Italy) has unique and very interesting features facilitating the field testing of mosquito integrated vector management (IVM) approaches and control methods, including the Sterile Insect Technique (SIT). With the help of the local municipal administration, the Procida citizens are actively involved as volunteers in monitoring the seasonal and spatial distribution of the Asian tiger mosquito. The collected baseline data will be useful to implement a future island-wide integrated suppression trial of *Ae. albopictus,* including the release of sterile males, to be carried out in collaboration with the local municipal administration and with the technical support of the Joint FAO/IAEA Division in Vienna.

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Key Words: Baseline data, mosquito surveillance, *Aedes albopictus*, *Culex pipiens, Culex laticinctus,* IVM, SIT, Sterile Insect Technique, Italy

1. INTRODUCTION

According to the World Health Organization (WHO), mosquitoes are considered the deadliest animals on Earth, causing more than one million human deaths every year and representing a risk due to the diseases they transmit, and to which more than one third of the human population is exposed (WHO 2019).

In this scenario, during the last four decades, invasive mosquito species have played a significant role because of their confirmed or potential capabilities to vector an increasing number of diseases to humans and animals. Introductions of invasive mosquito species have increased world-wide as a result of the globalisation of trade and travel, climate change, and the capacity of the mosquitoes to adapt from their native areas to temperate regions, such as the European continent.

Among all the invasive mosquito species listed to date, *Aedes* species are of major concern, with at least five described species having become established in parts of Europe (Medlock et al. 2015). This includes the Asian tiger mosquito *Aedes albopictus* (Skuse 1894) (Diptera: Culicidae) that is considered a major threat to public health in Europe.

1.1. Aedes albopictus *Invades Europe*

Ae. albopictus originated from Southeast Asia and has spread world-wide during the last 40 years. In Europe, it was firstly recorded in Albania in 1979 and is present today in 24 European countries (ECDC 2018a). Species distribution models, combining eco-environmental and terrestrial cover variables, and future climatic scenarios, predict further spread of this species in Europe and other countries in the world (Fischer et al. 2011; Caminade et al. 2012; Cunze et al. 2016).

In Italy, the mosquito arrived in 1990 in Genova (Liguria region) (Sabatini et al. 1990) and has quickly spread over the whole Italian territory, in particular in the north-eastern area (Friuli-Venezia-Giulia region, and large parts of the Lombardia, Veneto and Emilia Romagna regions) and central and southern coastal areas, including major islands (Albieri et al. 2010; Marini et al. 2010; Valerio et al. 2010). *Ae. albopictus* has a very aggressive day-time human-biting behaviour (Valerio et al. 2010; Manica et al. 2016) and it is a competent vector for more than 20 arboviruses, including the dengue and chikungunya viruses, in addition to filarial nematodes of veterinary and zoonotic significance (Cancrini et al. 2003; Pietrobelli 2008; Bonizzoni et al. 2012).

The first European outbreak of chikungunya fever occurred in Italy in 2007 and was ascribed to the presence of the local *Ae. albopictus* populations. This event drastically increased awareness of the risk of new or re-emerging mosquito-borne diseases in Europe (Gasperi et al. 2012). In addition, the Zika virus (ZIKV) outbreak in South and North America starting in 2015, and the confirmation that *Ae. albopictus* is a competent vector of the disease (Grard et al. 2014; Di Luca et al. 2016; Heitmann et al. 2017), have further emphasised the importance to carefully monitor and sustainably manage this species also in European countries. During the summer of 2017, about 250 cases of chikungunya fever were reported in the urban and costal area of Lazio (Venturi et al. 2017).

In the Mediterranean region, this species is mainly active during the summer, and evidence has been collected that under specific climate conditions the populations show a bimodal distribution with peaks in July and September (Manica et al. 2016). Considering the heavy nuisance caused by the *Ae. albopictus* female day-time biting behaviour, the presence of this species is considered also a serious socio-economic threat for regions with a tourism-based economy (Roiz et al. 2008).

To face the increasing risk of the spread of vector-borne diseases, several European Union (EU) countries started mosquito monitoring, surveillance and control programmes and, in 2005, the European Centre for Disease Prevention and Control (ECDC) was established, i.e. an EU agency aimed at strengthening Europe's defences against infectious diseases, including vector-borne diseases (Zeller et al. 2013; ECDC 2018b).

Mosquito monitoring programmes usually are based on the use of special traps (ovitraps to collect mosquito eggs, gravitraps to collect gravid female mosquitoes, and adult traps to collect adult mosquitoes of both sexes) to determine the occurrence and the spatial-temporal distribution of the species. However, the management of an area-wide trap network, covering wide territories or a whole country, requires great financial resources as well as a significant labour force.

1.2. Involvement of Civil Society in Citizen Science

Recently, the involvement of civil society in research projects, also known as citizen science, has become increasingly popular (Dickinson et al. 2012; Bonney et al. 2014). To support mosquito monitoring performed by experts through conventional trapping (the so called "active" monitoring), community-based surveillance activities have been launched in some EU countries. These citizen science projects are based on the public participation through active monitoring, such as the "Mosquito Atlas" in Germany (Mückenatlas 2019) and the "Mosquito Recording Scheme" (MRS 2019) in the UK, or through smartphone-based mosquito data collection applications (the so called "passive" monitoring), such as the "Mosquito Radar" (Muggenradar 2019) in the Netherlands, the "Mosquito

Alert” (2019) (hunting the tiger) in Spain, the “iMoustique®” in France, the “MosquitoWEB” (2019) in Portugal, and the ZanzaMapp (2019) in Italy.

The results of some of these projects, recently reviewed by Kampen and colleagues, demonstrated that public mosquito surveillance, despite some limitations mainly represented by the inexperience of volunteers, can usefully supplement surveillance programmes by:

1. Substantially reducing the field work costs
2. Collecting data in such a quantity that conventional research groups would not be able to generate by themselves
3. Raising awareness and improving knowledge amongst citizens on invasive species and associated public health problems
4. Detecting the arrival and the spread of *Ae. albopictus* and other invasive mosquito species populations in various European areas (Kampen et al. 2015).

Hence, such citizen science projects can help public agencies with the monitoring and control efforts of invasive mosquito species (Jordan et al. 2017; Palmer at al. 2017).

Mosquito management activities are frequently ineffective because some mosquito species, as is the case of *Ae. albopictus*, breed in human-made water containers, mostly located within private-access properties and areas, making the required action within the target area (egg or adult monitoring, sanitization and control of larval breeding sites, etc.) very complex, if not impossible, to be achieved. A mosquito community-based monitoring network could facilitate the monitoring on such private properties, and also help in the successive implementation of area-wide mosquito population suppression programmes that can include eco-friendly approaches such as the Sterile Insect Technique (SIT) or the Incompatible Insect Technique (IIT).

The SIT, which is based on the mass-rearing and release of sterile male-only insects that induce sterility in the local population, has been successfully applied against the New World screwworm fly and several fruit flies, tsetse flies, and lepidopteran species (Dyck et al. 2021).

The IIT is an alternative population suppression strategy, based on cytoplasmic incompatibility (CI), widespread in many diplodiploid species. With IIT the sterility in the target population is achieved through the release and mating of males infected with a different *Wolbachia* strain, which results in embryonic lethality (Saridaki and Bourtzis 2010; Lees et al. 2015).

The absence of effective vaccines against mosquito-borne diseases and the problem of growing insecticide resistance in mosquito populations (Sokhna et al. 2013; Vontas et al. 2012) have made the SIT, the IIT, and related approaches potentially promising components of area-wide integrated vector management (AW-IVM) programmes for some key mosquito species (Lees et al. 2015; Bourtzis et al. 2016).

Ae. albopictus is a suitable candidate species for SIT and/or IIT application because of its relative ease of mass-rearing, its sexual dimorphism that facilitates sex separation, and its low biological dispersal potential (Bellini et al. 2007, 2013; Albieri et al. 2010; Marini et al. 2010; Balestrino et al. 2014; Gilles et al. 2014).

In this paper, we present a community-based mosquito monitoring approach that we are developing on Procida, a Mediterranean island in southern Italy. We are collecting, with citizen involvement, baseline data and setting-up the optimal social and technical working environment for future *Ae. albopictus* population suppression experiments by the SIT and/or the IIT (Bourtzis et al. 2016).

2. PROCIDA ISLAND

Procida is a small island of the Phlegraean archipelago, situated in the Naples gulf, about three km from both the mainland and Ischia Island. It is a flat volcanic island (average 27 m above sea level) with a 16 km-long jagged coastline which forms four capes and with a total surface area of only 4.1 km^2, including the uninhabited tiny satellite island of Vivara (0.4 km^2). Except for Vivara, which is a natural reserve, Procida's territory is quite urbanized and accessible.

Most of Procida's private properties include a garden with ornamental flowers, vegetable cultivations and/or orchards with citrus plants and family-type farming of chickens and rabbits. Despite its small surface, Procida has a very high and urban-like population density with 10.477 inhabitants (2459 inhabitants/km^2 - ISTAT 28/02/2017). This human population density approximately doubles during the summer months, because of tourism, which is the current main local economic activity.

According to the perception of residents, *Ae. albopictus* arrived on the island around the year 2000, most probably introduced by tourists and/or maritime transport of goods. Thanks to very favourable host and climatic conditions, with an average annual temperature of 16.2°C and an average annual precipitation of 797 mm (http://bit.ly/ecdata_procida; accessed: 07th May 2018), and very abundant availability of water containers in private gardens, *Ae. albopictus* spread quickly over the entire island, reaching high population densities in some areas and becoming a serious nuisance in the last years.

Procida has unique and very interesting features for field testing of mosquito IVM including the SIT or the IIT: a very small size, a completely urbanized and accessible territory, a high human population density and year-round presence of *Ae. albopictus*. The island has obtained a world-famous reputation, due to several novels and films that were set there, which could help provide wide media coverage in the case of very positive population suppression results, that could facilitate fundraising for future larger population control tests.

Furthermore, many Procida citizens are aware of the SIT approach and of its advantages and effectiveness in insect pest control programmes. In fact, during the 1970's and 1980's, Procida island was chosen as an experimental area to study the field performance of sterilized male Mediterranean fruit fly *Ceratitis capitata* from a genetic sexing strain (Robinson 2002) in a cooperative programme between the Italian National Committee for Research and Development of Nuclear Energy (ENEA) and the International Atomic Energy Agency (IAEA) (Cirio 1975; Cirio et al. 1987). About 20 million sterile Mediterranean fruit fly males were released on the island from April to July 1986 and the population suppression obtained, linked with the protection of citrus fruits, was positively perceived by the residents for several years thereafter.

3. FIRST RECORD OF *Aedes albopictus* ON PROCIDA ISLAND

Official data about Asian tiger mosquito presence in the Phlegraean islands (islands in the Gulf of Naples and the Campania region of southern Italy) are available only for the satellite island of Vivara, where *Ae. albopictus* was detected for the first time in 2002 (D'Antonio and Zeccolella 2007).

In September 2015, an entomological field survey was undertaken in five private properties and five tourist facilities to confirm the presence of *Ae. albopictus* on Procida island and to obtain preliminary data about its distribution (Fig. 1A).

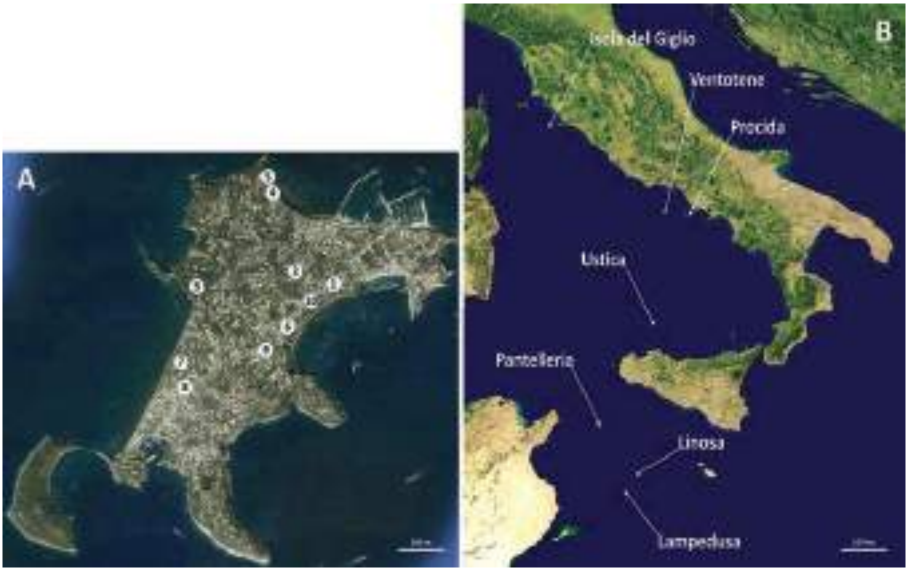

Figure 1. A) Aedes albopictus *field sampling sites on Procida island in 1-3 September 2015. Locations 1-10 are listed in Table 1. B) Italian minor islands were the presence of* Ae. albopictus *has been officially reported.*

Modified CDC light traps (CDC-LT) were used for the field sampling (Reisen et al. 2000; Li et al. 2016), without standard light source and baited with dry ice. CDC-LTs were placed in shaded locations in courtyards of private houses with rich vegetation for three days, in the period 1-3 September 2015 (26.3°C average temperature, 75% average humidity, 7.6 km/h average wind speed). Traps were activated each day from 8.00 h till 20.00 h. Deployment and inspection of the CDC-LTs were hampered by the frequent bites of *Ae. albopictus* to the trap operators.

A total of 240 adult mosquitoes were collected in eight out of 10 CDC-LTs. Adult *Aedes* mosquito specimens were identified using morphological characteristics (Schaffner et al. 2001) and a summary of the number and sex of *Ae. albopictus* trapped at each site is given in Table 1. A total of 216 *Ae. albopictus* adults were sampled (169 females and 47 males) with the remaining 24 mosquitoes identified as males and females of *Culex pipiens* L. and *Culex laticinctus* Edwards (Di Marco and Severini, unpublished results).

Table 1. Aedes albopictus *sampled with CDC-LTs in 1-3 September 2015 on Procida island*

Trap No.	Site Name	Coord. N.	Coord. E.	*Aedes albopictus* males	*Aedes albopictus* females	Other Mosquitoes*
1	Edificio Scolastico	40°45’ 39.1”	14°01’ 27.8”	3	68	22
2	Madonna della Libera	40°45’ 43.1”	14°01’ 13.1”	2	7	0
3	Camping_ Punta Serra	40°45’ 38.6”	14°00’ 36.3”	0	0	0
4	Tirreno Residence	40°46’ 05.1”	14°01’ 03.7”	1	15	1
5	Via Faro	40°46’ 09.0”	14°01’ 03.0”	1	2	0
6	Olmo	40°45’ 27.0”	14°01’ 10.3”	37	48	0
7	Chiaio-lella	40°45’ 17.5”	14°00’ 30.7”	0	3	1
8	Hotel Riviera	40°45’ 10.9”	14°00’ 32.3”	2	19	0
9	Camping Vivara	40°45’ 21.2”	14°01’ 02.2”	0	0	0
10	Via dei Bagni	40°45’ 34.3”	14°01’ 19.0”	1	7	0
			Total	47	169	24

* Culex pipiens *L. and* Culex laticinctus

Trap site number 6, named “Olmo”, represents an interesting hot spot of *Ae. albopictus* density as well as a “prototype” of the typical family-type garden on Procida island. This site is comprised of a 0.09 ha garden with cultivation of vegetables and farming of chickens and rabbits. In this garden three big water containers were identified that were utilized for the irrigation of the vegetables. The containers contained hundreds of mosquito larvae of various developmental stages and pupae.

Larvae and pupae were collected, transported alive to the laboratory at Department of Biology, University of Naples Federico II, and reared until adulthood resulting in 57 males and 118 females of *Ae. albopictus*.

Our *Ae. albopictus* record represents the first official report of this species on Procida island and these data are added to the recent record of the occurrence of the Asian tiger mosquito on other six Italian minor islands: Isola del Giglio (Toscana), Ventotene (Lazio), and Ustica, Lampedusa, Linosa and Pantelleria (Sicilia) (Fig. 1B) (Romi et al. 2016; Toma et al. 2017).

Other Mediterranean islands with reported presence of *Ae. albopictus* mosquitoes are the Maltese islands (Gatt et al. 2009) and the Balearic Islands of Mallorca, Ibiza and Minorca (Spain) (Miquel et al. 2013; Barceló et al. 2015; Bengoa et al. 2016).

4. THE PUBLIC SURVEY ON PROCIDA ISLAND OF THE MOSQUITO PROBLEM

During the entomological survey on Procida Island in September 2015, a public survey was likewise conducted to evaluate the perception by Procida inhabitants of the Asian tiger mosquito problem and their interest to support and to participate in area-wide programmes to control this insect. The public survey was a crucial step to start informing citizens about our project and to create the first positive relationship with local people interested and sensitive to the mosquito problem.

We interviewed, using a paper-based questionnaire, 200 randomly selected people (about 2% of the total island population; see Table 2). We obtained a very high participation rate with 200 out of 213 people who accepted to participate in our survey (94%). A list of the questions of the survey and their responses is reported in Table 3.

According to 70% of respondents, the abundance and hence the problem of mosquitoes in Procida has increased in the past 10 years. Most inhabitants spend on average more than one hour a day in green areas or outdoors, and about 50% of respondents were forced to limit the time spent outdoor activities because of the mosquitoes. Seventy-seven percent of respondents were aware of the nature of mosquitoes as human disease vectors and about a quarter of respondents know people who needed medical assistance because of a mosquito bite. Eighty five percent of respondents attempted to limit mosquito bites using electric diffusers, mosquito nets or chemical repellents.

Table 2. Sample classification from the survey of Procida inhabitants on the Asian tiger mosquito problem

Survey sample characteristics (N = 200)				
Gender	Male	Female		
	103	97		
Residence	Local resident	Tourist		
	171	29		
Age	18-39 years	40-64 years	64+ years	
	85	90	25	
Occupation	Unemployed	Employed	Retired	Student
	40	120	26	14

By contrast, very few respondents make active efforts to curtail the proliferation of these insects through the reduction of larval breeding sites (only 3% of respondents use larvicide products to dissolve in water and only 11% remove water containers from their houses or gardens).

Eighty eight percent of respondents were in favour of a mosquito control programme on Procida, although only 44% of people surveyed agreed to the installation of monitoring traps on their private properties. A third of respondents agreed to contribute economically to the project, and 25% of respondents would be interested to commit themselves as volunteers to the realization of the project (contributing from one to 24 hours per week).

5. THE PROCIDA COMMUNITY-BASED APPROACH

Considering the specific features of Procida island and the positive response from locally interviewed inhabitants and tourists, in 2016 we decided to start an *Ae. albopictus* systematic monitoring project, involving the local municipal administration and citizens, with the aim to collect baseline data to fully characterize the Procida site for a future SIT-based area-wide suppression programme.

We developed a multi-step approach plan, to progressively increase the citizen and administrator participation in the project, which includes three main phases:

- Phase 1: Monitoring over one year using ovitraps to define the temporal dynamic of the *Ae. albopictus* population on the island.
- Phase 2: Higher-density ovitrap monitoring to capture the spatial distribution of the mosquito on the island, possibly identifying hot spots.
- Phase 3: Estimation of local mosquito population density by mark-release-recapture (MRR) experiments.

Table 3. Responses to survey questions on the Asian tiger mosquito problem by Procida inhabitants

Survey question	Responses (percentages)			
1. Over the past decade, the mosquito problem in Procida *has*:	increased	decreased	the same	don't know
	70.5	2.0	15.5	12.0
2. Is there a garden or green area in your estate?	Yes	No		
	77.5	22.5		
3. In your estate the presence of mosquitoes is:	abundant	medium	scarce	absent
	35.0	44.5	17.0	3.5
4. How much time do you spend on average every day in a green area/garden?	< 1 hour	> 1 hour	no time	
	18.0	71.5	10.5	
	Yes	**No**		
5. During summer, are you forced to give up outdoor activities because of the mosquitoes?	50.5	49.5		
6. Did It happen that you or any of your relatives and/or friends had to go to the doctor for a mosquito bite?	75.0	25.0		
7. Do you know that the Asian tiger mosquito can transmit viral diseases to humans?	76.5	23.5		
8. Do you use protective measures against mosquitoes?	84.5	15.5		
8a. Do you use electric diffusers?	57.5	42.5		
8b. Do you remove standing water?	10.5	89.5		
8c. Do you use mosquito nets?	53.5	46.5		
8d. Do you use larvicides?	3.0	97.0		
8e. Do you use insect repellents?	44.5	55.5		
9. Would you welcome a regional/municipal mosquito control programme?	88.0	12.0		
10. Would you agree to the installation in your property of traps for the capture and monitoring of mosquitoes?	43.5	56.5		
11. Would you agree to contribute personally to the financing of a mosquito control project?	33.0	67.0		
12. Are you interested in participating, as a volunteer, to a mosquito monitoring and control programme in Procida?	25.0	75.0		

Following a numbered list of planned steps to be carried out, the main results obtained to date are:

1. *Contact with local administration:* Consultations with the Procida major and municipal counsellors to explain the various aspects of the project and to request logistic support from the Procida municipal administration.
2. *Information campaign:* Implemented on the island by the distribution of pamphlets to inform Procida citizen about the project and to invite them to participate.
3. *Active mosquito monitoring by citizens – 1:* A public assembly was organized in collaboration with the Procida municipal administration to select volunteers for the first project phase. Twelve persons, including the major and two municipal counsellors were involved as volunteers in the ovitrap monitoring programme (April 2016-May 2017). These volunteers were trained over a one-week period after which they managed 16 out of 26 ovitraps autonomously over 13 months, reporting weekly mosquito collections (Fig. 2). During the 13 months of monitoring, a total of 44 245 *Ae. albopictus* eggs were collected that were subsequently transported to the Department of Biology of the University of Naples Federico II for counting and species identification.
4. *Media coverage:* In collaboration with the press office of the Procida municipal administration press releases about the project were issued. (http://bit.ly/press_release1; http://bit.ly/press_release2; http://bit.ly/press_release3; http://bit.ly/press_release4; http://bit.ly/press_release5).
5. *Official agreement between institutions:* An official memorandum of understanding (MoU) was signed between the Procida municipal administration and the Department of Biology of the University of Naples Federico II for the implementation of the project on the island.
6. *Crowdfunding campaign:* A crowdfunding campaign was launched to collect funds and to further diffuse the project between Procida inhabitants (http://bit.ly/crowdfunding_procida).
7. *Active mosquito monitoring by citizens - 2:* A second public assembly was organized in collaboration with the Procida municipal administration to select volunteers for project phase 2 from July 2016 to September 2016. We successfully involved 79 families to allow deployment of 101 ovitraps on their private properties all over the island. A total of 40 811 eggs were collected during two weeks in July and two weeks in September 2016.
8. *Passive mosquito monitoring by citizens:* We utilized the mobile application ZanzaMapp (2019) developed at the University of Rome La Sapienza, to involve citizen as well as tourists in the passive monitoring of mosquitoes. An entomological survey was performed on the island at the same time to validate the data obtained by the citizens and tourists using the ZanzaMapp app (September 2016).

Figure 2. Volunteers and researchers during Aedes albopictus *monitoring phase 1 on Procida island.*

9. *Active mosquito monitoring by citizens - 3:* A third public assembly was organized with citizens in collaboration with the Procida municipal administration to select volunteers for project phase 3. We successfully involved 12 families to allow placement in their gardens of ovitraps, CDC-light traps, Biogents BG-Sentinel traps, sticky traps and human landing catches stations to set up optimal conditions to perform a mark-release-recapture test in an area of the island selected as a potential SIT testing site (September 2017).
10. *School involvement:* Procida primary and secondary school students have been involved in a collaborative didactic project aimed at the diffusion of knowledge about Asian tiger mosquito. Students were asked to actively participate in a sanitation campaign to reduce *Ae. albopictus* larval breeding sites in their home properties (October 2017-June 2018).
11. *Active mosquito monitoring by citizens - 4:* Ten volunteers were selected for the management of a permanent mosquito monitoring network on the island using 20 ovitrap and 20 low-cost weather stations (April 2018-May 2019). Volunteers collected eggs weekly and eggs were counted using stereo-microscopes. Data resulting from counting were uploaded by students in an *ad-hoc* online database. The collected eggs were sent monthly to the University of Naples Federico II to validate the counts.

6. CONCLUSIONS

Mosquitoes are considered among the deadliest animals on earth and invasive mosquito species, such as the Asian tiger mosquito, represent a special concern in view of their ability to adapt to new habitats world-wide and to transmit several serious diseases to humans. Managing the public health threat represented by mosquitoes is not only a matter of vector control but also of influencing or modifying public behaviour.

The involvement of civil society in research projects, the so-called citizen science, represents an interesting opportunity in this context to mobilize support, with the possible reductions in working costs, as well as increasing capillarity in the target area. At the same time, it could increase public awareness about the mosquito problem and about virtuous control efforts that could limit mosquito spread (Fig. 2).

Procida Island has a unique combination of key features that makes it an ideal "open-space laboratory" to study the effectiveness of surveillance and suppression methods, including the SIT and similar tools as part of an AW-IVM approach against *Ae. albopictus*. The project on Procida Island began as a pilot study in 2015, with a limited group of volunteers and resources. We successfully involved the Procida administration and about 200 local inhabitants.

Through the action of a dedicated team of full-time professionals and the participation of an increased numbers of volunteers, we started the collection of baseline data about the spatial and temporal population dynamics of *Ae. albopictus*.

We aim in the near future to utilize these collected data, the know-how obtained, and the network of established interactions with citizen and the local municipal administration, to extend the approach to the whole territory of the island. Our long-term and most ambitious objective is to obtain a consistent suppression of the population of this invasive species on Procida to help the local economy, which is mainly based on tourism. Furthermore, we aim to promote the application of a SIT-based mosquito integrated management approach, empowered by active community engagement and participation, on other islands of Campania Region (Ischia and Capri), and eventually on the mainland.

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COMMUNITY ENGAGEMENT FOR *Wolbachia*-BASED *Aedes aegypti* POPULATION SUPPRESSION FOR DENGUE CONTROL: THE SINGAPORE EXPERIENCE

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SUMMARY

Despite a long-running and comprehensive national dengue control programme, Singapore continues to experience regular outbreaks of dengue. The Environmental Health Institute of the Singapore National Environment Agency (NEA) is thus evaluating a *Wolbachia*-based combined Incompatible and Sterile Insect Technique approach (IIT/SIT) as a dengue control strategy. This approach involves field releases of irradiated male *Wolbachia*-carrying *Aedes aegypti* (L.) mosquitoes, with the aim of further suppressing the urban *Aedes aegypti* mosquito population and reducing dengue transmission. Since the beginning of our project, we considered community education and engagement as a key factor for the success of the field studies. We have therefore conducted extensive groundwork to share and consult with, and engage a wide range of stakeholders, including residents at the study sites, the general public, the medical and scientific communities, and government agencies. In this chapter, we outline our strategy for engaging residents at the study sites and the general public (two primary stakeholder groups), focusing on the key principles around which we have built our approach.

Key Words: Mosquitoes, *Aedes albopictus, Wolbachia,* cytoplasmic incompatibility, Incompatible Insect Technique, Sterile Insect Technique, outreach, community education, public mobilisation, high-rise urban environment, public housing apartments

1. INTRODUCTION

Alongside rapid urbanisation and increased global travel, the worldwide incidence of dengue has risen dramatically in recent decades. Today, around 3900 million people in 128 countries are at risk of contracting the disease, with an estimated 390 million annual infections worldwide (Bhatt et al. 2013).

In Singapore, the four serotypes of dengue virus are transmitted between humans, mainly by *Aedes aegypti* (L.), the yellow fever mosquito, with *Aedes albopictus* (Skuse), the Asian tiger mosquito, as a secondary vector. Reflecting the global situation, dengue transmission in Singapore correlates with the presence of *Ae.*

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aegypti, whilst areas with *Ae. albopictus* alone are associated only with isolated dengue cases (Hapuarachchi et al. 2016; Ong et al. 2019).

Singapore's long-running and comprehensive dengue management programme, which has a strong focus on source reduction and environmental management, now maintains the *Aedes* House Index (the percentage of properties in which breeding sites are detected, Ong et al. 2019) at low levels of around 2%. Yet, despite effective suppression of the *Aedes* mosquito vector populations, Singapore continues to experience regular dengue outbreaks, with explosive ones—associated with switching of the predominant dengue virus serotype (Lee et al. 2010) —occurring in 2005, 2007, and 2013-2014.

The continued susceptibility of Singapore's population to dengue outbreaks may be attributed to multiple factors, including: a highly urbanised, high-density, and high-rise environment; reduced herd immunity after decades of low local dengue transmission; transmission outside homes, such as at schools and workplaces; and the presence of cryptic (or unusual) *Aedes* mosquito breeding sites that are difficult to detect (Dieng et al. 2012; Low et al. 2015), amongst others. These challenges, coupled with the absence of approved antivirals and an effective vaccine against dengue, highlight the urgent need for novel and sustainable vector and dengue control approaches.

One new approach, currently being tested in field trials in Asia, the Americas, Australia, and the Pacific islands, involves the release of *Ae. aegypti* mosquitoes carrying the *Wolbachia* bacterium (McMeniman et al. 2009). In Singapore, the Environmental Health Institute (EHI) of the National Environment Agency (NEA) is trialling since 2016 a *Wolbachia*-based combined Incompatible and Sterile Insect Technique (IIT/SIT) approach, involving releases of irradiated male *Wolbachia*-carrying *Ae. aegypti* (*Wolbachia-Aedes*) mosquitoes (Lees et al. 2015; Bourtzis et al. 2016; Dyck et al. 2021). As the eggs resulting from mating between released male *Wolbachia-Aedes* mosquitoes and female urban *Ae. aegypti* mosquitoes are non-viable, the initiative, termed 'Project *Wolbachia* – Singapore', ultimately aims to further suppress mosquito populations and hence the risk of dengue transmission (Liew and Ng 2019; NEA 2019a). Irradiation is used to sterilise the small percentage of female *Wolbachia-Aedes* mosquitoes remaining after sorting mass-reared pupae, thus preventing population replacement stemming from their release (Bourtzis et al. 2016; Lees et al. 2021).

Project *Wolbachia* – Singapore's IIT/SIT approach aligns well with Singapore's long-standing emphasis on mosquito population suppression, and is intended to complement traditional vector control measures, such as mosquito breeding habitat removal, space spraying with insecticides, and entomological surveillance. Existing Singapore legislation provides for NEA to produce *Wolbachia-Aedes* mosquitoes for research purposes, and the approval for releases of these male mosquitoes was obtained at the ministerial level.

Singapore has a high human population density of almost 8000 people per km^2, and more than 80% of the population lives in public housing apartments (SingStat 2019). This makes Project *Wolbachia* – Singapore the first trial in the world to use the technology in such a high-rise, high-density urban environment.

In addition to rigorous laboratory studies and risk assessment, community engagement has been an integral component of Project *Wolbachia* – Singapore since its inception. Since 2012 (well before field releases commenced in 2016), EHI has carried out extensive groundwork to share and to consult with, and engage stakeholders, including residents at the study sites, the general public, the medical and scientific communities, schools and tertiary institutions, and government agencies.

These efforts continue today, as Project *Wolbachia* – Singapore trials advance in stage and scope. As of December 2019, trial releases accompanied by community engagement activities have been conducted in two study sites of 163 and 121 high-rise apartment blocks respectively (Fig. 1), covering a total of 27 600 households. Here, we present an overview of our strategies for dialoguing with residents at the study sites, and the public, with an emphasis on the key community engagement principles that have guided our approach.

2. PROJECT *WOLBACHIA* – SINGAPORE COMMUNITY ENGAGEMENT PRINCIPLES

Community engagement is essential to the testing and future use of novel vector control technologies involving the release of modified mosquitoes. While there are differences in country contexts and technologies used, studies on the design, implementation and evaluation of such community engagement programmes emphasise common principles, such as inclusive public engagement and consultation, transparency, and tailoring the engagement to the local audience (Subramaniam et al. 2012; McNaughton and Duong 2014; Ernst et al. 2015; Kolopack et al. 2015).

To achieve effective and respectful outreach for Project *Wolbachia* – Singapore, we developed a framework for engaging residents and the public based on the principles laid out in this Section. This framework also builds upon existing professional collaborations in research and dengue management, as well as long-standing outreach efforts urging the general public to remove and destroy *Aedes* mosquito breeding habitats in their homes (NEA 2019b).

Whilst this chapter focuses on residents at the study sites and the general public, it should be noted that our community engagement efforts also extend to other stakeholder groups. For example, we conduct scientific seminars, lectures, and workshops to inform and consult the scientific and medical communities about *Wolbachia-Aedes* technology. We additionally consult with international experts to share knowledge and key findings, and also hold seminars and workshops to engage stakeholders in the government, including agencies with functions in public health and the environment. The feedback received from all stakeholder groups has been instrumental in shaping our outreach and deployment strategies.

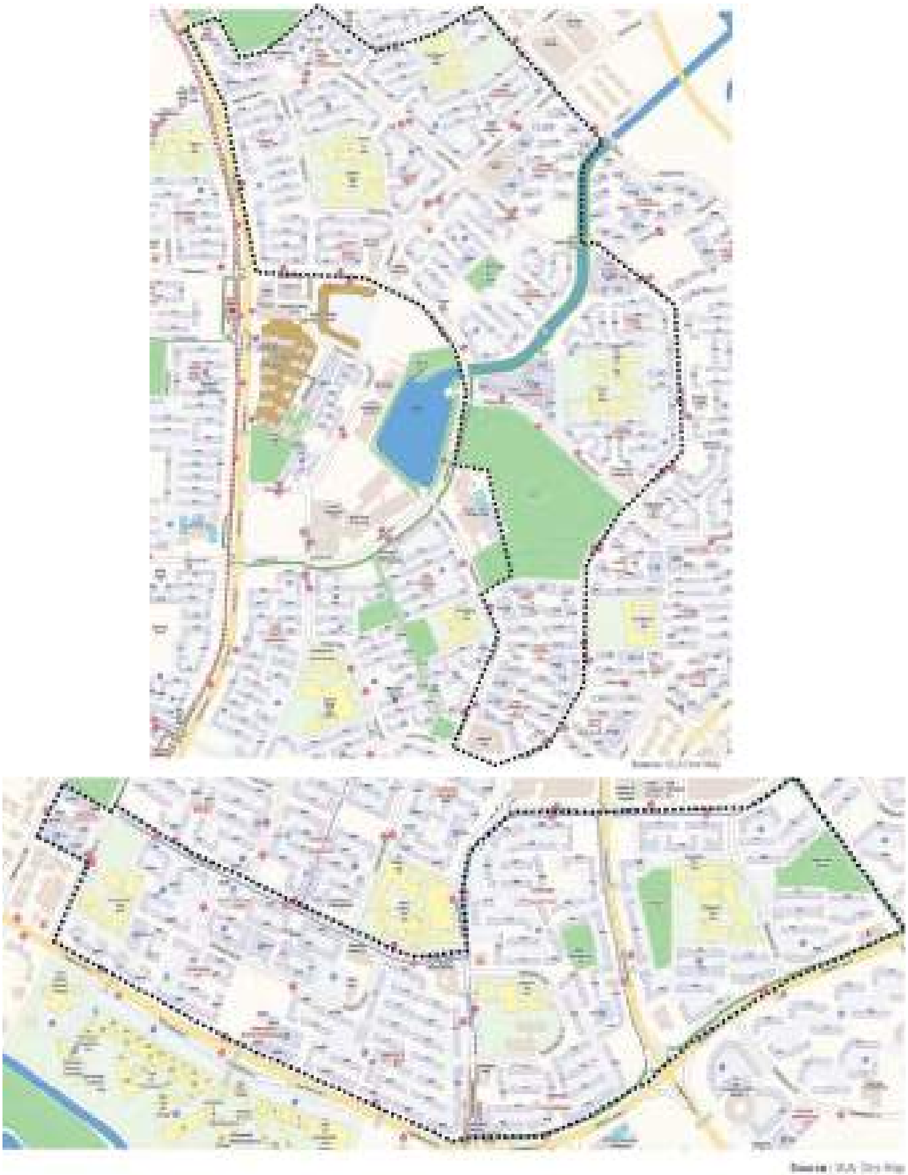

Figure 1. Project Wolbachia *– Singapore study sites as of December 2019. Trial releases of male* Wolbachia*-carrying* Aedes aegypti *mosquitoes, accompanied by community engagement activities, have been conducted in 163 high-rise apartment blocks at Nee Soon East (top) and 121 at Tampines West (bottom), covering a total of 27 600 households.*

2.1. Transparent, Clear and Consistent

Project *Wolbachia* – Singapore involves the release of male *Wolbachia-Aedes* mosquitoes in close proximity to residential dwellings. We thus endeavoured to make our community engagement as accurate and clear as possible, so that residents and the public are well informed about the intervention taking place in their neighbourhoods and the possible effects that this may have on their households.

Importantly, our community engagement got off to an early start. We initiated outreach activities in 2012, in parallel with our laboratory studies and risk assessment of the *Wolbachia-Aedes* technology, well before the first male *Wolbachia-Aedes* mosquitoes were released in 2016. This allowed time for members of the public to familiarise themselves with *Aedes* mosquito biology and behaviour, such as the fact that male mosquitoes do not bite, and with *Wolbachia-Aedes* technology in general. We also had sufficient time to address potential issues raised by stakeholders; for example, we conducted laboratory studies to confirm that male *Wolbachia-Aedes* mosquitoes do not lose the *Wolbachia* bacterium as they age, a concern that was raised by an expert entomologist.

Given that public messaging in Singapore has for decades emphasised the importance of keeping mosquito populations low, the proposed strategy of releasing large numbers of male *Wolbachia-Aedes* mosquitoes may be seen by some as counterintuitive. Thus, we sought to ensure that Project *Wolbachia* – Singapore's purpose and goals—that the mosquito releases, together with existing control methods being applied, are compatible and intended to further suppress mosquito populations—were clearly communicated to the public.

To avoid conflicting messaging, consistency is also key. For example, residents at the release sites are encouraged to remain vigilant and continue practising standard mosquito control activities, such as turning over pails and flowerpots, and clearing roof gutters. Residents are also advised to kill adult mosquitoes as they normally would.

As Project *Wolbachia* – Singapore scaled up and progressed to more advanced stages (NEA 2018a, 2019c), we also delivered prompt updates to keep the public abreast of new developments. These developments included the releases of male *Wolbachia-Aedes* mosquitoes at higher floors, in addition to the releases at the ground floors; collaborations with private sector companies to incorporate technologies such as automated devices for mosquito production, sorting, and release (NEA 2018b); and the use of irradiation post-pupal sorting to sterilise any female *Wolbachia-Aedes* mosquitoes remaining from the rearing process among the males to be released (NEA 2018a).

We have endeavoured to provide members of the public with adequate information, so that they can develop informed opinions on the risks and benefits of Project *Wolbachia* – Singapore. At the same time, we also sought to present this information in a manner that is accessible to individuals without scientific training. Striking this balance is important so that key messages are not obscured by technical details and scientific jargon yet remain accurate.

2.2. Science-based and Educational

Misinformation is a common source of fear and doubt surrounding new technologies. Our community engagement thus aims to demystify the *Wolbachia-Aedes* technology, by equipping the public with a strong understanding of the scientific concepts behind it.

In our outreach materials and engagement sessions, we use accessible language and infographics to explain concepts such as the origin and natural occurrence of the *Wolbachia* bacterium*,* cytoplasmic incompatibility, and how the release of male *Wolbachia-Aedes* mosquitoes will lead to a reduction in *Ae. aegypti* mosquito populations. Project *Wolbachia* – Singapore scientists and technicians were also heavily involved in outreach and were often on-site during door-to-door house visits, dialogue/outreach sessions and roadshows, to answer any questions related to the technology.

Where possible, we also created hands-on experiences for the public to learn about *Wolbachia-Aedes* technology. For example, participants at our roadshows and dialogue/ outreach sessions were encouraged to place their hands into transparent Perspex boxes containing male *Wolbachia-Aedes* mosquitoes, so that they could experience for themselves that male mosquitoes do not bite. We also organise regular tours of NEA's *Wolbachia-Aedes* mosquito production facility (Fig. 2), where members of the public learn how mosquitoes are reared, sorted, and prepared for release.

Figure 2. Environmental Health Institute (EHI) researchers conducting a tour of the National Environment Agency's (NEA's) Wolbachia-Aedes *mosquito production facility for members of the public, during a Project* Wolbachia *– Singapore learning journey.*

As children and the youth community are often readily engaged and may also proactively help convey information to their families, we also hold sharing sessions at schools and tertiary institutions. Additionally, we have been engaging childcare centres (Fig. 3), and have been working with schools located within the release sites, to provide hands-on lessons related to Project *Wolbachia* – Singapore, including the release of male *Wolbachia-Aedes* pupae around school premises, and the identification of mosquitoes trapped by Gravitraps.

Figure 3. A Project Wolbachia *– Singapore show-and-tell session for young children at a childcare centre.*

Such citizen science initiatives have increased scientific understanding, and, by allowing participants to contribute directly, have helped build acceptance and a sense of ownership for the project.

2.3. Comprehensive and Inclusive

Given the need to reach out to people with diverse backgrounds, needs, and concerns, we set out to make our engagement programme as comprehensive and inclusive as possible. To reach a greater number of target groups, we employed multiple tiers of communication strategies, with varying levels of detail and engagement.

Informational brochures, distributed to all residents and stakeholders at study/ release sites (Fig. 4), are used to convey short, key messages explaining the *Wolbachia-Aedes* technology and addressing what residents can do to help keep mosquito populations low.

Figure 4. Informational brochures for distribution to residents and all stakeholders at the study/release sites.

These brochures use accessible language and infographics, and, given Singapore's multicultural landscape, are available in English, Mandarin, Malay and Tamil - the country's four official languages (Fig. 5).

More detailed information on the technology, releases, and trial results was made available through media releases (NEA 2018a, b, 2019c, d, e) and features in mainstream media (Co 2019; The Straits Times 2019), as well as via social media and other public communication channels (e.g. documentary features) (National Geographic Asia 2019). Members of the public interested in learning more can also visit NEA's *Wolbachia* website (NEA 2019a) for technical details, including scientific literature.

Besides disseminating information, we also conduct numerous face-to-face engagement activities, to engage residents more thoroughly and better understand their concerns. These activities include educational sessions in schools and pre-schools, door-to-door house visits, coffee chat sessions, apartment block and garden parties, roadshows in locations with heavy footfall (e.g. marketplaces, community centres, and shopping malls) (Fig. 6), and mosquito production facility tours.

‘Project *Wolbachia* – Singapore’ progresses to
Phase 3 field study in February 2019

‘Projek *Wolbachia* – Singapura’ akan melangkah ke kajian lapangan
Fasa 3, bermula Februari 2019

新加坡沃尔巴克氏体计划将在2019年
二月步入第三阶段的实地研究

‘சிங்கப்பூரின் ஓல்பாக்கியா திட்டத்தின்’ மூன்றாம் கட்ட கள
ஆய்வு பிப்ரவரி 2019 முதல் தொடங்கவிருக்கிறது

Figure 5. A brochure for Phase 3 of Project Wolbachia *– Singapore, with information translated into Singapore’s four official languages: English, Mandarin, Malay and Tamil.*

Figure 6. Outreach booths at public events/roadshows to showcase Project Wolbachia *– Singapore. Top: At a shopping mall. Bottom: At a community centre, with Singapore's Minister for the Environment and Water Resources, Mr. Masagos Zulkifli (far right).*

At the time of writing, we have held more than 100 public engagement sessions to raise awareness, answer queries, and solicit feedback about Project *Wolbachia* – Singapore. To further extend the reach of our message, we engaged members of the public who are interested to help out as NEA Dengue Prevention Volunteers (DPVs), and who can then help to convey accurate information on *Wolbachia-Aedes* technology to their own networks within the community.

Leveraging the increasing pervasiveness of digital media, we used websites, social media platforms, and videos (NEA 2019f, g, h) to complement our face-to-face outreach activities and increase exposure. As Project *Wolbachia* – Singapore scales up and the number of affected members of the public involved in the project increases, we foresee digital media playing an even larger role in our community engagement strategy.

In developing activities to engage different groups and individuals, we relied strongly on our own local knowledge and experience gained, as well as that of leaders and residents in the community. At the same time, over the course of our outreach, we also learned through experience which engagement methods are the most effective and adjusted our strategy accordingly. For example, after one dialogue session with poor attendance, we shifted our strategy towards bringing our roadshows to where people are, rather than getting residents to go to a specific dialogue.

2.4. Consultative and Responsive

To promote Project *Wolbachia* – Singapore, an important scientific initiative that may result in tangible public health benefits, we adopted a consultative approach early on to respect the concerns and opinions of the public, taking seriously any feedback received from residents, members of the public, experts, and other stakeholders. Valid concerns (such as safety of the technology, potential negative ecological impacts, niche replacement by other mosquitoes, and unintentional release of female mosquitoes) were channelled into our risk assessment track, a parallel effort that has resulted in the publication of a risk assessment of the technology (Ng et al. 2017; NEA 2019g).

Through house visits, roadshows, and dialogue sessions, we actively solicited feedback from residents and the public. We also consulted community or grassroots leaders, who have strong networks on the ground and a thorough understanding of residents' concerns. Following detailed engagement sessions to explain *Wolbachia-Aedes* technology and understand and address concerns, we were able to obtain leaders' support, approval and advocacy. The spectrum of views and concerns we received informed our release and engagement strategies later on.

We encouraged the community to learn more about the project and have also established mechanisms for the public to pose queries, voice concerns, and report incidents related to Project *Wolbachia* – Singapore. Feedback can be submitted via email, telephone hotline, an online reporting system, or verbally to our field officers. Our site managers also carry a dedicated mobile phone by which residents can reach them.

Our officers follow up on feedback received by contacting each case through house visits, telephone calls, or email, to learn more about and address their individual situations. During such follow-ups, we engage the respondents with information about Project *Wolbachia* – Singapore, as well as attempt to address the issues raised. For example, a few residents who reported increased irritation from male mosquitoes were advised to keep their windows and doors closed, or to kill the mosquitoes as they normally would. If residents report bites, field officers are typically deployed to inspect the area for mosquito breeding sites.

Whilst these mosquitoes resulting from local breeding sites are unrelated to the release of male *Wolbachia-Aedes* mosquitoes, such feedback from residents has enabled us to find and eliminate mosquito breeding sites, an outcome that is consistent with the aims of Project *Wolbachia* – Singapore.

By respectfully engaging dissenting views and responding promptly to public feedback, we hope to improve the ability of the public to make informed opinions, thereby enhancing acceptance of Project *Wolbachia* – Singapore.

3. IMPORTANCE OF SITUATIONAL AWARENESS

Like most field studies, trials of *Wolbachia-Aedes* technology may be affected by environmental factors such as the presence of mosquito breeding habitats, temperature and rainfall variations, and imported mosquito-borne infections. Proper situational awareness is therefore important for community engagement efforts to swiftly address concerns from the public when they arise.

For example, a coincidental rise in the populations of other mosquito species (such as *Culex quinquefasciatus* Say) in the community, and a resulting increase in bites experienced, could lead to public doubts concerning Project *Wolbachia* – Singapore. In such cases, data on the population trends of the other mosquito species, together with an understanding of their biting behaviour and the detection of their breeding locations on site, could provide the evidence needed to be able to dissociate these experiences from the project.

Dengue cases may also occur within study sites, complicating the messaging that Project *Wolbachia* – Singapore suppresses the dengue-transmitting mosquito population and hence potentially also the risk of disease transmission. A good analysis of the situation within and around each of these sites is thus critical, as most of these reported dengue cases may have resulted from infections outside the release sites — perhaps at school or at work— but were tagged to residential addresses within the sites.

Thus, our community engagement team is working closely with field officers to gather and respond to all information received on mosquito populations, mosquito sightings, and dengue cases within and around study sites, with the aim of assessing the cause of all issues raised by the community.

4. FUTURE DIRECTIONS AND CONCLUSION

Thanks to early and comprehensive community engagement, nationwide online and face-to-face street surveys conducted in 2016 (prior to the launch of Project *Wolbachia* – Singapore) found high levels of acceptance for *Wolbachia-Aedes* technology, with a high number of respondents indicating that they had no concerns with the release of male *Wolbachia-Aedes* mosquitoes in their neighbourhoods. As Project *Wolbachia* – Singapore scales up (NEA 2019c), an ongoing challenge will be to expand community engagement accordingly, whilst still maintaining its quality. Whilst face-to-face interactions will remain a mainstay of our engagement strategy, this is highly labour-intensive; in the future, engagement through digital and social media will be scaled up to reach larger segments of the population. To maintain quality and impact, it will be critical to continually evaluate our community education and engagement strategies, and to modify them when necessary (Jayawardene et al. 2011; Healy et al. 2014). To this end, Project *Wolbachia* – Singapore as a whole, including the community engagement component, is regularly reviewed by an external panel comprising local and international experts (NEA 2019c).

We will also continue to conduct population surveys to ascertain public awareness, perceptions and attitudes towards Project *Wolbachia* – Singapore, which will allow us to further fine-tune our outreach and deployment strategies. Prior to the release of male *Wolbachia-Aedes* mosquitoes, a nationwide online survey conducted in 2016 revealed that 94% of the population had no objections to the release of male *Wolbachia-Aedes* mosquitoes in their neighbourhoods, and a face-to-face street survey targeting older respondents (aged 40 years and above) revealed similar results – 89% had no objections to such releases in their neighbourhoods, and 31% had heard of the project through mainstream media. During the Phase 1 field study in 2016, a survey conducted showed that more than 70% of the households interviewed had heard of our project, and more than 90% had no concerns with the releases.

In summary, community engagement is increasingly recognised as a critical dimension of biomedical and global health research, as well as the social sciences, especially where novel technologies such as *Wolbachia*-based control methods are concerned. We believe that respectful, impactful community engagement is crucial for the success of *Wolbachia*-based technologies and is recognised by senior leadership as a key project priority.

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SECTION 5

NEW DEVELOPMENTS AND TOOLS FOR AREA-WIDE INTEGRATED PEST MANAGEMENT PROGRAMMES

TECHNICAL INNOVATIONS IN GLOBAL EARLY WARNING IN SUPPORT OF DESERT LOCUST AREA-WIDE MANAGEMENT

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SUMMARY

Technical innovations can play an important role in the effective management of transboundary pests if they are well integrated with participation and collaboration by affected countries and coordinated by a centralised body. This is particularly relevant to those migratory pests that can easily and rapidly move across regions and continents to simultaneously threaten food security and livelihoods in numerous countries. Innovations to FAO's successful desert locust global monitoring and early warning system are highlighted and the lessons learned can be applied and adapted to other emerging transboundary threats such as the fall armyworm in Africa and the red palm weevil.

Key Words: Schistocerca gregaria, transboundary plant pests and diseases, remote sensing, surveillance, forecasting, early warning, geographic information systems, unmanned aerial vehicles

1. INTRODUCTION

Transboundary pests are migratory insects and disease vectors that easily move from one country to another and can rapidly traverse regions and travel great distances to threaten crop production throughout the world. While the desert locust *Schistocerca gregaria* Forskål is probably the most well-known and best studied of the migratory insect pests, there are other notable transboundary pests such as the migratory locust *Locusta migratoria* (L.), the Moroccan locust *Dociostaurus maroccanus* (Thunberg), and Italian locust *Calliptamus italicus* (L.), as well as the red palm weevil *Rhynchophorus ferrugineus* (Olivier) and the fall armyworm *Spodoptera frugiperda* (Smith) that are becoming an increasing threat to agriculture and livelihoods in Africa and Asia (Fig. 1).

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The desert locust is an ancient insect, dating from millions of years and coexisting with early man until the advent of cultivation, when it became what is considered today as the world's most dangerous migratory insect pest. The unique behaviour of the desert locust allows it to quickly take advantage of optimal environmental conditions by rapidly increasing in number and forming highly migratory swarms that can affect some 20 percent of the earth's land surface and livelihoods of millions of people in Africa, the Middle East and Asia.

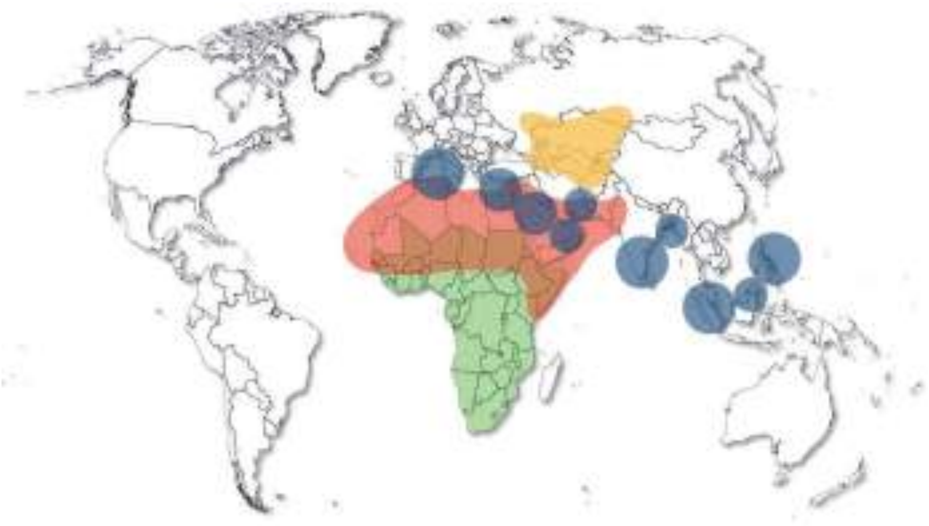

Figure 1. Major transboundary pests in Africa and Asia: Desert locust distribution area (red), fall armyworm (green), red palm weevil (blue), and migratory / Moroccan / Italian locusts in Central Asia (yellow) (source FAO DLIS).

It was not until the early twentieth century that efforts began in earnest to systematically monitor the pest across its vast desertic habitat stretching from West Africa to India, consisting of some of the world's driest and remotest areas. In 1943, the Desert Locust Information Service (DLIS) was established in the UK, which was the basis for the future global forecasting and early warning system. The DLIS was responsible for the systematic collection and mapping of desert locust infestations so that seasonal breeding areas could be identified, and a better understanding could be gained about the formation of swarms and their migratory patterns. In 1978, FAO assumed the centralised responsibility of the DLIS.

1.1. Desert Locust Biology and Behaviour

Low numbers of isolated desert locust are present somewhere within its vast habitat throughout the year. This area includes about 30 countries and covers some 16 million km^2 of desert in North Africa, the Arabian Peninsula and south-western Asia (Fig. 2).

The individualistic solitarious adults and hoppers (the nymphal stage) are well-camouflaged to blend in with their environment as a means of protection from predators. The adults are passive fliers at night, drifting up to 400 km downwind. Often the winds bring the adults into areas of recent rainfall, which wets the sandy soil sufficiently for females to lay eggs that hatch after about two weeks. The resulting hoppers shed their skins (moulting) on about a weekly basis six times before becoming an adult. The entire lifecycle lasts about three months, but may last up to a half year, as adults may remain immature for months in low temperatures or in the absence of regular rains. Desert locust do not have a dormant stage, they do not overwinter, and eggs cannot survive from one year to the next.

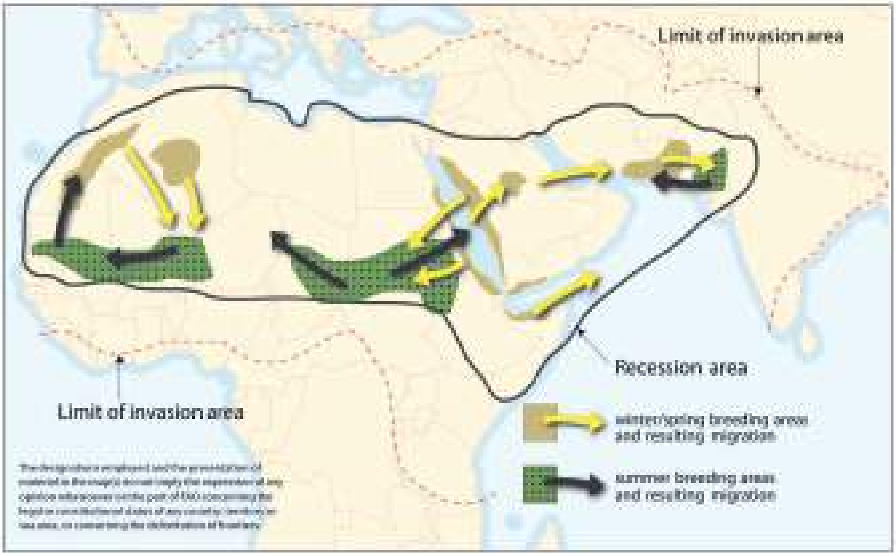

Figure 2. Desert locust recession and invasion areas, and their seasonal migration patterns (source FAO).

Locusts will quickly take advantage of exceptionally heavy and often short-lasting rainfall, whether localized or widespread, that cause ecological conditions to become favourable for breeding and survival. Once annual vegetation dries out, locusts tend to concentrate in those areas that still remain green, increase in density and form small groups that may fuse together and develop into hopper bands and adult swarms. This behavioural phase change is referred to as gregarisation. Swarms fly during the daylight hours, moving more than 200 km in a single day. This allows them to easily traverse Africa and to cross water bodies such as the Red Sea, the Mediterranean Sea and the Arabian Sea. They also can extend further north, south and east from their normal habitat and invade several dozen countries (Fig. 2). In October 1988, swarms migrated some 5,000 km across the Atlantic Ocean from West Africa to the Caribbean (Rosenberg and Burt 1999).

A single desert locust is able to consume its own weight, approximately 2 grams of food every day. A 1 km^2 sized swarm generally contains about 40 million adults (Pedgley 1981). The high number of locusts and their voracious appetite can pose a serious threat to agriculture and food security. To put this into perspective, a swarm the size of Bamako, Mali will eat the same amount of food in one day as half the entire population of Mali. Similarly, a swarm the size of Vienna will consume the same amount of food in one day as everyone in Austria and Switzerland, while a swarm the size of New York City will eat as much food in a single day as all the residents in the states of New York and Pennsylvania.

Desert locust plagues do not develop overnight. Instead, they evolve from a serious of events in which locust numbers steadily increase. It starts with a calm period of recession, when locusts are normally present at low densities in semi-arid or arid areas, causing no significant crop damage, and hopper bands and swarms are absent. This is followed by localized outbreaks that may cover only a few hundred square kilometres within a single country. If an outbreak is not detected or controlled and if good rains continue to fall, then the outbreak could increase in size and expand into several neighbouring countries, leading to an upsurge. Similarly, if an upsurge is not controlled and rains continue, then a plague could develop on a regional, continental, or global level. Normally, gregarisation occurs after at least two successful generations of breeding. Several more successive generations must take place before the majority of the populations consist only of hopper bands and adult swarms. Therefore, it takes at least one year for a plague to develop. On the contrary, a plague can collapse very rapidly in a matter of months due to effective control, unusually low temperatures and a lack of rainfall.

Desert locust recession and plague periods occur irregularly in response to the sporadic nature of rainfall in the desert. Since 1860, there have been nine plagues and major upsurges that were interrupted by periods of recessions and localised outbreaks (Symmons and Cressman 2001). These lasted from several months to several years or more (Fig. 3).

The last major upsurge or regional plague occurred in 2003-2005. In addition to an unusually cold winter, it took nearly USD 600 million and 13 million litres of insecticide sprayed by ground and aerial campaigns to bring the plague under control (Brader et al. 2006). In West Africa, more than 8 million people were affected, up to 100% of cereal crops were lost in some areas, and some 60% of household heads became indebted in Mauritania, while 90% of households in Burkina Faso received food aid.

1.2. Climate Change Impact

Changes in the climate will affect desert locust habitats, breeding, migration, and plague dynamics. It is well known that the current climate change is causing temperatures to increase. Warmer temperatures will extend the length of the summer, winter and spring breeding periods and allow desert locust eggs and hoppers to develop faster as long as this is associated with a continuation or increase of good rains. This is likely to be most pronounced during the winter and may allow an extra generation of breeding to take place (Cressman 2013).

Warmer temperatures could also potentially affect desert locust migration by allowing solitarious adults to fly longer during nights, especially during the colder portions of the year. Consequently, adults may arrive at a destination sooner or reach new areas further away. Warm temperatures could allow swarms to take off earlier in the morning, resulting in a longer period of flight during the day and a greater displacement distance. In other words, swarms could reach new places that have not been reachable up to now. Climate change could also allow swarms to fly higher than 1800 m, which is the general limit of flight due to temperature. If this is the case, then the Atlas Mountains in north-western Africa, the Hoggar Mountains of Algeria, the Jebel Akhdar Mountains in northern Oman, the mountains in the interior of Iran, and the mountain ranges along both sides of the Red Sea may no longer be natural barriers that impede migration. On the other hand, if warmer temperature regimes were to become extremely hot, for example above 50°C, then desert locust presence and survival could become limited in some areas of the Sahara and the Arabian Peninsula (Meynard et al. 2017).

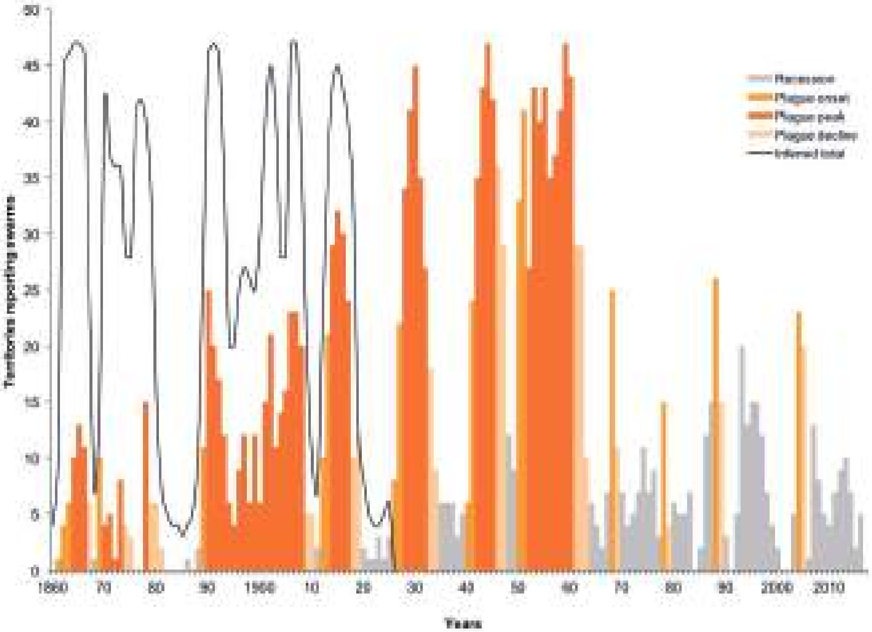

Figure 3. Desert locust plagues and recessions between 1860 and 2017 (source FAO DLIS).

The impact of climate change on the spatial variability of rainfall in desert locust breeding areas remains unclear. There is some evidence that rainfall could increase during the summer in seasonal breeding areas of the northern Sahel in West Africa and in the Yemen interior that could cause locust numbers to increase faster than normal. There appears to be more widespread agreement concerning an increase in extreme rainfall events.

It is worthy to note that unusually heavy rains have been responsible for locust plagues in the past, such as a cyclone in 1968 in Oman that caused a plague the following year and widespread heavy rains from Senegal to Morocco in October 2003 that led to a regional plague for the next two years.

The effects of wind are less certain, but any changes in wind speed, direction and circulation flows are likely to affect desert locust migration and could allow adults and swarms to reach new areas. Warmer temperatures in combination with shifting wind patterns and decreased rainfall could permit new migration routes into Southern Europe and Central Asia. In general, however, further research is required to better understand the impacts of climate change and variability on the desert locust population dynamics and migration.

2. MONITORING AND EARLY WARNING

Locust-affected countries and FAO have adopted a preventive control strategy for the area-wide management of desert locust in order to reduce the frequency, duration and intensity of plagues (Fig. 4). Successful preventive control requires effective early detection and warning, rapid response, good communications and contingency planning. The former consists of monitoring weather, ecological conditions and desert locust populations on a regular basis throughout the vast recession area that stretches from the Atlantic coast in West Africa to western India. This is accomplished through observations made by ground teams during survey and control operations that are recorded and sent to the national locust control centres and, from there, to FAO's centralised global system operated by the DLIS in Rome (Fig. 5).

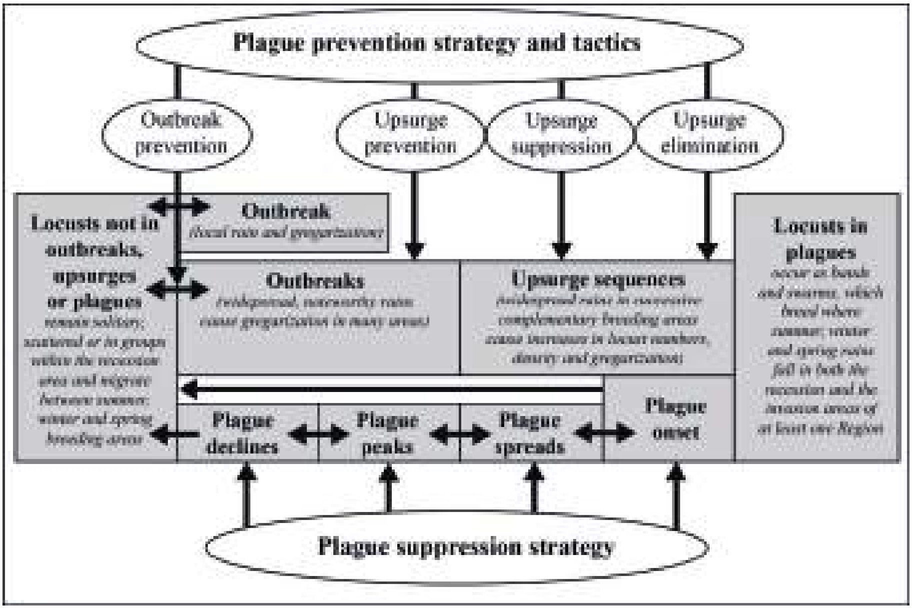

Figure 4. Desert locust preventive control strategy (source Magor et al. 2007).

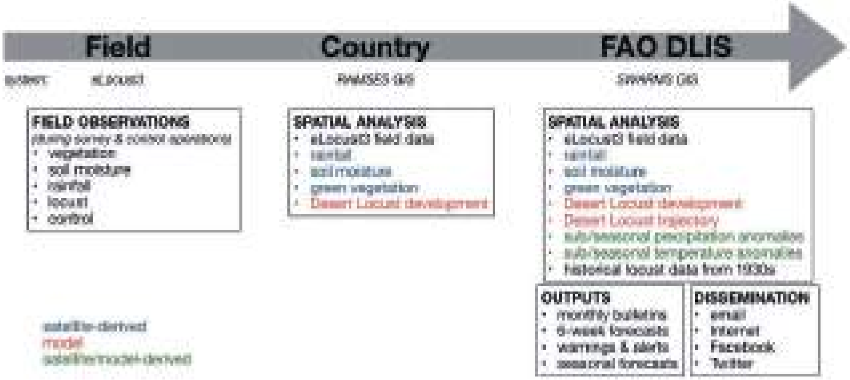

Figure 5. Information flow in the FAO global desert locust early warning system (source FAO DLIS).

Each locust-affected country is responsible for monitoring and controlling desert locust in its own territory. In most countries, a dedicated national locust centre within the Ministry of Agriculture and Plant Protection Department assumes these responsibilities. Specialized teams undertake ground surveys using four-wheeled-drive vehicles in the desert, looking for annual vegetation that may be green and harbouring desert locust hoppers or adults.

If important infestations are detected, then control operations are immediately implemented to prevent the locust population from increasing further and spreading. The obvious challenge is to find and treat these relatively small localities within a vast and remote area that is devoid of infrastructure and inhabitants. This is further complicated by increased conflict and insecurity in many places, preventing national locust teams from undertaking the necessary survey and control operations. The DLIS plays an important role to guarantee a coordinated action amongst countries during periods of simultaneous outbreaks and the presence of swarms in more than one country.

In the past, early warning was hampered by difficulties in accessing remote desert locust habitats and sending timely information. Although tremendous efforts were made to systematically map desert locust infestations and analyse changes in population dynamics, this was often hampered by information that usually took weeks or months to arrive from the field. As a result, it was impossible to provide advice for decisions to be made to allow responding on time.

An effective early warning system for any transboundary pest relies on the transmission of geo-referenced data in real- or near real-time from the field to a centralised collection point where data can be analysed rapidly in order to provide timely and meaningful advice for decision makers. Timeliness becomes even more essential when dealing with a transboundary pest such as the desert locust that has the ability to rapidly increase in number and form swarms that can quickly move from one area to another.

3. INNOVATIONS

The present state of the global FAO desert locust monitoring and early warning system is a direct function of continuous innovation. It has evolved alongside advances in communication, computer and spatial technologies during the past several decades.

3.1. Advances in Communications, Geographic Information Systems and Transport

At the beginning of the last major desert locust plague in 1987, telex was the primary communications means for countries to transmit data and reports from the field to the DLIS in Rome. Similarly, FAO used telex to send advice, warnings and monthly bulletins and forecasts. By the end of the plague in 1989, telex had been replaced by facsimile. Although this was an improvement because additional information formats such as tables and photos could be transmitted, it remained somewhat limited since not everyone had a fax machine. These limitations were overcome with the introduction of email in the mid-1990s and its widespread use from 2000 onwards. This was further expanded as a greater number of individuals began using personal computers and mobile phones.

Until the mid-1990s, field data were plotted and analysed manually using paper maps, transparencies and coloured pencils. This tedious and labour-intensive system was replaced by custom-made geographic information systems (GIS) to allow rapid mapping and detailed spatial data analysis. The *Schistocerca Warning and Management System* (SWARMS) in the DLIS was one of the first uses of GIS for operational monitoring rather than production of one-time static maps (Healey et al. 1996). In 2000, FAO developed a standard custom GIS, *Reconnaissance and Management System of the Environment of Schistocerca* (RAMSES), for locust-affected countries to manage and analyse their own survey and control results with remote sensing imagery and historical data. In 2014, RAMSES was redesigned as open-source software with a spatial database.

The remote locust habitats in the desert have become progressively easier to access in most countries due to improvements in transport and infrastructure. Four-wheeled-drive vehicles have replaced camels that were used by locust survey and control teams in the past. A proliferation of tracks and roads in the desert associated with increased development allow access to a greater number of places in a shorter amount of time. In this way, survey teams can monitor a larger area with the same amount of resources.

Even though countries could send and receive information faster and ground teams could reach desert locust habitats easier, the field teams were unable to transmit high quality data in real-time from the location of the survey or the control operations, often in the middle of nowhere, to national locust centres and the DLIS. FAO addressed this issue by developing a custom tool, *eLocust*, that is a rugged handheld tablet for recording field observations and sending them by satellite in real-time to the national locust centre. This was introduced in 2006 and it revolutionized desert locust early warning overnight. Suddenly, weather, ecological, locust and control data were available within a few minutes from anywhere in the desert between West Africa and India. National locust directors now could know at any given time the exact location of every field team and results of the survey and control operations.

An upgraded version in 2015 of the custom tool, *eLocust3*, allowed users in the field to enter more survey and control data, navigate to potential areas of green vegetation without the need for internet connectivity, and take photos and videos of the situation.

3.2. Satellite-based Remote Sensing

Despite such advances in communications and transport, the desert continues to be huge and vast. It remains unattainable for any single locust-affected country to have sufficient resources to scour each and every hectare in search of desert locust. Therefore, all efforts must be made to somehow delimit the large areas that need to be searched and prioritise them to those that have the greatest potential of containing important locust populations. Satellite-based remotely sensed imagery is routinely used to help guide field teams to such places. This is undertaken in a systematic manner by first determining those regions or areas where rains may have fallen by using satellite-based rainfall estimates. While model-derived estimates may be more accurate in terms of rainfall quantity, satellite-based estimates are a better spatial indicator of rainfall (Dinku et al. 2010).

Once a region of possible rainfall has been identified, then multi-temporal and multi-spectral image analysis that exploits the mid-infrared, near-infrared and red wavelengths is applied to daily observations from NASA's 250 m resolution Moderate-Resolution Imaging Spectroradiometer (MODIS, Aqua and Terra) to determine if there was a response of annual vegetation to the rainfall (Pekel et al. 2011). This is the vegetation required by desert locust for food and shelter. An automatic processing chain combines the daily imagery into a ten-day dynamic vegetation greenness map that shows the three-month greenness history of each 250 m pixel in order to monitor the development of green vegetation in those areas that received rainfall or runoff (Fig. 6). These maps are used by locust-affected countries to position and prioritize surveys and by FAO DLIS for analysis and forecasting. They can also be used by teams that are equipped with *eLocust3* to help navigate to green areas in the field.

The internet has become the *de facto* means of delivering imagery and other data to analysts and decision-makers. The DLIS is constantly seeking new ways to improve the timely distribution of remote sensing products. While some delays may be due to satellite reception, there are other delays that are attributed to processing the data into map products. The latter is being actively addressed by utilizing Google Earth Engine technology whereby the user can process the image online in less than a few minutes by taking advantage of parallel and cloud computer technologies. This on-demand system is not only faster but may be more sustainable in the future compared to traditional processing and delivery chains.

3.3. Unmanned Aerial Vehicles for Surveillance and Focused Control

In the past few years, unmanned aerial vehicles (UAVs or drones) have become increasingly available for public use, and the technology is rapidly improving and expanding to new fields of use. The use of fixed- and rotary-wing drones could potentially improve desert locust monitoring, early warning and rapid response control, while reducing the costs of survey and control operations. Drones could supplement current tools utilized for monitoring in order to help guide ground teams to green vegetation and locust infestations. For example, the latest satellite imagery would be analysed to identify regions or areas within a country where ecological conditions may be favourable for locusts, specifically, where recent rains have occurred and where green vegetation may be present.

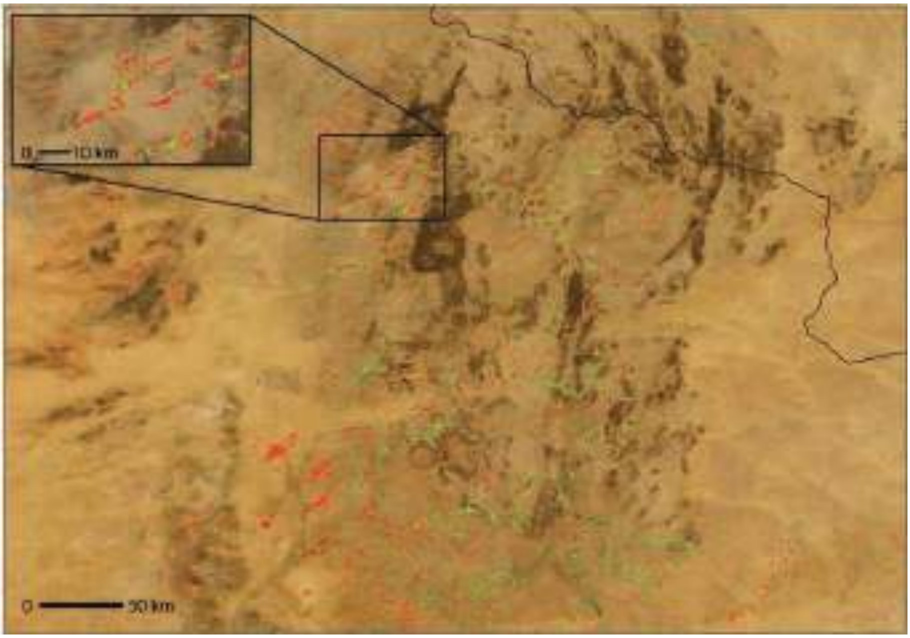

Figure 6. A 10-day dynamic NDVI map for 1–10 September 2017 indicating where annual vegetation in northern Mali has become green within the last 10 days (red), 20 days (orange) and 30 days (yellow) along dry river beds (wadis) in between the Adrar des Iforas hills. Green and darker colours indicate vegetation that has been green for several months, probably perennial vegetation such as trees, oases and forests. Each pixel is 250×250 m (0.0625 km^2) (source FAO DLIS).

This initial filtering will help to reduce the large and vast areas that need to be checked by ground teams. A team would then be deployed to this area to undertake surveys. The team would be equipped with a small, portable long-range fixed-wing drone. This drone should cover a radius of about 50-100 km. The team would programme the route itinerary of the drone and launch it. The drone would capture and process information along the route using optical and/or hyperspectral sensors to detect areas of green vegetation and moist soil, as well as the presence of any sizeable hopper or adult concentrations (groups, bands, and swarms). Once the drone returns to the survey team, the processed data would then be transferred to *eLocust3* and the team would use the results to go directly to the areas of interest or change direction if the results of the flight do not indicate the presence of favourable conditions for desert locust.

A ground team could also carry a small portable rotary drone with them during surveys to a specific location identified from above or an area that may contain vegetation or locusts. The drone would help the team get a better idea of the ecological conditions and the locust situation by taking low-level images of the area to identify the presence of green vegetation and locusts. If the team stops in an area with green vegetation or crops, the drone could look *in situ* for locusts and also determine the size of the potentially infested area. If the location is less precise, then the team could launch the rotary drone to look for any signs of green vegetation or favourable breeding areas within a 5 km radius. The drone could also be used to collect information from areas that are not accessible to the ground team due to topography or insecurity.

Lastly, a rotary drone could be used to undertake targeted control treatments of small infested areas and in areas that are difficult to access by ground teams (Benavente-Sánchez et al., this volume). This is highly desirable and advantageous because it makes control operations much safer and more precise. Field officers would avoid coming into contact with the chemicals as drones would be doing the spraying. Spot control would involve spraying only the specific locust infestation rather than treating the entire area, thus reducing pesticide usage and protecting the environment.

3.4. Forecasting the Time and Scale of Breeding and Early Warning Systems

The innovative tools mentioned up to this point are primarily utilized for directing national locust field teams in managing the current situation. Another set of technologies are exploited for locust prediction that involve the adaptation of cutting-edge methodologies to operational monitoring and forecasting.

Remote sensing imagery has traditionally been used for monitoring rainfall and vegetation. More recently, a new product has been developed to monitor ground soil moisture down to 10-15 cm below the surface. This moisture is critical for breeding to allow egg-laying and hatching, as well as the growth of annual vegetation necessary for locust feeding and shelter. Similar to other satellite-derived products, soil moisture maps are updated every ten days. This facilitates comparative analysis with rainfall vegetation imagery. The product will be another field tool to help delimit the large areas to be surveyed by guiding teams to specific places where breeding may be underway.

The DLIS utilizes seasonal predictions of monthly precipitation and temperature anomalies that are issued six months in advance and updated every month. These maps can help to forecast the timing and scale of locust breeding several months ahead, giving extra time for national locust programmes for planning and pre-positioning resources. Sub-seasonal predictions for up to four weeks in advance and updated twice a week can be used for short-term advice and warnings to assist in managing operations, especially during control campaigns. Both products are derived from several sophisticated weather models and may vary considerably from month to month. Hence, they can be less reliable at times and must be interpreted with caution.

At present, two models are used in desert locust early warning, an egg and hopper development model and a trajectory model. The former estimates the time required for egg and hopper development based on location. This is useful for forecasting the timing of breeding and planning field operations. The trajectory model is used to estimate the displacement of locust adults and swarms forward and backward in time. The model relies on meteorological data such as wind speed and direction, pressure and temperature provided by the European Centre for Medium-Range Weather Forecasts (ECMWF) every 12 hours for the desert locust recession area (Fig. 2.). This allows the forecaster to select the height or temperature level of flight and, by indicating the take-off or landing date and time, estimate where the adults or swarm came from or will go to.

4. SUCCESSES AND LESSONS LEARNED

The global desert locust early warning system operated by FAO is one example of successful area-wide pest management. Outbreaks, the precursors to upsurges and plagues, are now easier to detect and respond to on time due to improvements in monitoring and early warning (Fig. 4). This has resulted in a significant decrease in the duration, intensity and frequency of desert locust plagues. As mentioned previously, there were nine major plagues and ten major upsurges from 1860 to 2000, some of which lasted up to 14 years and affected 50 or more countries. Since 2000, there has been only one major upsurge and no plagues (Fig. 3).

This success can be attributed to a number of important factors. As the desert locust is such a well-known and old pest with a long history, countries are exceptionally aware of its importance and potential threat. This high visibility facilitates national interest at all levels and helps to engage the relevant stakeholders. Locust-affected countries understand the transboundary nature of the desert locust and, hence, willingly engage in joint monitoring and area-wide control activities as well as development projects to strengthen national capacities. Regardless of political relations, strong networks exist amongst countries that contribute to the regular and timely sharing of high-quality data and exchange of reliable information. Without this, an early warning system would not be possible.

The latest technologies are utilized and adapted in an innovative manner for the development of useful and well-focused tools that can be used by those involved in locust survey, control, reporting and forecasting. A bottom-up approach is used for these developments based on user requirements and feedback. The products, methodologies and tools are constantly updated to reflect changes in latest technologies and user needs. Substantial training and retraining are provided to each country using the train-the-trainers strategy, in which national master trainers are trained in the technology as well as in teaching and communications, so that they can provide essential training at all levels to national locust staff. Clear, concise, and imaginative training material and programmes are designed and updated to complement this process, supplemented by online videos for self-learning.

It is important to develop products and tools that are sustainable, can be maintained locally and used for as long as possible. Whenever possible, existing infrastructure, resources and expertise should be utilized and, if necessary, expanded rather than reinventing something that already exists. When developing an area-wide pest management programme, for example, that has a requirement for data collection in the field, it is far better to take advantage of the mobile phones that most farmers and extension agents already have, as well as the know-how to use them. This eliminates the need to develop, procure, distribute, and maintain a different device and to provide relevant training. Instead, it would only be necessary to develop an app for data collection and recording that works on the mobile phones. If data transmission is required, and there exists sufficient mobile telephone network coverage, then there is no need to rely on satellite communication, which is not only more expense but also requires additional hardware, maintenance, and training.

It is crucial to use technologies that are appropriate and relevant. For example, the Sterile Insect Technique (Dyck et al. 2021), requested by some countries, is not an appropriate control strategy for desert locust because of the size of its vast recession area and the fact that the released locusts would cause damage consuming crops. On the other hand, it has been extremely successful in contributing to the eradicating other pests such as the pink bollworm *Pectinophora gossypiella* (Saunders) in the south-western USA and northern Mexico (Staten and Walters, this volume), because it is not migratory, the released moths do not affect the cotton crop, and it was largely restricted to this host plant within this large geographic area.

Lastly, it is important to remain relevant by never ceasing to innovate in order to take advantage of the potential benefits and applications offered by the latest technologies. One example is the integration of drones and high-resolution satellite imagery into area-wide pest management programmes for monitoring and control. While this should not be a substitute for ground means, it can certainly supplement current efforts to monitor more effectively large areas and undertake safer and more precise control operations.

The innovative use of new technologies and tools in managing transboundary pests is unlikely to be entirely successful if it is not coupled with strong collaboration amongst beneficiary countries that is overseen by a centralised coordinating body. For example, 30 frontline countries affected by the desert locust share field data amongst themselves and with the DLIS. This allows DLIS to continual monitor the situation, forecast its developments, and provide timely and accurate early warning. A centralised coordinating structure also allows the implementation of standardized methodologies and training material in all countries as well as coordinated action between countries during outbreaks, upsurges, and plagues.

All of the lessons learned from the desert locust experience can be applied and adapted to other transboundary pests such as other locusts, as well as the red palm weevil and the fall armyworm, as a means to better manage these pests, protect crops, and enhance food security.

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MATING DISRUPTION WITH PHEROMONES FOR CONTROL OF MOTH PESTS IN AREA-WIDE MANAGEMENT PROGRAMMES

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SUMMARY

Mate finding in many insect groups is mediated by pheromones, particularly among moths where the male flies upwind along the pheromone plume to a calling female. The use of a formulated, synthetic copy of this message to disrupt this process dates to 1973 with the demonstration of protection of cotton from the pink bollworm *Pectinophora gossypiella* (Saunders). This method has been expanded to many moth pests, particularly tortricid moths that infest high-value pome fruits and grapes. Because most applications of mating disruptant are not directly lethal, an operational assumption is that efficacy is enhanced when the area under disruption is large enough to mitigate the effects of mated females immigrating into the protected area. Area-wide protocols thus should optimize efficacy of disruption and possibly permit effective control for some highly dispersive species such as heliothine moths that are unlikely to be controlled only by mating disruption in farms of even multiple hectare size. Successful area-wide programmes considered include those for the pink bollworm, codling moth *Cydia pomonella* (L.), oriental fruit moth *Grapholita molesta* (Busck), navel orangeworm *Amyelois transitella* (Walker), European grapevine moth *Lobesia botrana* (Denis & Schiffermüller), and gypsy moth *Lymantria dispar* (L.). Efficacious control, regardless of the magnitude of the crop area, typically requires an initially low population and therefore integration with other control measures.

Key Words: Lepidoptera, mate finding, pheromone plume, codling moth, pink bollworm, European grapevine moth, gypsy moth, oriental fruit moth, navel orangeworm, Sterile Insect Technique

1. INTRODUCTION

The French naturalist Jean-Henri Fabre, working with the giant peacock moth *Saturnia pyri* (Denis & Schiffermüller) in the 1870s, was one of the first to document that females release an alluring odour that draws in males from many metres downwind. In 1882 the New York state entomologist Joseph Albert Lintner, who was also amazed at the ability of a single female saturniid moth to attract many suitors, proposed that synthetic copies of these chemical messages might serve as a means of

J. Hendrichs, R. Pereira and M. J. B. Vreysen (eds.), Area-Wide Integrated Pest Management: Development and Field Application, pp. 779–794. CRC Press, Boca Raton, Florida, USA.

direct control of moth pests. The realisation of this method, however, had to await development of micro-analytical methods capable of identifying pheromones present in billionths of a gram per female (Roelofs 2016).

At present, female-produced pheromones have been chemically defined for several hundred moth species in many lineages (El-Sayed 2016; Löfstedt et al. 2016). Generally, these messages are comprised of blends of 2-3 components, although some moths apparently use a single component and a few species have blends as complex as 6 chemicals. Using these compounds to interrupt chemically triggered mate finding by males requires methods to gradually release these compounds into the crop to be protected. There are now many kinds of slow-release formulations that protect these usually labile chemicals until their release into the atmosphere.

Harry Shorey and his colleagues at the University of California, Riverside (Gaston et al. 1977) were the first to demonstrate in field trials that application of formulated synthetic pheromone could control a moth pest. In the 1970s, the pink bollworm, *Pectinophora gossypiella* (Saunders) (Gelechiidae), was a principal pest of cotton grown in southern California's Coachella Valley. Synthetic pheromone was formulated into open-ended, hollow plastic fibres and released at 10 µg per fibre per day; fibres deployed by hand at a density of approximately 1 per m^2 provided control by reduction in boll damage comparable to conventional practice in the insecticide-treated controls. This success provided an impetus for efforts to develop mating disruption for other key moth pests. Today this method has been commercialized for many of the most economically important moth pests (Evenden 2016) and we term this method of pest control 'mating disruption'. The successes and constraints of the mating disruption technique have been summarized by Cardé and Minks (1995), Cardé (2007), and Evenden (2016). Witzgall et al. (2010) reported that an estimated 770 000 ha yearly received mating disruption treatments for moths.

This review will explore the utility of this approach in large-scale applications where the goal of management is either to suppress a pest directly or in some cases, to eradicate a population over an entire region. To understand the prospects for successful disruption in area-wide programmes, it will be useful to consider how a broadcast application of a disruptant interferes with mate finding and how formulation type and a precise matching of the disruptant to the natural pheromone influences efficacy of disruption.

Most moth pheromones are straight-chain, C_{10} to C_{18} compounds with either an acetate, alcohol, or aldehyde moiety, and 1-3 positions of unsaturation (El-Sayed 2016; Löfstedt et al. 2016). These chemicals, and generally most other moth pheromones, are subject in the field to degradation by processes such as isomerization, oxidation and polymerization, and consequently one objective of all formulations is to provide protection against degradation while releasing the active ingredients into the atmosphere, ideally at a fairly constant rate until depletion. Most formulations use a plastic matrix (e.g. microcapsules, open-ended hollow fibres, sealed plastic tubes ('ropes'), PVC capsules, laminates, etc.) to retain the disruptant. Other formulations use dollops of a waxy emulsion into which the disruptant is mixed or the disruptant is released as an aerosol in timed puffs from widely spaced, sealed cans (Table 1).

Table 1. Examples of formulations used in mating disruption of moths (modified from Cardé 2007); the densities and methods of application, field longevities, and probable modes of action are general examples

Formulation*	Density ha^{-1}	Application method	Longevity	Mode of Action
Atomizer ‘puffer’	<1 to several	hand-placed	season-long	sensory impairment; camouflage; competition
Sealed plastic tubes	hundreds	hand-placed	season-long	sensory impairment; camouflage; competition
Open-ended, hollow fibres, laminate ‘flakes’	≈10 000	aerial	weeks	sensory impairment; camouflage; competition
Waxy dollops	100-10 000	hand-applied, aerial, specialised equipment	weeks to season-long	sensory impairment; camouflage; competition
Microcapsules spray	millions	conventional	days to several weeks	sensory impairment; camouflage
‘Attracticide’ (e.g. fibres with insecticide in sticker)	≈1000	specialized equipment	weeks	direct toxicity; impairment of orientation; competition

* *When the formulation is not comprised of the full (attractive) pheromone blend or it contains an antagonist, it may not evoke competition (for an example see Section 3.3)*

In field applications these differing formulation types produce a large range of densities of disruptant sources and release rates and consequently in the atmospheric concentrations and spatial distributions they generate. Some formulations are point sources that match or exceed the attractiveness of a calling (pheromone-emitting) female. Other disruptant formulations are either intrinsically non-attractive because they are an incomplete copy of the pheromone blend (lacking components required for good attraction), or they contain synthetic by-products that are antagonistic and so either reduce or even eliminate attractiveness of the formulated product (Cardé 2007).

As formulations can be expected to be applied repeatedly to the same area and therefore themselves could be a source of pollution, a formulation’s degradability over time should be a factor in its selection.

2. MECHANISMS OF MATING DISRUPTION

Disruptants can interfere with mate location in 3 principal ways:

1. *Competition*: Males may spend time and energy orienting to sources of formulation. The efficacy of this mechanism should be dependent on the ratio and comparative attractiveness of these sources to calling females (see Miller et al. 2006 for a theoretical consideration of this mechanism). A variant on this method adds insecticide to point sources of pheromone, an “attract and kill” strategy (Cork 2016).

2. *Sensory Impairment*: Exposure of males to disruptant causes either a diminution in responsiveness by raising the threshold for response, or by altering navigational ability, or exposure may simply eliminate responsiveness to the pheromone. Generally, such impairment can be due to adaptation of either sensory receptors or habituation, which is a central nervous system phenomenon, or both factors.

3. *Camouflage*: The pheromone plume from a calling female becomes imperceptible amongst the background of disruptant.

When the formulation mimics the natural pheromone, all of these mechanisms could contribute to efficacy and they could act additively or synergistically. There are other supplementary ('minor') mechanisms such as delay in mating that also could contribute to efficacy (see for examples: Cardé 2007 and Evenden 2016).

As will be documented, the susceptibility to disruption seems to vary with species, formulation type and application rate (Table 1), and whether the active ingredients match the full natural pheromone. Some of the behavioural traits that promote or interfere with successful mating disruption are listed in Tables 2 and 3.

Table 2. Male behavioural traits expected to confer higher or lower susceptibility to mating disruption matched with moths thought to possess these traits (see Cardé 2007 for further details on mechanisms)

Higher Susceptibility	Lower Susceptibility
Readily habituated [oriental fruit moth]	Difficulty to habituate [pink bollworm]
Slow to dishabituate [oriental fruit moth, codling moth]	Rapid dishabituation [pink bollworm]
Poor ability to navigate along plumes within a background of pheromone	Good ability to navigate along plumes within a background of pheromone
Rhythm of response imprecisely coordinated with female calling [pink bollworm]	Male and females mating rhythms well-coordinated
Males rely principally on pheromone for orientation and mating [pink bollworm]	Visual and tactile cues facilitate orientation and mating [oriental fruit moth]

Although formulation type, the match of its active ingredient to the natural pheromone, and its application rate all are quite important to disruption efficacy, ecological factors also are crucial: what is the pest's density at the start of application, does it need to be suppressed to a suitable level before application, and how migratory are mated and unmated females?

The first issue influences efficacy in two ways: the higher the density of moths, the closer they are to each other spatially and phenologically; at very low population densities, both factors should diminish the probability of females mating, essentially an Allee effect, in which a population at very low density could collapse because of a failure to find a mate (Liebhold and Bascompte 2003).

In most management protocols, it will be necessary to use additional control tactics such as cultural methods (e.g. sanitation) or insecticide application to reduce the population to a level amenable to mating disruption. For those moths that are notable adult nectar feeders (such as noctuids), a bait of adult feeding stimulants laced with insecticide provides another tactic to integrate with mating disruption (Gregg et al. 2018).

Moth movement is a second ecological factor to consider. If the species is highly migratory (such as many heliothine moths) and the area under management is near a source population, then the area under mating disruption may suffer crop damage due to the immigration of mated females. Possibly too, virgin females could leave an area under disruption, mate, and then reinvade the crop. The density and proximity of the outside population and the tendency of mated females to migrate should govern in part the programme's success. Migratory tendency and capability vary considerably among moth species and, even within a species, migration can be modulated by changes in crop suitability and season. Application of the principles of density and movement will be useful to interpreting several case studies.

Table 3. Female behavioural traits expected to confer higher or lower susceptibility to mating disruption matched with moths thought to possess these traits (see Cardé 2007 for further details on mechanisms)

Higher Susceptibility	Lower Susceptibility
Low pheromone emission rate [oriental fruit moth]	High pheromone emission rate
Calling from within plant canopy	Calling from top of canopy [pink bollworm]
Rhythm of response imprecisely -coordinated with female calling [pink bollworm]	Male and females mating rhythms well-coordinated
Low migratory tendency [North American gypsy moth]	Migratory [navel orangeworm]

Of particular relevance to the use of mating disruption in area-wide programmes will be assessing how size and isolation or distance of treated areas from outside infestation influences efficacy. Given the logistical difficulties and cost of evaluating whether area-wide programmes enhance management of a 'keystone' moth pest over conventional field-by-field or grower-by-grower practices, much of what we can conclude will be by inference rather than by referring to empirical tests.

The following case studies are examples of successful management using mating disruption, often combined with other techniques. Each example is considered briefly and specific documentation of the field evidence pertinent to area-wide use is cited. Mating disruption has been studied in all of the following pest species over many years.

3. CASE STUDIES

3.1. Pink Bollworm

The first field-scale use of mating disruption was against the pink bollworm, *Pectinophora gossypiella* (Gaston et al. 1977), and in 1978 it was the first pheromone to be registered by the USA Environmental Protection Agency (US-EPA) as an insecticide. The active ingredients are a 1:1 mix of the two components of the female's pheromone, (*Z,Z*)- and (*Z,*E)-7,11-hexadecadienyl acetates. There are now several formulation products in use (e.g. hollow fibres, sealed plastic 'ropes').

There is one notable field study of its use in area-wide management. In Arizona in the 1970s, control of this pest relied largely on frequent night-time aerial sprays of insecticide aimed at the adult moth. This approach was becoming untenable, because of increasing insecticide resistance and costs. The Parker Valley is isolated from other cotton-growing areas by surrounding desert (Fig. 1), thereby eliminating influx of mated females from outside the treated area and thus constituting an ideal location to evaluate an area-wide programme. Growers in the Parker Valley of Arizona, in collaboration with the Animal and Plant Health Inspection Service (APHIS) arm of the United States Department of Agriculture (USDA), devised a multi-year programme to attempt to directly manage pink bollworm with mating disruption and some use of insecticide in all cotton fields (detailed in Staten et al. 1996).

Figure 1. Aerial view of the Parker Valley agricultural area along the Colorado River in Arizona. The growing area was well isolated from other cotton production by surrounding desert (courtesy Google Maps).

Parker Valley's 11 250 ha of cotton comprised a significant size and, prior to this programme, intensive application of insecticide provided unacceptable levels of control (> 25% boll infestation in the year prior to the programme's start). Mating disruptant applications began in 1990 and the programme ended in 1993. Throughout, approximately one third of the cotton received a hand application of rope dispensers and the rest of the applications were by air and used fibres or fibres plus an insecticide overspray.

Over the project's four-year span, the use of insecticide dropped from half of the aerial applications to less than a tenth. There were approximately 650 fields in the programme and 45 of these were selected at random for assessing boll damage. From 2000 to 3000 cotton bolls were cracked weekly to check infestation. The highest recorded weekly larval count per boll was 21% in 1990, dropping to 7% in 1991, 2% in 1992 and zero in 1993, when over 20 000 bolls were examined season-long and not a single pink bollworm larva was found. Clearly this technology offered a new paradigm for management of a pest that was becoming very difficult to control with conventional insecticides. One remarkable feature of this demonstration was that it succeeded even though initial infestation levels were high and this species is considered rather migratory (Stern 1979). These sorts of programmes lack replication and direct experimental controls, but the substantial infestation levels across the Parker Valley in the years prior to the programme serve to verify efficacy. Despite the success of this programme, mating disruption for pink bollworm is now in limited use worldwide (≈ 50 000 ha annually according to Witzgall et al. (2010), or ≈3% of cotton worldwide), mainly due to the availability of *Bacillus thuringiensis (Bt)*-cotton, which has supplanted mating disruption and conventional insecticides for control of this and other moths.

Mating disruption also played a central role in the successful area-wide effort to eradicate pink bollworm from the south-western USA (Texas to California) and adjoining areas in northern Mexico. This audacious undertaking was reviewed by Lance et al. (2016). The programme began in 1994 and involved at various stages and regions the application of mating disruptant, release of sterile moths, some application of insecticides and, beginning 1997-1998, planting of *Bt*-cotton. Since 2008 no larvae have been detected anywhere in the entire cotton-growing regions of the south-western USA and northern Mexico (Staten and Walters, this volume). The success of this programme (which continues with monitoring using pheromone traps) is difficult to attribute to any single control technique and it is most probable that the combination of methods was needed to suppress reproduction. Its success was likely also guaranteed by the integration of these methods on an area-wide basis over all cotton growing areas in the region and the fact that this moth is a cotton specialist.

3.2. Codling Moth

Cydia pomonella (L.) (Tortricidae), infests many kinds of pome fruits and walnuts, and it is particularly troublesome in apple orchards. Most work has characterised its pheromone as a single component (*E,E*)-8,10, dodecadienol. Probably more field tests on mating disruption have been conducted on this species than any other moth. As of 2010, about 210 000 ha were treated yearly with mating disruptant for this pest worldwide (Witzgall et al. 2010). A general conclusion is that this species is difficult to disrupt in the sense that for success initial populations need to be low and isolated from the influx of mated females from untreated areas; in any case, growers will not tolerate more than a small percentage of fruit damage.

Witzgall et al. (2008) reviewed these studies and provided two general conclusions: 1) prior to treating with disruptant, initial populations must be low and therefore a remedial application of insecticide or some other method could be necessary to achieve the desired population level; and 2) the larger the area under pheromone management and the greater its isolation from outside sources of infestation, the greater the level of protection and economic benefit per hectare (McGhee et al. 2011). Given that apples typically are produced in orchards of varying size and proximity to other orchards and non-commercial hosts (a 'patchwork' pattern), coordination of a common strategy across many growers in a region is a logistical and sociological challenge (Brunner et al. 2002).

No definitive field studies have established the minimum plot size for maximum achievable efficacy (i.e. the point where no further reduction in mating can be expected) and certainly efficacy will vary somewhat with application rate and formulation type (e.g. puffers vs. hand-applied, point-source), how thoroughly the formulation disperses disruptant throughout the canopy, canopy structure, initial population density, climate, and topography (see Witzgall et al. 2008). The principal lessons to be drawn from numerous field studies with codling moth are that for mating disruption to succeed, initial populations must be very low (if need be requiring remedial treatment with an insecticide) and sufficiently isolated from sources of mated females.

3.3. Oriental Fruit Moth

Grapholita molesta (Busck) (Tortricidae) is a troublesome pest on peaches, pears, nectarines, plums and apples; its first generation also can inflict significant damage to shoot tips. Its pheromone is a 3-component blend: (*Z*)-8- and (*E*)-8-dodecenyl acetates (95:5) with 3-10% of (Z)-8-dodecenol added. This moth seems especially susceptible to mating disruption, with a wide variety of formulation types (microdispersibles to aerosol puffers) of the pheromone providing excellent fruit protection (Cardé 2007; Evenden 2016). As of 2010, about 50 000 ha were treated yearly with mating disruptant for this moth (Witzgall et al. 2010).

In Australia, Il'ichev et al. (2002) set out to establish that an area-wide approach would provide enhanced control over an orchard-by-orchard management. In the project's first year an area of over 800 ha including 18 orchards were treated with hand-applied dispensers at 1000 per ha; in the second year over 1000 ha comprising 40 orchards were treated with 500 dispensers per ha.

In the first year, growers decreased insecticide application by half and during the second year most growers did not apply any insecticides for this pest. Areas previously identified as 'hot spots' of infestation also were brought under control. Il'ichev and colleagues concluded that area-wide management with pheromone was highly effective for oriental fruit moth, but cautioned that infestations could linger at the edges of disruptant-treated blocks if the permeation of air-borne disruptant there was incomplete due to wind patterns or if neighbouring orchards harboured oriental fruit moths (Il'ichev et al. 2002).

3.4. European Grapevine Moth

The pheromone of *Lobesia botrana* (Denis & Schiffermüller) (Tortricidae) is usually considered to be a single compound, (*Z*,*E*)-7-9-dodecadienyl acetate. Historically this moth has been a grape pest in Southern Europe, North Africa, Anatolia and the Caucasus; it was recently introduced into South America (Taret et al., this volume). As of 2010, Witzgall et al. indicated that 100 000 ha in the European Union, Argentina and Chile were treated yearly with mating disruptant for this moth.

In 2009, it was discovered in northern California with a nexus in Napa County. Following the discovery of this pest, its distribution was mapped in 2010 with a network of traps throughout the grape growing regions of California. Over 100 000 males were trapped, mainly in Napa County, but with significant populations in adjoining Sonoma County, and isolated pockets elsewhere, these being attributed to movement of material such as stakes from the original area of infestation rather than natural dispersal. A multi-pronged eradication programme with support from growers and organizational direction from federal, state, county and extension agencies was initiated in 2011 (for detailed reviews see Lance et al. 2016, and Simmons et al., this volume).

In 2011 and 2012, mating disruptant was applied to ≈160 km^2 in Napa County, generally in the core-infested area, and to ≈16 km^2 in Sonoma County, with smaller application areas in 2013 and 2014. Since 2015, extensive pheromone trapping has not caught any males, and the moth has been declared eradicated. It is fair to note that conventional insecticides were used in many but not all vineyards. Therefore, the area-wide demise of this pest cannot be attributed solely to mating disruption, but it was viewed as a key component of successful eradication (Lance et al. 2016).

3.5. Navel Orangeworm Moth

Amyelois transitella (Walker) (Pyralidae) is a key pest of almonds, pistachios and walnuts in California. This moth can be controlled using mating disruption as part of an integrated pest management programme. Aerosol dispensers, deployed at one per 0.8 ha, emit (*Z*,*Z*)-11-13-hexadecadienal, one of the 4-components of its pheromone, but which alone is at best slightly attractive compared to the full blend. Mating disruptant generally provides control levels similar to insecticide-alone regimes and the combination of disruption and insecticide generally resulted in better pest management and even lower levels of damage (Higbee and Burks 2008).

Based on flight mill studies, virgin navel orangeworm females appear capable of migrating several km (Sappington and Burks 2014), although whether mated females would naturally migrate such distances when they are within a host orchard remains to be determined. Evidence that such moderate-distance migration is an important consideration stems from the field study of Higbee and Seigel (2009), who found that navel orangeworm damage in almond blocks was correlated with distance from pistachios (with traditonally higher populations): some spillover was detectable even with a 5-km separation. The migratory capacity of navel orangeworms demonstrated so far suggests that crop protection should be enhanced as the area under treament is scaled up.

In California, almond and pistachios orchards often extend in contiguous plantings over many km and therefore movement of mated females from untreated orchards to adjoining blocks treated with disruptant is probable. This pest is an ideal candidate for an area-wide management programme using mating disruption. Careful monitoring in a 4-year demonstration project in 1050 ha of almonds, showed that 3 insecticide sprays yielding 5-10% damage could be replaced by mating disruption, producing only 0.5% damage (Higbee 2010). Registration and use of its complete pheromone blend might provide higher levels of nut protection than the currently used aldehyde (Higbee et al. 2017). Another rationale for area-wide application is that several percent nut damage occured in 16-ha mating disruptant plots (Higbee et al. 2017); presumably, increasing greatly the area under disruption would enhace protection by limiting influx of mated females. Overall, in 2016 mating distuption was used in California on over 80 000 ha of almonds and pistachios (Higbee personal communication).

3.6. Gypsy Moth

The potentially expanding range of the gypsy moth, *Lymantria dispar* (L.) (Erebidae), in North America and introductions of its Asian form are monitored by a network of more than 200 000 pheromone traps (Lance et al. 2016). Traps are baited with the pheromone, (*7R,8S*)-cis-7,8-epoxy-2-methyloctadecane, called '(+)-disparlure', but, as optically pure disparlure is quite expensive, the (+)- enantiomer is only used in survey lures. Mating disruption uses the inexpensive racemate as a disruptant. The racemate is about one tenth as attractive because its (-)-enantiomer is an antagonist of attraction.

Aerially applied racemic disparlure is being used to retard the advance of gypsy moths to the south and west of low-level populations along the edge of its advancing USA range. Mating disruption is deployed in a 100-km-wide band from North Carolina to Minnesota in a programme called "Slow the Spread" (reviewed by Lance et al. 2016; Liebhold et al., this volume). In this zone, trap capture is low (between 0-1 per trap yearly throughout most of the band, rising to several or more per trap near the edge adjoining the established population). Prior studies have established efficacy of aerially applied formulations (e.g. Thorpe et al. 2006), verified by substantial reductions in capture in pheromone-baited traps in subsequent years and mating of tethered, sentinel females.

From 2000 to the present, an average of about 180 000 ha have been treated yearly (Lance et al. 2016). The migration biology of the North American gypsy moth differs from most moths in that the female is flightless and natural population spread occurs through wind-borne 'ballooning' of first instar larvae. (Anthropogenic transport, particularly of egg masses, remains an important regulatory and practical issue.) Thus, female movement does not influence the success of mating disruption in a given area. Larval movement (ballooning 1st instars), however, will influence the size of the area wherein mating disruption will be effective.

4. IS EFFICACY INFLUENCED BY THE SIZE OF THE AREA TO BE TREATED?

A reoccurring theme in all studies of mating disruption is the assumption that the larger the plot size, the more efficacious mating disruption, because large size mitigates the influx of mated females from outside the treated area. Experimental evaluation of formulations and active ingredients for efficacy can be accomplished, at least with some moths, with replicated small plots (ca. 25 by 15 m) (Roelofs and Novak 1981) and with small field cages (ca. 8 m^3) (Koch et al. 2009) by testing for disruption of attraction to pheromone lures and to females. It is generally accepted that proof of crop protection, however, needs to be assessed in replicated plots that are large enough to minimize or eliminate the immigration of mated females from untreated areas, whereas disruption of attraction to a lure or female in a trap are insufficient metrics. There have been several notable studies using replicated large plots [Brockerhoff et al. 2012 with the light brown apple moth, *Epiphyas postvittana* (Walker); Onufrieva et al. 2018 with the gypsy moth; and Higbee and Burks 2008, Higbee et al. 2017 with the navel orangeworm moth]. With such large experimental plots, some useful information on the infiltration of mated females may be found by monitoring infestation or disruption levels from a plot's edge to its centre.

As the distance that mated females migrate varies with species and also can be influenced by habitat, host availability and season, there is no set answer as to how large a plot needs to be to guard against such immigration from the periphery. What is clear is that efficacy should be enhanced with enlarged treatment areas and becomes optimal if all areas that harbour the population of the pest are treated. Crucial to understanding these interactions will be characterisation of the moth's migratory capacity and the conditions in the field that trigger this behaviour. There are not, however, replicated field experiments that have defined optimal plot size directly, and given the sizes required, these are unlikely to be undertaken.

5. INTEGRATION OF MATING DISRUPTION WITH STERILE INSECT RELEASE

The Sterile Insect Technique or SIT is another environment-friendly method for insect suppression and in some cases eradication (Dyck et al. 2021). The SIT efficacy is largely contingent on the ratio of sterile to native insects (the overflooding ratio) and the competitiveness of sterile insects. Because mating disruption seeks to diminish mating success, it seems counterintuitive to combine these two approaches, as application of disruptant would diminish the probability that sterile insects would mate with native insects. However, because both techniques reduce the number of fertile females, in combination they may enhance population suppression over either method used alone. Furthermore, where use of either mating disruption or SIT fails to provide sufficient control (e.g. because of gaps in coverage), then the other method could be employed. There are many examples of incomplete population suppression using mating disruption, even with prior application of conventional insecticides to suppress the population to a low level (e.g. Witzgall et al. 2008; Evenden 2016; Higbee et al. 2017).

The SIT generally aims to achieve a particular overflooding ratio (e.g. a 10 to 1 ratio of sterile insects to native ones) and one challenge is determining the density of the native population. One method for evaluating a SIT programme is to compare the ratio of internally-marked sterile to native males captured in pheromone traps (Vreysen 2021). A difficulty, however, in combining mating disruption and SIT is that application of disruptants makes the monitoring of population levels and the overflooding ratio with pheromone traps problematic. Of course, absence or very low numbers of trap catch in pheromone-baited traps often is used to verify that male orientation to females is being suppressed and therefore that mating disruption is protecting the crop.

For evaluation of population density in some species (e.g. pink bollworm and codling moth), males can be trapped in mating disruption areas by using a lure with high rate of pheromone emission, that is, 10-fold higher than would normally be used in population monitoring (Doane and Brooks 1981; Witzgall et al. 2008), and so these high-dose traps could be used for evaluation of the SIT component. In a few cases, there are kairomone lures that are effective in sampling adult moths in mating disruption plots (e.g. Knight 2010; Burks 2017).

The combination of these two methods has of course been limited to those few moth species for which the SIT package and mating disruption have been fully developed and tested for field efficacy. With pink bollworm, aerial release of sterile insects was long used to suppress this pest in the San Joaquin Valley of California, with daily releases of up to 18 million moths. As considered earlier in Section 3.1, mating disruption, SIT and other measures were combined in an ambitious and successful area-wide eradication programme in the south-western USA and northern Mexico (Lance et el. 2016; Staten and Walters, this volume). As both mating disruption, SIT and in the later years *Bt*-cotton were combined, it is impossible to parse out the precise contribution of each tactic and especially the extent to which either mating disruption or SIT contribute to suppression. It also is possible that sequential application of these techniques (SIT preceding mating disruption or vice versa) would yield higher levels of eventual suppression than simultaneous application.

The SIT is the mainstay in the management of the codling moth in the Okanagan Valley of British Columbia, Canada (Nelson et al., this volume). Over the past 20 years, this programme has produced a dramatic reduction in insecticide use in apples (some growers have not applied an insecticide for codling moth in 15 years). Fruit infestation is extremely low (<0.2%) in >90% of the orchards. Like successful programmes using mating disruption with codling moth (Section 3.2), a key factor is early intervention with insecticides to reduce populations to the low levels where SIT can provide a final reduction and continued control (Bloem et al. 2007).

An effort in the same region to combine SIT with mating disruption, Judd and Gardiner (2005) established in organic orchards that these two measures coupled with removal of overwintering larvae using tree bands (cultural control) in concert suppressed codling moths to non-detectable levels within several years. Judd and Gardiner (2005) proposed that mating disruption and SIT together was as effective as SIT and some initial insecticide.

The area-wide integration of mating disruption with SIT application was also effective south of the Canadian-USA border (Calkins et al. 2000). This finding as well as the successful eradication efforts with pink bollworm both point to a favourable interaction of mating disruption with SIT, but precisely how these two seemingly competitive approaches either complement or interfere with each other remains to be determined.

Whether combining mating disruption and SIT enhances crop protection over either method alone thus remains an outstanding question. Before widespread implementation of these combined tactics, it would be valuable for future programmes to compare in replicated plots the levels of crop protection provided by each technique alone and in combination. Perhaps modelling how these two processes interact would provide insight into the value of combining SIT and mating disruption.

It also might be feasible to develop through either conventional selection or genetic engineering strains for SIT application that have males that are 'resistant' to mating disruption (e.g. by reducing their tendency to habituate response to pheromone, possibly by altering their biogenic amines levels—see Linn and Roelofs 1986). Males from such a strain would be more apt than their wild counterparts to find females amidst a background of mating disruptant. How such males perform in a non-disruptant environment would be important to understand, but presumably such a trait would render modified moths less competitive than their wild counterparts, because this is a trait that would not be favoured under the constraints of traditional natural selection in the field. Presumably, as well, interbreeding with wild moths would not produce offspring.

In field-cage observations using laboratory strains of codling moth, a single encounter with a point source of pheromone can render a moth unresponsive to pheromone for 24 hours (Stelinski et al. 2006). In contrast, the strain of pink bollworm used in the SIT programme remains pheromone responsive ('resistant' to habituation) after a 24-hour exposure to pheromone (Cardé et al.1998). Although we do not know how released sterile pink bollworm males behave in disruptant plots, these observations suggest that they should search for females and therefore the field efficacy of mating disruptants in this strain should be at least partially contingent on a mechanism of competition between pheromone sources from deployed formulations and those from wild and also sterile females.

Cardé et al. (1998), interpreting the mechanisms of mating disruption in the pink bollworm, assumed that wild and sterile pink bollworm males were behaviourally equivalent. An open question, however, is whether continuous exposure to high pheromone levels during many generations under mass-rearing has altered its pheromone response characteristics.

An obvious criterion for evaluating sterile moths is how competitive they are in finding mates. If the SIT is to be integrated with mating disruption, then another criterion may be the ability of sterile males to find wild females amidst a background of disruptant and whether this is a trait that can be enhanced by selection.

6. CONCLUSIONS

The principle and its practical application that formulated pheromone can control many kinds of moth pests are well established. The susceptibility of a given moth pest to mating disruption, however, varies with characteristics of its communication system (e.g. rate of pheromone release from the female, male sensitivity to sensory interference) and formulation rate of release, its match to the natural pheromone, and type (e.g. widely-spaced aerosol dispensers, point sources mimicking females, non-attractive disruptants). The acceptance of this method for management of many species of moths signifies both its efficacy and cost-effectiveness.

Incorporation of mating disruption into area-wide integrated management programmes would seem to be a straightforward process, simply requiring scale-up of established methods, with the benefit that efficacy is expected to be enhanced, given that a large area of application (hundreds of ha) mitigates the influx of mated females from the periphery. One concern in widespread use of mating disruption is that the goal, suppression of mating, also suppresses capture in pheromone-baited traps, which can be a useful tool for population monitoring.

Replicated field demonstrations that the larger the disruptant-treated area, the greater the efficacy of mating disruption generally, however, will remain an elusive and perhaps intractable experimental goal. We will continue to rely on replicated, small-scale trials to establish that formulations are sufficiently efficacious to warrant area-wide application. Ultimately, the success of any area-wide approach depends not just on effective tools for insect control, but cost-effectiveness, environment-friendliness, social acceptance, and management protocols for implementation (Hendrichs et al. 2007).

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CRISPR-BASED GENE DRIVES FOR COMBATTING MALARIA: NEED FOR AN EARLY STAGE TECHNOLOGY ASSESSMENT

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SUMMARY

The potential power of CRISPR-based gene drives makes it necessary to engage in science and technology assessment already in early stages of research and development. In order to argue for efforts to address this urgent need, gene drives to combat malaria-transmitting mosquitoes are discussed using the concept of prospective technology assessment. First, development risks are described, followed by considerations about anticipatable risks and irreversible consequences, as well as unforeseeable effects and uncertainties. Afterwards, fundamental problems in connection with the development of gene drives against malaria mosquitoes are raised. Opportunities for shaping technology are briefly discussed, before alternatives, in particular the World Health Organization's elimination strategy, are considered. Finally, several normative questions are put forward.

Key Words: Anticipation, gene drive, gene flow, malaria, malaria vector, mutagenic chain reaction, normative questions, prospective technology assessment, risk, selfish genetic element

1. THE MALARIA CHALLENGE

Malaria is caused by *Plasmodium* parasites which infected female mosquitoes can transmit to humans. Most of the malaria control strategies have been aimed at managing the vector of the malaria pathogen, i.e. the relevant mosquitoes of the genus *Anopheles*. However, not only the main malaria vector *Anopheles gambiae* Giles can transmit malaria, but in sum more than 30 *Anopheles* species (with highly varying regional distribution), i.e. roughly one percent of all known mosquito species can do so (NAS 2016).

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The *Plasmodium* cycle first takes place in an infected mosquito (in particular inside the salivary glands) and after transmission in the liver and blood of a human. Some of the pathogens reach a sexual stage so that the whole malaria-parasite cycle (mosquito–human–mosquito) can start again after a bite of another mosquito which gets infected (White et al. 2014). Malaria is still endemic in many regions of the world. The World Health Organization (WHO) estimates that currently about 200 million humans are infected annually. In 2015, death cases due to malaria were in the range of 438 000, primarily in Africa (WHO 2015a).

The fight against malaria is regarded as one of the grand global challenges and was appropriately included in the UN Agenda for Sustainable Development in 2015. Control of the mosquito vector is mainly hampered by the development of resistance against the commonly used insecticides. Therefore, novel biotechnological strategies are under development and are coming into operation (for an overview cf. e.g. Alphey 2014). One such novel approach is a new tool of genetic engineering, i.e. the mutagenic chain reaction, mostly called *gene drive,* which comprises a variety of different technologies.

2. GENE DRIVE AND PROSPECTIVE TECHNOLOGY ASSESSMENT

Sexual reproduction provides, in principle, a 50 % probability for the propagation of the parental genotypes to the progeny in accordance with Mendelian heredity rules. This evolutionary mechanism can be circumvented if specific parts of the genome can be transformed from heterozygote to homozygote, which has been observed in nature in some exceptional cases. Then, certain genotypes can be inherited with quite a high probability (significantly above 50% and up to 100%) even if the fitness of the offspring is adversely affected.

In 2003, the British researcher Austin Burt proposed to genetically engineer such gene drives and to use them against malaria-transmitting mosquitoes (Burt 2003). The key idea was "to drive novel genes or mutations into wild populations" (Bull 2015). Theoretically, a rapid and targeted manipulation of entire mosquito populations would be possible while circumventing Mendelian rules. First attempts were made in the laboratory (e.g. Windbichler et al. 2011), but a research boom started only in 2013 with the discovery of the novel gene scissor CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats–CRISPR-associated 9). The first CRISPR-based manipulation of the fruit fly *Drosophila melanogaster* Meigen in the laboratory was named as a mutagenic chain reaction by the researchers involved (Gantz and Bier 2015). Meanwhile, gene drives have been technically realized in the laboratory in yeast (DiCarlo et al. 2015) and in the malaria-transmitting mosquitoes *Anopheles stephensi* Liston (Gantz et al. 2015) and *An. gambiae* (Hammond et al. 2016).

Considering the malaria case in particular, gene drive approaches can be divided into *suppression drives* and *modification/manipulation drives*. Suppression drives aim at dramatically reducing or eradicating malaria-transmitting insect populations, regionally or globally. Modification/manipulation drives strive to genetically modify or manipulate mosquitoes in a way that malaria infection of humans is reduced or disabled.

Gene drives could become extremely powerful tools for humans to make dramatic intentional or unintentional changes in populations and entire ecosystems. Therefore, it is necessary to engage in appropriate procedures of science and technology assessment in good time before such technologies are mature. An appropriate concept to guide such efforts is the approach of *prospective technology assessment* (ProTA) (Liebert and Schmidt 2010, 2015), which includes:

- analysis of scientific-technological development at an early stage, anticipating what might be relevant for science-based mid-term assessments and for (participatory) discourse inside and outside science
- assessment of intentions/visions, potentials, risks and unintended consequences, realistic potentials versus unrealistic visions and promises, uncertainties (and ignorance)
- characterization of the type of technology involved
- analysis/assessment of opportunities for shaping science and technology and of technical or socio-technical alternatives, so that desired potentials can be exploited
- reflection on normative issues, values and interests involved.

ProTA is more than an accompanying exercise of socio-economic research on societal or industrial acceptability, but it is partly impossible without analysing the scientific-technological core itself. ProTA differs also from outlining a development pathway for gene drive mosquitoes from research to use in the wild, including safe and efficient field-testing, regulations and post-implementation monitoring (James et al. 2018). In the following, above mentioned aspects of ProTA will be elaborated on. In doing so, sometimes more questions will be raised than answered.

3. INTENTIONS, POTENTIAL AND DEVELOPMENT RISKS

In general, research aiming to realize the potential of CRISPR-Cas9-based gene drives to reduce or eradicate malaria seems to be justified. The suffering of malaria victims worldwide, and in several African regions in particular, cannot be ignored by the international community. However, malaria is not only a naturally occurring phenomenon and a serious plague for humankind, but also an unpleasant result of human evolution that has provoked a long-lasting human and humanitarian struggle against it.

It is not only a question of scientific understanding and development of technical tools. Social, political and economic factors are also of great importance. Social organization and behaviour, access to modern healthcare, a functioning healthcare system in the regions concerned, preventive and curative measures and availability of suitable means are of utmost importance when trying to manage malaria. What we currently observe is an unjust societal and global divide with respect to the malaria burden, with prevention and medication affordable for the rich, but not for the poor. It is important to recognize that the remarkable social stratification of the malaria burden has almost nothing to do with new technological approaches to fight against malaria.

Nevertheless, new biotechnical tools might provide helpful contributions to better manage malaria. Production and release of sterilized or *Wolbachia*-infected mosquitoes are already being validated in pilot projects to locally manage mosquito populations (Bourtzis et al. 2016). At first glance, CRISPR-based gene drives manipulating the genetic code of mosquitoes, at least theoretically, seem to be most promising in terms of efficacy. The release of one single modified organism could, in the medium-term, result in the modification of all individuals of a specific mosquito population (Noble et al. 2017). However, it turned out that in all of the above-mentioned laboratory experiments the chain reactions are being reduced after a few generations. The engineered gene drives effectively became unstable (Gantz et al. 2015; Champer et al. 2016, 2017), which is not the case in naturally occurring selfish genes. Indeed, in the drive systems described so far, resistance alleles were detected after a few generations, highlighting a fundamental instability of engineered homing-based systems[1]. Thus, the potential of gene drives, which could be exploited in principle, has been made visible, but not more. Instead, development risks have emerged questioning whether the objective of a functional gene drive can be achieved. The observed slowdown of gene drives is partly due to the fact that, after a cleavage of targeted DNA due to CRISPR-Cas9, competing repair mechanisms are coming into play. With relevant probability non-homologous end joining (NHEJ) is sometimes faster and more prevalent than homology-directed repair (HDR), which only leads to the engineered homing endonuclease with self-replicating characteristics passing on the new genetic information from generation to generation. NHEJ can produce resistance against the drive and eventually stop the mutagenic chain reaction (Unckless et al. 2017; Champer et al. 2017).

It is not clear whether an evolutionary stable homing-based gene drive can be engineered (Bull 2015). Limited efficiency of gene drives could be regarded as something positive, in particular if one is afraid of a virtually unlimited efficacy of such engineered systems. However, it is possible to precisely predict the non-linear behaviour of gene drives in the wild? How exactly can mathematical modelling reproduce the actual complex dynamics? Can a persistent behaviour of a gene drive construct, which was originally predicted as eventually self-limiting, be excluded? It is being proposed to overcome resistance phenomena by combining several drives and targeting different DNA sites simultaneously. But that has not been demonstrated so far and it could turn out that complexity and instability would again be increased instead.

It is also questioned whether homologous recombination, which is essential for gene drives, is error-free (Guirouilh-Barbat et al. 2014). There are also doubts that gene drives in wild mosquito populations will be feasible.[2] Although CRISPR-Cas9 is much easier, cheaper, faster, and more precise in usage than older tools of targeted genetic engineering, e.g. ZINC finger or TALEN (NAS 2016, p. 24-31; Häcker and Schetelig, this volume), off-target effects due to an incomplete specificity of constructed guide-RNAs cannot be excluded, which are associated with off-target DNA cleavages. These can contribute to an evolutionary instability of gene drives. Furthermore, cross-fertilization has been reported among various mosquito species (Tripet et al. 2011). Thus, one insect species could take the role of transmitting diseases from another.

At the same time, it is also well known that *Anopheles* mosquitoes possess a large genetic diversity and evolutionary adaptability. In particular, that is the case for *An. gambiae*, the primary carrier of *Plasmodium* in sub-Saharan Africa (The *Anopheles gambiae* 1000 Genomes Consortium 2017). Hence, the genetic variability of targeted mosquito species is a hurdle for an efficient gene drive strategy and a single gene drive might not be effective so that the development and use of many gene drive constructs might be needed to cover the range of genetic variants of *Anopheles*. Hence, functioning of mutagenic chain reactions or gene drives in malaria-transmitting mosquito populations is questioned from various sides. Development risks are numerous, but scientists eager to take on the challenges could ultimately overcome the obstacles. Therefore, one should be careful in stating that gene drives are not feasible; rather, one should anticipate that they can become a reality in the near future, and therefore there is an urgent need to study the risks and consequences involved.

4. RISKS, CONSEQUENCES, AND UNCERTAINTIES

What risks would be involved using gene drives to suppress insect pests in the wild? Unleashing a highly potent mutagenic chain reaction in insects like malaria-transmitting mosquitoes could eventually spread across national borders. The population extinction programme could potentially work globally. This begs the following pertinent questions: who has the legitimate right to decide the initiation of such a mission and who will regulate it? Who will take part in the decision-making? What to do if approval is obtained in one country but not in the neighbouring country? Fundamentally, this would concern all living beings, and humankind as a whole should be asked. Could that be organized?

What, if modified mosquito genes mutate and further evolve, creating possibly unwanted variants? Could that be induced by off-target effects due to the fact that the specificity of guide RNAs targeting cleavage points in the DNA is not 100%? Polymorphism as a genetic variation inside a population could be induced causing unclear consequences (Araki et al. 2014). Recently, a scientific debate has started over the importance of off-target effects. The creation of unintended single-nucleotide variants – not only small insertions and deletions (indels) – would fuel concerns. That can only be detected by whole-genome sequencing after gene-editing with CRISPR-Cas9. Unfortunately, that is seldom done by the researchers so far.[3]

Is it possible that after implementing the genetically engineered population suppression or modification against *An. gambiae*, *Plasmodium falciparum* Welch and/or other malaria parasites find a way to change their currently favoured or most important host mosquito? The ecological niche left open could be filled again. We already know about 30 mosquito species that can transmit malaria, but what would be the next move? Would this require efforts to eradicate more and more mosquito populations or species by engineering and unleashing more and more gene drives in nature? Moreover, the reduction of transmitting rates could lead to increased selection pressure on the pathogen itself, which could in turn develop an increased virulence (David et al. 2013).

As cross-fertilization between different mosquito species has been observed (Fang 2010), an interspecific gene flow could affect other non-target mosquitoes or insects. This entails the potential of a gene drive-based eradication programme to jump over to other species (David et al. 2013), in particular if target sequences are equal. An effective elimination programme, originally targeting one species, could then result in significant consequences for ecosystems.

If targeted mosquito species play important roles in ecosystems or if other species are non-intentionally also affected, unwanted cascading effects in ecosystems are possible. Is it scientifically irrefutably clarified that malaria mosquitoes only have a damaging function in nature by infecting humans and other hosts? Do they have instead also important beneficial or indispensable roles in the food chain or in pollination processes? For mosquitoes in general, there are examples showing such vital purposes, like in the Camargue, in Nordic Arctic, or in aquatic systems (Fang 2010), but mainly we have to admit ignorance about side effects of eradicating malaria-transmitting *Anopheles* mosquitoes (David et al. 2013). However, a comparison in this respect with other methods of malaria vector control must not turn out negatively for gene drives.

Questions related to modification/manipulation drives indirectly aimed at the *Plasmodium* parasite or rendering the parasite harmless for humans are quite similar, even though the strategy would be different, i.e. not suppression or eradication. Off-target effects could also have negative consequences and unwanted genetically engineered variants could emerge and reproduce. Accidentally, also the pathogenicity of the parasite could be increased.

It is well known that parasites evolve quickly and in unforeseen ways in relation to their hosts (Wijayawardena et al. 2013. A genetically engineered intervention into this interplay could result in unexpected or even damaging consequences. Could a mutating parasite like *Plasmodium* escape the grasp of newly engineered characteristics of the host mosquito? Would that just cheat the gene drive or probably even facilitate malaria transmission pathways or render the infections´ impact on humans more likely or worse? Would that maybe provoke the engineering of another gene drive attempt and after the next one, another one, and so on?

Some scientists involved in gene drive research and development are warning that modified/manipulated drives are highly invasive in wild populations, even if their efficiency is low or resistance against the drive occurs (Noble et al. 2018). The question, among others, is what consequences will the spread of newly introduced alleles have? Is it possible to clarify this in advance? Is that also relevant for suppression drives, even if they are more or less limited in their potential to drive to fixation?

Thus, several risks and consequences of gene drives can be anticipated in all clarity. However, there are also plenty of uncertainties and unknowns due to the dynamic complexity of natural systems. Some important dynamical features are probably unknowable. A report of the US National Academy of Sciences (NAS 2016) has confirmed that many gaps exist in our knowledge on off-target effects of gene drives inside targeted organisms and on non-intended effects on other species and the environment.

5. FUNDAMENTAL PROBLEMS AND QUESTIONS RELATED TO GENE DRIVES

If it is possible to engineer gene drives working effectively in a natural environment, then any single release of one gene drive modified organism could have irreversible consequences. The characteristics of the modified organism or the ramifications of the induced mutagenic chain reaction could also turn out as "wrong" or become detrimental, what is probably unknown prior to its release. Therefore, there is no tolerable limited release, as long as unwanted impacts cannot be completely excluded. The dual-use potential of gene drives seems to be obvious. Once it is sufficiently clarified that gene drives can be reliably engineered, small competent groups could (with only a limited amount of money) covertly pursue a strategy to engineer and use gene drives to the harm of others. One example could be an attacker (state or non-state actor) who decides to manipulate an important organism that is beneficial for agriculture and that can only be found regionally or locally, with the aim to harm an adversary. Also, weapon-like effects of engineered gene drives are imaginable.[4] Studying such possibilities in detail is therefore necessary prior to major investments in the development of gene drive technology.

With mutagenic chain reactions becoming a reality, the already significant depth of intervention by humans into natural processes would be massively increased. It has been proposed to differentiate genetic methods of mosquito control into more or less *self-limiting* approaches, and in increasingly *self-sustaining* invasive tools, which can or should persist in nature (Alphey 2014). Gene drives, in principle, correspond to the latter category and could become extremely powerful technologies, but with a high risk as unintended changes could become fully irreversible.

The precautionary principle, which is a guideline at least in the European context, would require an in-depth study of the risks of this new technology prior to any development in the laboratory and even more so prior to any consideration about application in nature. A number of serious risks and probable hazardous events due to gene drives have been already identified (cf. e.g. Hayes et al. 2018, p. S143). Those must be scrutinized and "Scientists must remain mindful that great power entails equally great responsibility, and take precautions accordingly" (Min et al. 2018, p. S54).

Unlike genetically modified organisms (GMOs), animals manipulated by gene drives are engineered with selfish genetic elements deliberately designed to spread in the environment and to operate autonomously in nature. Could these constructed or affected organisms evolve in a way not anticipated or even not anticipatable by their designers? Must we realize that a new form of technology is emerging?

Jan Schmidt (2015, 2016) has coined the term *late-modern technology* ("nach-moderne Technik") to identify a remarkable paradigm-shift. This new type of technology is based on the concept of self-organization and is linked to instabilities which can be triggered in non-linear dynamical systems. In contrast to the classic-modern type of technology, which is related to concepts of stability, linearization, predictability and controllability of functions and outcomes, in late-modern technology evolutionary, self-organizational, and non-linear features are exploited, which could lead in principle to intrinsic limits with respect to predictability and controllability.

Organisms manipulated by an engineered gene drive are not only intentionally self-replicating but also capable of further evolutionary changes. This technology has to go through instabilities and has to trigger instabilities. It is a type of technology which acts nature-like in nature. However, this kind of built-in dynamic is provoking limitations with regard to the possibility of a stable construction of the techno-biological system and also with regard to subsequent monitoring and control of the technology. A remarkable difference to classical modern and rational technology concepts is emerging.

Engineering gene drives to fight malaria could turn out as a harbinger of much more. Could gene drives against other mosquitoes, or against other insects in general or rodents transmitting infectious diseases be next? Gene drives could also be engineered against any so-called pest animals. Various tephritid fruit flies or plague locusts (Acrididae) could be a target. Also, non-native invasive species (plants as well as animals) could be attacked, eventually eradicated. All that is already mentioned in the NAS (2016) report. The research, development and use of gene drives against malaria-transmitting mosquitoes, which can be argued for based on convincing humanitarian objectives, could be the door-opener for a new dimension of human campaigns in and probably against nature or its biodiversity.

What starts with the fight against malaria could end up with nature being totally in the hands of humans (“Natur unter Menschenhand”), nature under complete management of humans, as the biologist and influential science manager Hubert Markl propagated already 20 years ago (Markl 1998, p. 147). Gene drives will dramatically change the way humans can interact with nature. Humans will have a tool then, to deliberately steer evolution – with less time for the ecosystems to adapt to the induced turbulence, in contrast to changes due to current tools and naturally occurring mutations. Humans can decide what species they like or dislike, which one has the right to survive in a given form, etc.

One of the young shooting-stars on the scientific scene (emerging from the group around George B. Church), Kevin Esvelt, already named his new working group at the Massachusetts Institute of Technology “Sculpting Evolution”. Despite the far-reaching visions, however, we are faced with huge unknowns of the complex, non-linear interactions in genetic transfer, living cells and organisms, populations, ecosystems, and sensitive global life connections.

It appears that an intrinsic logic might underlie gene drive approaches against malaria-transmitting mosquitoes, as well as the other above-mentioned objectives: it is conceivable that after a first gene drive attempt more and more gene drives have to be engineered and released to correct or improve what wasn´t achieved in the first step. As one gene drive will not suffice, the pressure for more will “naturally” be generated to bring about human control – which could turn out as being unachievable in the end. Do we have to expect a chain reaction of mutagenic chain reactions? A pathway towards life on earth totally in the hand of humans, prone to human errors and ignorance?

6. SHAPING OPPORTUNITIES FOR GENE DRIVES?

Is there a chance to shape gene drives in a way that serious risks associated with them could be eliminated? Engineering “reversal drives” has been proposed that can undo results of a drive if necessary. But how to deal with off-target effects of both the original and the reversal drives? One is stuck in a principle problem. Therefore, even for several scientists involved in gene drive research, engineering reversal drives is not a convincing concept because a potential fundamental irreversibility of gene drives is admitted, and a reversal drive could only be a second drive which again could be ill-targeted.

Recently, self-limiting CRISPR-based drives have been proposed (Noble et al. 2019). But, so-called *daisy-chain drives* are just theoretical models and do not reflect on the real complex dynamics in real life. Furthermore, this concept seems to be more a test-bed approach, where drives at first (hopefully) are locally and timely restricted, but later a full-fleshed global release is intended. Furthermore, my impression is that it is more likely that all attempts to improve gene drives by additional features, proposed so far, increase the complexity and non-linearity of the engineered constructs and thus it is more likely that they would increase concerns.

7. ALTERNATIVES TO GENE DRIVES

If one hesitates to believe in gene drives as the new silver bullet against malaria, one has to refer to the alternatives. Several ideas for technical alternatives are currently discussed, researched, validated and are partly in use (Alphey 2014; Bourtzis et al. 2016; Fasulo et al., this volume; Häcker and Schetelig, this volume). One example is the infection of *Aedes* or *Anopheles* mosquitoes with *Wolbachia* bacteria (Incompatible Insect Technique or IIT), which are maternally inherited and affect reproduction capabilities, leading to strategies to suppress or replace mosquito populations (Bourtzis et al. 2016). Another example of population suppression is the release of genetically modified mosquitoes passing on dominant lethal factors to their offspring (RIDL) (Alphey 2014; but see Evans et al. (2019).

Another approach, which has been in use for over 50 years on all continents against major agricultural pest insects, is the Sterile Insect Technique (SIT), where large numbers of the target insects are mass-reared, sterilized by radiation, and then released in order to negatively influence the reproduction of insect populations (Dyck et al. 2021).

Multiple releases of sufficient quantities of manipulated mosquitoes are necessary in all these cases (when population suppression rather than replacement is the objective), to obtain the wanted results. Such approaches can also be debated and must be carefully assessed. Obeying the precautionary principle is also mandatory for these technologies. But, in principle, one could say, that unpleasant risks and other ramifications of these alternative technologies might be less severe than in the case of gene drives. In the case of the SIT, there are decades of track record of successful large-scale application against many pest insects. As the released insects are sterile, they cannot become established, and thus there is no irreversibility.[5]

8. GLOBAL PROGRAMME TO ELIMINATE MALARIA

Of particular importance with regard to alternatives is the global programme to eliminate malaria. The United Nations and the WHO have declared the intent of reaching this goal by 2030 (WHO 2015b) using the following classical methods:

- vector control, in particular by distribution of long-lasting insecticidal bed nets; indoor residual spraying; mosquito screening, surveillance and monitoring; and education of citizens in endemic regions
- prevention, in particular preventive intermittent chemoprophylactic treatment of pregnant women and children under 5 years, especially in many African regions
- better access and use of diagnostic testing and appropriate medical treatment (e.g. artemisinin-based combination therapy).

In this century, important successes in the fight against malaria have already been recorded: a reduction of malaria incidence by 37% and of mortality rate by 60% (WHO 2015a). Therefore, the hopes are high that the malaria elimination strategy can be accomplished, if sufficient funding can be raised (much more than USD 2.5 billion annually, which was the global financing for malaria control in 2014, will be needed) and if a concerted effort and enduring engagement of all stakeholders can be achieved over the next coming years. In this struggle, unglamorous tasks such as improvement or set up a minimally functional health care system are critical, including durable and affordable access to diagnostics and pharmaceuticals, as well as educating and empowering communities so that they can reduce the risk themselves. A success with this strategy would also have further positive ramifications not just with respect to malaria.

It is clear that the global programme to eliminate malaria involves much more than just high technological means such as gene drives. The malaria challenge is not only a problem solvable with scientific-technological approaches, but also social, political and economic factors have to be addressed. Not only is vector control crucial, but in the end, control of *P. falciparum* and other parasites is of utmost importance. The parasite cycle has to be interrupted in a sustainable manner, which is much more an issue of health care, access to suitable simple measures, societal development, etc. than using sophisticated novel technologies. Without a somewhat stable (minimal) health care system in the endemic regions of concern, elimination of malaria is impossible. Also, the socio-economic conditions of the disease's origin, besides the natural-scientific causes for malaria, need to be scrutinized in order to find appropriate means to support the transition process towards malaria elimination. Population growth of mosquitoes has also to do with socio-cultural or techno-economic change, for example the rapid increase of plastic containers used for food distribution or scrap tyres provide some mosquitoes with ideal breeding grounds.

One should also remark that in several countries past (successful) elimination campaigns also had harmful side effects on the environment by massive use of DDT; furthermore, first resistances of mosquitoes against insecticides developed at that time. Improper use of malaria drugs, which mostly have to be taken in suitable combinations, has also led to resistance of the malaria parasite.

9. NORMATIVE QUESTIONS

Many issues and values involved in the necessary debate on gene drives are mentioned in the NAS (2016) study “Gene drives on the horizon”. Many scientists state: “risk has to be balanced against benefit”, which can lead to a purely utilitarian position. Clearly, weighing positive and negative consequences is a relevant part, but ethical reflection should heed all ramifications and also fundamental problematics.

Derived from Hans Jonas´ principle of *responsibility* (Jonas 1979), which aims at achieving a “conservative” preservation of our lifeworld, the precautionary principle focuses mainly on objective reasoning in respect to serious risks, notwithstanding that benefits might also be possible. On the other hand, an unfolding principle, which strives for “*alliance technology*” serving humankind and being concurrently in harmony with nature (Bloch 1959), requests a positive and socially just developmental progression of humankind by using new technology that is bound to its alliance with nature, in harmony with nature. Both principles which may be contradictory at a first glance can be used as normative orientation[6], already in the process of research and development, when striving for the eradication of malaria (probably using gene drives).

The perceived role of humans in nature is highly relevant: what position has humankind within nature, still being a central part of our common lifeworld which we share with other living beings? One position claims a human role as the manager of all life on earth, man as “master of nature” (Descartes), the other sees humankind embedded into nature and as partner of life on earth, or as Albert Schweitzer has formulated it in his principle *respect for life* (Schweitzer 1966)*: I am life that wants to live in the middle of life that wants to live.*

How should members of the scientific community and of our societies behave in between these diametrically opposite positions? Schweitzer´s position seems to be incompatible with an approach where humankind feels entitled to steer evolution on earth. Who is entitled to change nature in a way that it could irreversibly affect all life on earth? A single researcher, the scientific community, a competent national agency, a nation?

In any case, the whole fabric of risks, uncertainties and ignorance, and the possible dramatic consequences of mutagenic chain reactions, will (and must) have a massive influence on ethical discourse debating responsible conduct in gene drive R&D directed towards practical use, and other technology-based malaria elimination strategies. In the end, it has to be assessed which procedure for malaria elimination seems to be promising, associated with low risk, and is globally, societally and ethically acceptable. I deem it as obvious that the WHO strategy cannot be replaced by anything else. But maybe additional new measures or tools could be helpful.

All potentials, risks, uncertainties, ramifications of gene drive R&D have to be made transparent within science and to the broader public as early as possible. When a gene drive technology against malaria-transmitting mosquitoes seems to be mature – and that could soon be the intuition of several researchers and funding organizations (like the Gates Foundation) involved – it would probably be too late to be withheld. Then it will be no longer possible to stop its use in a region with serious malaria burden.

The hope of promoters is that some advantages will predominate notwithstanding those arguments describing possible or anticipatable negative side effects or long-term consequences, serious uncertainties and ramifications that have been put forward. All the concerns will then be covered-up by promises and hopes.

In conclusion, using the example of combatting malaria, this contribution tried to substantiate why prospective technology assessment in the field of gene drive research and development is urgent and what issues should be assessed in order to provide input for decision making inside and outside science. The pertinent questions to be answered are going beyond tailored disciplinary research fields of scientific specialty. As scientists involved in gene drive research have put it:

> *“Determining whether, when, and how to develop gene drive interventions responsibly will be a defining challenge of our time”* (Min et al. 2018, p. S40).

10. NOTES

(1) More precisely: homing endonuclease genes (HEGs) or homing-based gene drives.

(2) The entomologist Flaminia Catteruccia is cited with: “…you can have the fanciest technology on earth, the perfect gene drive, but if your laboratory mosquitoes can´t mate with wild mosquitoes, then it´s not going to work at all” (Shaw 2016).

(3) Schaefer et al. (2017) had reported in Nature an unexpected high number of unintended single-nucleotide variants after genome editing with CRISPR-Cas9 in a laboratory population of mice. After several criticisms, the Nature editors have withdrawn the paper because it could not be shown beyond any doubt that the use of the gene scissor was the cause of these effects. However, they admitted that Schaefer et al. “did not examine only predicted sites”, as in many other studies, “but looked at the entire genome” and that the “work of Schaefer et al. highlights limitations in the current literature that should be considered” (Nature Methods 15 (4): 229-230).

(4) At end of 2017, it has been revealed that the US Defense Advanced Research Projects Agency (DARPA) is investing 100 million USD in gene drive technology (http://genedrivefiles.synbiowatch.org/).

(5) Therefore, sterile insects are accepted as beneficial organisms by the International Plant Protection Convention (FAO 2005), to which 183 countries are signatories.

(6) More about these viewpoints can be found in Liebert and Schmidt (2015).

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GENOME EDITING AND ITS APPLICATIONS FOR INSECT PEST CONTROL: CURSE OR BLESSING?

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SUMMARY

Gene and genome editing are described as cutting-edge research tools with the potential to tackle urgent global challenges in the management of agricultural pests and human disease vectors such as mosquitoes. The field is defined by the chances and challenges to interlink the disciplines of insect genomics, molecular biology, and pest control together with the need for clear risk assessment, policy development and public approval of the application of such novel technologies. The goal is to generate innovative and sustainable pest control solutions applied in the best interest for the environment and human society. Here, starting from available genome editing technologies, the current strategies and applications for insect pest control are discussed, including approaches to overcome the evolution of resistance alleles and other potential pitfalls to be expected from selective pressures resulting from gene drive applications. They are supplemented by views on regulatory, policy and ethical considerations that in our opinion will be necessary to define how the different tools can be used in the future in a safe and responsible way.

Key Words: CRISPR-Cas9, gene editing, gene drive, genome engineering, GMO, transgenic, insect genomics, engineered nucleases, meganucleases, zinc finger nucleases, TALEN, homing endonucleases, regulations, risk assessment, ethics

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1. INTRODUCTION

Europe faces serious problems related to insect pests in two key areas: health and agriculture. The European Centre for Disease Prevention and Control has warned European authorities of the increased risk of local transmission cycles and epidemics of mosquito-borne infectious diseases (ECDC 2018). Locally transmitted cases of malaria, for instance, have already been reported in Greece, while in other regions in the world the disease claims nearly half a million lives annually. Similar threats are emerging with viral diseases such as chikungunya and viral haemorrhagic fevers including Hantaan, dengue, and yellow fever. European authorities have responded to this new threat with recommendations for vector control measures, as well as increased financing for relevant research, demonstrating the interest and concern of the European Union (EU).

Additionally, endemic and invasive agricultural insect pests cause tremendous losses in agricultural yield and revenues. Worldwide, insects cause up to 40% loss of potential crop yields, and the European Environment Agency reported the presence of over 10 000 invasive pest species for Europe in 2017 (EEA 2017). Significant damage caused by these pests affect the economy and negatively impact biodiversity. Invasive species directly cost Europe billions of Euros per year. Additionally, heavy reliance on pesticides to control them generates significant unquantified costs. These are costs associated with environmental pollution, e.g. soil and water. Other costs arise from resistant pests and include those required to develop alternative new effective pesticides. Also, loss in biodiversity, reintroducing biodiversity, and the impact on public health from pesticide residues or direct exposure to pesticides add to the expenses.

For these reasons, innovative pest management methods and strategies are urgently needed. Numerous stakeholders have expressed this urgency to prevent total crop losses and combat diseases transferred by mosquito vectors, but, at the same time, demand clear information about the risks and benefits of such novel technologies.

Novel vector and pest control solutions based on genome editing tools have the potential to improve the lives of millions of people, both by offering adequate protection against insect-borne diseases and by preventing crop and livestock damages caused by invasive agricultural pests. By using these novel technologies, different approaches are now possible that were only fiction in the past. While they sound promising, their possible side effects have to be considered and detailed evaluations prepared concerning the applicability of such new systems. Moreover, societal questions need to be answered like ‘Should we use some of the technologies at all?’ In the end, it is a combination of science, technology, ethics, policy, and communication that will define the feasibility and applicability of one or the other technology.

In this chapter, we want to review the technological genome editing options that are available and have been developed in the field so far. We also discuss their 'curse or blessing', together with considerations for their safe and responsible application for insect pest control tactics and strategies that do not pose risks and are friendly to the environment. The views presented here are the personal opinion of the authors on this important topic, without the claim for completeness or being the only or best possible solution. More considerations on the evaluation of gene editing and gene drive technologies are presented in the chapters by Liebert (this volume) and Nielsen (this volume).

2. AVAILABLE GENOME EDITING TECHNOLOGIES

Genome editing technologies can be divided into three categories: homologous recombination, engineered nucleases, and the CRISPR/Cas system.

2.1. Homologous Recombination

The early genome editing trials were performed by *homologous recombination* (HR), based on observations from the yeast *Saccharomyces cerevisiae* Meyen ex E.C. Hansen, where HR occurs at a high frequency. A specific sequence can be inserted into the genome at a defined position by flanking the sequence with homology arms identical to the sequences left and right of the selected insertion position. The construct is then injected into cells and inserted into the genome by recombination of the homology arms with the corresponding genomic sequences. However, the recombination rate in most cell types is meagre. In higher plants, it is estimated to be in the range of 0.01-0.1% (Puchta 2002; Hanin and Paszkowski 2003; Reiss 2003). In mammalian cells such as mouse embryonic stem cells it can be 1% or higher, but it can as well be as low as one in more than a million events (Vasquez et al. 2001). Moreover, the integration often occurs at unspecific sites, leading to off-targeting with a frequency of one in 10^2 to 10^4 treated cells (Vasquez et al. 2001). Design of the homology arms (sequence and length) seems crucial for the off-target rate in human cells, as the genome contains a lot of repetitive elements such as LINEs and SINEs (Long and Short Interspersed Nuclear Elements), which comprise 36% of the human genome. The longer the arms, the higher the probability of including such repetitive elements, which can cause recombination at unspecific sites (Ishii et al. 2014).

2.2. Engineered Nucleases

A more efficient way to edit genomes was established with the use of *engineered nucleases*. These nucleases act like molecular scissors, inducing double strand breaks at specific genomic positions that are then repaired by one of the two cellular repair pathways: non-homologous end joining (NHEJ) or homology-directed repair (HDR).

Three different nuclease families have been engineered for genome editing: meganucleases, zinc finger nucleases (ZFNs), and transcription activator-like effector-based nucleases (TALEN).

Meganucleases are predominantly found in microbes, and it is almost impossible to find a natural meganuclease targeting the specified sequence. Therefore, scientists applied different strategies, including random mutagenesis and high throughput screening, fusion of different nucleases, as well as rational design, to modify the binding specificity of the enzymes to expand the rather limited choice of target sequences (Sussman et al. 2004; Arnould et al. 2006; Rosen et al. 2006). With a recognition sequence of 14-40 nucleotides, meganucleases have a high target site specificity.

The ZFNs and TALENs are fusion proteins consisting of a non-specific DNA cutting enzyme, the restriction endonuclease Fok1, which is linked to a zinc finger (ZF) or transcription activator-like effector (TALE) domain. These peptides recognize specific DNA sequences and thereby confer sequence specificity to the endonuclease. Like meganucleases, the choice of naturally occurring target sites of ZFs and TALEs is limited and can be extended by protein engineering to theoretically bind nearly any desired sequence.

Targeted genome editing including TALENs was named the 2011 method of the year by Nature Methods (Becker 2012). Protein engineering, however, is time consuming, cumbersome and costly. Therefore, while ZFNs and TALENs were successfully applied to modify different insect genomes (Bozas et al. 2009; Takasu et al. 2010; Liu et al. 2012; Aryan et al. 2013; Smidler et al. 2013; Takasu et al. 2013), a widespread use of these endonucleases was prevented by the need for a new engineered protein for each new genomic target site.

2.3. The CRISPR-Cas System

The discovery of *Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)* has tremendously advanced the field of genome editing. CRISPR sequences were first discovered in bacteria and archaea in the 1990s and were identified as a prokaryotic equivalent to the eukaryotic acquired immune system. Upon infection of a bacterial or archaeal cell with a pathogen, the cell incorporates a short sequence of the foreign DNA (e.g. virus or plasmid) into its genome. Such foreign DNA sequences are collected in clusters and separated by short repeat sequences. Small clusters of Cas (CRISPR associated) genes are located next to the repeat-spacer arrays (for comprehensive explanations and illustrations see Horvath and Barrangou 2010; Marraffini and Sontheimer 2010; Bhaya et al. 2011). Upon reinfection, the arrays are transcribed and processed by one family of Cas proteins into short CRISPR RNAs (crRNA), which are bound by another class of Cas proteins.

The crRNAs then guide these Cas proteins to the foreign DNA for degradation by endonucleolytic cleavage in a mechanism similar to RNA interference in higher eukaryotes (Marraffini and Sontheimer 2010). The target site specificity of the Cas endonuclease is determined by its bound crRNA. Therefore, Cas proteins can be programmed to target nearly any genomic site by adjusting the crRNA sequence. As the crRNA sequence adjustment is easy and cost-effective, CRISPR-Cas is an ideal and versatile tool for genome editing and has essentially abolished the use of ZFNs and TALENs.

The Cas proteins are categorized into two classes. The class I systems use multiple Cas proteins for the degradation of foreign nucleic acids, whereas the class II systems consist of one single protein. Therefore, the class II systems are more suitable for research and application purposes. The most used nuclease for CRISPR genome editing is the multifunctional class II protein *Cas9*. In addition to the crRNA, Cas9 requires a transactivating CRISPR RNA (tracrRNA) to function. To streamline its application in the laboratory, scientist fused the crRNA and tracrRNA into one single-guide RNA (sgRNA) (Jinek et al. 2012). CRISPR-Cas can be used to knock out existing genes making use of the cell's NHEJ repair pathway or to introduce new DNA sequences including whole genes and transgene constructs by adding a repair template containing the respective sequence information and homology regions to the Cas9 target site. The cell's HDR pathway then uses the repair template to repair the Cas-induced double-strand break.

Since its first application for genome editing in 2012/2013, more class II Cas proteins suitable for genome editing purposes have been identified (Zetsche et al. 2015; Abudayyeh et al. 2016; Yang et al. 2016), and the system is continuously being adjusted and optimized for different applications and purposes. These efforts include modifications to decrease the off-target rate of the CRISPR-Cas system (Fu et al. 2014; Kleinstiver et al. 2016; Nowak et al. 2016).

Shortly after its first application for genome editing, CRISPR-Cas was used in *Drosophila melanogaster* Meigen (Ren et al. 2013; Yu et al. 2013; Bassett et al. 2014). Within just four years, it was subsequently applied to several other insect species including the lepidopterans *Bombyx mori* L. (Wang et al. 2013; Ma et al. 2014), *Spodoptera litura* (F.) (Bi et al. 2016), and *Danaus plexippus* (L.) (Markert et al. 2016), the orthopteran *Gryllus bimaculatus* (De Geer) (Awata et al. 2015), and importantly also to several dipteran vector and pest species, namely *Aedes aegypti* (L.) (Kistler et al. 2015), *Anopheles gambiae* (Giles) (Hammond et al. 2016), *Ceratitis capitata* (Wiedemann) (Meccariello et al. 2017; Aumann et al. 2018) and *Drosophila suzukii* (Matsumura) (Li and Scott 2016; Kalajdzic and Schetelig 2017; Li and Handler 2017), as well as in the non-pest dipteran, *Musca domestica* L. (Heinze et al. 2017).

The CRISPR-Cas genome editing system also transformed gene drive research and development. Gene drives are genetic drive mechanisms that can be used to spread a genetic trait through a population by biasing its inheritance beyond the Mendelian inheritance rate of 50%. Gene drives were initially designed using naturally occurring selfish genetic elements like *Medea* (maternal-effect dominant embryonic arrest) (Chen et al. 2007; Buchman et al. 2018) or homing endonucleases

(HE) (Burt 2003; Windbichler et al. 2011). HE recognize and cut short DNA sequences. These sequences are only located on chromosomes different from the one on which the HE is located, and additionally on the homologous chromosome exactly at the location of the HE. In a heterozygous individual, after introduction of a double-strand brake by the HE on the homologous chromosome, the cell's repair mechanism uses the chromosome containing the HE to repair the brake, thereby copying the HE gene onto the homologous chromosome and converting the heterozygote into a homozygote. This process is called *homing*.

The CRISPR-Cas system can be programmed to act like an HE by targeting its genomic integration site on the homologous chromosome using a respective homing guide RNA. Identical to the HE, the induced cut is repaired using the allele containing the CRISPR construct as a template, thereby copying it onto the homologous chromosome. Thus, in contrast to genome editing via CRISPR, where only the CRISPR-induced molecular changes, but not the CRISPR components themselves are passed on to the next generation, in CRISPR gene drives also the genes coding for the CRISPR components are incorporated into the genome and passed on to the offspring. With the inherent programmable target site specificity of the CRISPR-Cas system, nearly any genomic position can be selected for the placement of the CRISPR homing construct. If homing occurs in the germline, then theoretically all the offspring will carry the CRISPR construct instead of only 50% like in normal Mendelian inheritance.

The potential use of these genome editing technologies, especially of CRISPR-Cas gene drives for insect pest control, is discussed below.

3. CURRENT STRATEGIES AND APPLICATIONS INVOLVING GENOME EDITING FOR INSECT PEST CONTROL

A promising and proven, sustainable and species-specific method to manage insect pest populations is the Sterile Insect Technique (SIT) (Dyck et al. 2021). It is based on the mass-production and release of sterilized males of the target species in the affected area. The sterile males mate with wild females in the field, which will not result in viable offspring, thereby reducing the wild population size of the pest in the next generation. By repeated releases, the population can be decreased to a manageable level. The SIT is most successfully applied in area-wide programmes against several pest species of agricultural importance, including the Mediterranean fruit fly, the Mexican fruit fly, *Anastrepha ludens* Loew, or the New World screwworm, *Cochliomyia hominivorax* (Coquerel).

The effectiveness of such programmes can in some cases be increased with the establishment of so-called sexing systems to eliminate females to allow male-only releases. Male-only releases are desirable for some agricultural pests and are a prerequisite for vector insects such as mosquitoes. In both cases, early elimination of females enables more cost-effective mass-rearing and release.

Most importantly, the efficiency of male-only releases is superior due to the lack of undesired mating between sterilized males and sterilized females (Franz et al. 2021).

In mosquito control programmes, the release of only male insects is essential as it precludes the risk of increasing the number of disease-transmitting individuals by releasing females. Therefore, mass-reared males and females must be separated at large-scale or females be eliminated at some point during the production process. This sexing of the insects (needed up to one billion male insects per week) is a significant bottleneck for the application of the SIT to new insect species. There is, for example, no effective sexing system to date for any of the vector mosquitoes.

For recent field trials with transgenic *Ae. aegypti* mosquitoes carrying a conditional lethal RIDL system (Release of Insects carrying a Dominant Lethal) that kills the offspring in the late larval stage (Thomas et al. 2000; Phuc et al. 2007), male and female pupae were separated mechanically by hand, resulting in the production of 0.5-1.5 million males per week and a female contamination of the released insects of less than 1% (Carvalho et al. 2015). This labour-intensive and time-consuming method, however, does not allow large-scale programmes beyond the field trial scale. Therefore, coordinated international research efforts are ongoing to establish sexing systems in different *Anopheles* and *Aedes* species (Gilles et al. 2014; Bourtzis and Tu 2018). Genome editing, combined with available classical genetics or transgenic technologies, can help to solve these and other issues related to insect pest control.

3.1. Unravelling Sex Determination Pathways in Insects

Genome editing is being used in basic research of insect pests, for example, to uncover gene functions and thereby better understand the target insect's biology and physiology. One major point of interest concerning sexing is the elucidation of the sex determination pathways in pest species to identify the responsible gene(s) for male/female development. CRISPR technology was used to knock out the candidate gene for the male-determining factor *Nix* in *Ae. aegypti* (Hall et al. 2015). Knockout of *Nix* resulted in feminized males, whereas ectopic expression resulted in masculinized females, identifying *Nix* as necessary and sufficient for determining maleness in the yellow fever mosquito.

Similarly, the Cas9-mediated knockout of the candidate M-factor gene *Mdmd* in *M. domestica* confirmed the key role of this gene for male gonadal and germline development in the house fly (Sharma et al. 2017). Once the sex determination pathways are understood for pest species of interest, the knockout or overexpression of the sex determination genes via CRISPR-Cas could be used to create female lethality, or for the conversion of females into phenotypic males to create strains for large-scale sexing (Meccariello et al. 2019).

3.2. Site-directed Mutagenesis in Pest Insects to Enable Population Control

For several economically important insect species, transgenic insect strains have been established to demonstrate the ability to generate male-only populations for control programmes. The first strains consisted of transgenic conditional female-lethal systems in agricultural and livestock pests like *C. capitata* (Fu et al. 2007; Ogaugwu et al. 2013), *Anastrepha suspensa* Loew (Schetelig and Handler 2012b), *A. ludens* (Schetelig et al. 2016), and *L. cuprina* (Yan et al. 2017). In these systems, under permissive conditions, a lethal gene is explicitly expressed in females to kill them at an early embryonic stage via apoptosis (Schetelig and Handler 2012b; Ogaugwu et al. 2013; Schetelig et al. 2016; Yan et al. 2017), or later in development by the ubiquitous accumulation of tTA (Fu et al. 2007), resulting in a 100% male cohort for release. Similar systems have been created expressing the lethal cassette in both sexes to produce genetic sterility (Gong et al. 2005; Schetelig et al. 2009; Schetelig and Handler 2012a). A release of these insects would result in biologically fertile matings, but the offspring would die between the early embryonic (Schetelig et al. 2009; Schetelig and Handler 2012a) and the late larval stage (Gong et al. 2005). All the transgenic systems described here, and many others, have commonly been introduced into the insect genomes via transposable elements, which integrate into the genome randomly. Integration at an unfavourable genomic site, however, can have adverse effects on insect fitness due to insertional mutagenesis, as well as on transgene expression levels due to nearby regulatory genomic elements. Genome editing technologies now allow inserting the transgene construct at specific, characterized genomic positions, thus avoiding harmful side effects of random integrations and improving the quality of transgenic strains for pest control.

Genome editing technologies furthermore offer the possibility of recreating a genetic trait from one species in another, which is not possible with any other technology with similar efficiency and ease. Mediterranean fruit fly SIT programmes, for example, rely on a conditional, temperature sensitive lethal (*tsl*) mutation, obtained by classical mutagenesis and breeding, to eliminate female embryos during mass-rearing via heat shock, resulting in the release of only sterile males (Franz et al. 2021). It has been tried to generate such *tsl* strains in other insect pests via classical mutagenesis (Ndo et al. 2018), or to link other selectable makers to one sex via radiation-induced translocations. Most successful have been pupal colour markers in the Mediterranean fruit fly and the Mexican fruit fly, as well as dieldrin resistance in *An. gambiae* (Curtis et al. 1976), *An. arabiensis* (Curtis 1978) and *Anopheles albimanus* Wiedemann (Seawright et al. 1978). In *An. arabiensis*, classical mutagenesis was used again years later to induce insecticide resistance in males as a tool for female elimination (Yamada et al. 2012). All these sexing tools have been developed either by classical mutagenesis or selection of a naturally occurring phenotype and linking it to one sex. The underlying mutations and mechanism of the corresponding phenotype in most cases are unknown, although it would be extremely valuable to know. Once such a mutation created by classical mutagenesis (e.g. the conditional *tsl* in the Mediterranean fruit fly) is identified, CRISPR-Cas genome editing could be used to precisely edit the genome of another pest species to create an identical mutation in the respective gene. The mutation created in this way would not be different from the mutations induced by the approved classical mutagenesis techniques, not involving any transgene or foreign DNA (but usually rather small deletions or single nucleotide changes).

Li and Handler (2017) recently used CRISPR-Cas to create point mutations into the transformer sex determination gene in the fruit pest *D. suzukii,* thereby recreating the temperature-sensitive *D. melanogaster transformer-2* mutations (*tra-2*^{ts1} and *tra-2*^{ts2}) in a proof-of-principle experiment. The CRISPR-Cas system was also used to create an X-shredder in *An. gambiae* mosquitoes to achieve female elimination. The Cas9 is expressed gonadally to target X-chromosomal ribosomal RNA sequences, thereby destroying the X chromosome in X gametes, resulting in predominantly Y gametes and therefore predominantly male offspring (Galizi et al. 2016). The Cas9-based X-shredder is based on the original idea from Galizi et al. (2014) using the endonuclease I-*Ppo*I to cut X-chromosomal ribosomal RNA sequences.

Besides the important topic of sexing, genome editing could be used to improve any other aspect of insect pest control programmes, for example, to enhance insect fitness to overcome deficits induced in the insects by the mass-rearing process such as low competitiveness and short life span. Alternatively, males could be modified to improve mate-seeking success, all of which could improve the efficacy of SIT control programmes.

3.3. Gene Drive Systems for Population Suppression or Replacement

Finally, genome editing technologies like CRISPR also open a new path to insect pest control via gene drives. Gene drives could be used for pest control in two ways, via insect population suppression or population replacement. Like other approaches based on the release of sterile insects, population suppression via gene drives results in population size reduction in the next generation. However, population suppression drives do not use reproductive or genetic sterility in the classical sense, as this would prevent the spread of the trait into the population, as all the offspring carrying the trait would die before reproduction. Therefore, the genetic trait conferring population size reduction is linked to a gene drive component. Upon a one-time release of a seed population, the gene drive component drives the trait into the population with each successive generation, abolishing the need of repeated mass-releases. A population suppression gene drive could, for example, be a genetic modification killing females and resulting in only male offspring. These males will carry the "sterility" construct and pass it on to their sons upon mating with wild type females, thereby decreasing the population size further with each generation as no females are produced, until the population collapses, at which point the gene drive construct would disappear.

Another approach could be a modification that reduces female fertility. Such a system has been developed in the vector mosquito *An. gambiae* by targeting different putative female fertility genes. The gene drive components were designed to home in the germline of both sexes to ensure that all female offspring is affected (Hammond et al. 2016). In a different project, female sterility was achieved by targeting a female-specific exon of the sex determination gene *doublesex* in *An. gambiae*. Disruption of this exon by Cas9 did not affect male development but resulted in females with an intersex phenotype that were completely sterile (Kyrou et al. 2018). Most gene drive research has been performed in *Anopheles* mosquitoes so far, where it might be applied first in a control programme. There is only one report in an agricultural pest, *D. suzukii*, showing the functionality of a synthetic *Medea* gene drive system that could be used to spread a cargo gene into the population, for example for population suppression purposes (Buchman et al. 2018).

Population replacement gene drives mostly make sense for vector insects. The idea is to replace the wild type population by insects that are refractory to the infection with the pathogen, thereby interrupting the disease transmission cycle. In the first CRISPR-based gene drive in mosquitoes, Gantz et al. (2015) developed a drive system in which the Cas9 homing construct is expressed in the male and female germline of *An. stephensi*. The construct further contains previously identified dual anti-pathogen effector genes conferring resistance to *Plasmodium* infection, which are expressed somatically (Isaacs et al. 2011, 2012).

Gene drive technology holds great promise to solve problems caused by harmful insects, and the initial enthusiasm expressed in view of the options for gene drive design that opened up with the discovery of CRISPR was huge. It triggered statements that there would be the first mosquito gene drives out in field trials within less than two years. This enthusiasm was dampened, however, when the technology hit a sudden roadblock, making it very clear that we have to understand much more about drive mechanisms and their potential pitfalls before releases could be considered.

Two predominant issues arose in laboratory experiments: first, loss of function due to the evolution of resistant alleles (Hammond et al. 2017; Marshall et al. 2017; Unckless et al. 2017; KaramiNejadRanjbar et al. 2018), which is mostly a problem for homing-based CRISPR-Cas gene drives (i.e. mutations evolving at the homing gRNA target site), and second, failure of the drive due to population genetic diversity (Drury et al. 2017; Buchman et al. 2018) (i.e. sequence variation at the gene drive target site throughout a population; this affects CRISPR-based as well as other gene drives). Evolution of resistance alleles increases with the selective pressure put onto the gene drive-carrying insect by the drive itself or a linked genetic construct and is inverse proportional to the conservation level of the target sequence (see Section 7.3.1. for approaches being followed to overcome the technological pitfalls identified). The CRISPR-Cas technology with its versatility will be instrumental to overcome the identified evolutionary pitfalls, and in developing new drive systems to study and improve this technology for safe applications in the future.

4. REGULATORY CONSIDERATIONS OF GENOME EDITING

Genome editing technologies did not play a significant role in the development of genetically modified organisms (GMO) for agriculture, the food industry or other applications before the discovery of CRISPR and its potential for large-scale and high throughput, affordable genome editing. Therefore, questions of how organisms resulting from genome editing should be regulated were not relevant. This changed with the introduction of CRISPR, triggering large-scale research not only in crop optimization but also in modifying farm animals to be leaner, to develop faster, have longer wool, or a differently coloured coat. It raised the urgent question of how such products should be evaluated and regulated for bringing them to the market, and how currently existing regulations apply to genome editing. Should genome-edited products be classified and treated as GMO or not?

In the European Union (EU), the deliberate release of GMO is regulated by the 2001 EU directive (2001/18/EC). The directive specifies the procedures required in the EU for the evaluation and authorization of GMO releases. While the directive covers all kinds of GMOs, it so far has been applied only for the regulation of genetically modified (GM) crops. This selective implementation has been described as the ‘plant paradigm’. The 2001 directive states that an organism is characterized as GMO if its genetic material has been altered in a way that could not have occurred naturally by mating or recombination. Therefore, also organisms developed by non-transgenic methods can be classified as GMO. At the same time, however, conventional mutagenesis techniques like radiation or chemical mutagenesis, that were considered safe in 2001, are exempt from the GMO directive as long as they do not involve recombinant DNA (the mutagenesis exemption). Genomic modifications created by genome editing technologies can be changes on the level of whole genes (including the insertion of transgenic constructs) but can also be the introduction of small mutations (insertions or deletions of a few base pairs) all the way down to single nucleotide changes, as they could also occur by classical mutagenesis and breeding techniques. Therefore, from a rational and scientific point of view, such small mutations induced by genome editing technologies should be treated similarly to mutations induced by classical mutagenesis.

To clarify if and to which extent the rules of the 2001 directive would apply to the new genome editing technologies, the French Council of State sent an inquiry to the European Court of Justice (ECOJ) to interpret the directive for the new technologies. The ECOJ’s answer was provided in the form of a complex ‘Advocate General’s opinion in Case C-528/16’ statement, released in January 2018. It principally stated that changes induced by genome editing that could also have occurred by conventional mutagenesis should be regulated in the same way. However, scientifically unexpected, the court ruled in July 2018 against the Advocate General’s advice and stated that all products resulting from genome editing are subject to the directive and are to be treated as GMOs.

This decision raises different questions and uncertainty for the application of the new technologies. Interestingly, however, from a scientific point of view, it also puts the regulations for the established and safe methods in question. In an open letter to the Federal Ministry of Education and Research in Germany, the German Life Sciences Association stated that the inquiry was answered by the judges according to the legal conditions, but was not based on scientific facts available from EU authorities (the Scientific Advice Mechanism (SAM) and the European Food Safety Authority (EFSA)) as well as from a large number of scientists worldwide.

This EU decision follows years of complicated communication attempts in the area of regulation of GM organisms and products, which is still not consistent between countries. Regulation of GMO in the EU considers and evaluates the *process*, not the *product*. Therefore, two products with identical traits developed by different technologies are regulated differently. The recent court ruling on genome-edited organisms is a perfect representative of this evaluation approach. Genome editing as a process would be regulated, not the resulting product, meaning that all genome-edited products would be regulated without exception, even if the same trait could have been obtained by approved technologies without regulatory requirements. Moreover, in the EU, only the potential risks of the GMO are considered, while the prospective benefits are not considered.

The USA essentially takes the opposite approach. There, only the product is evaluated, independent of the method used to create it (Global Legal Research Center 2014). Consequently, the USA does not regulate any products that could as well be the result of traditional mutagenesis or breeding techniques, summarized by the United States Department of Agriculture (USDA) in the following statement on agricultural products on its website:

> "*Under its biotechnology regulations, USDA does not currently regulate or have any plans to regulate plants that could otherwise have been developed through traditional breeding techniques as long as they are developed without the use of a plant pest as the donor or vector and they are not themselves plant pests*" (USDA 2018).

Thus, small deletions or single nucleotide substitutions are not regulated in the USA. Even the introduction of larger nucleotide sequences that could have also occurred by cross-breeding is not regulated. In a March 2018 press release, the USDA specifically stated that this includes changes made by genome editing technologies. This approach to product evaluation resulted in the recent clearing for commercialization of a mushroom edited by CRISPR-Cas (Waltz 2016). The clearance was given without the regulation for GMOs, as only a few base pairs in the polyphenol oxidase gene were removed to enhance the shelf-life. Another significant difference in the USA approach for risk assessment compared to the EU is that also potential benefits of the commercial GMO are considered.

The existence of globally diverse approaches to the evaluation and regulation of GMO not only causes problems in international trade, as products will be classified differently in different countries, but also pose an enormous challenge for the release of modified insects. With their big range of motion and dispersal, insects will not stop at borders. Therefore, especially for insects carrying a drive mechanism with the potential to spread through whole populations, a common international ground for evaluation and regulation has to be found.

5. THE IMPORTANCE OF INFORMED DECISION-MAKING ON GENOME EDITING TECHNOLOGIES

The long ongoing GM crop debate in the EU essentially led to a moratorium in the EU on GM crops from 1998 to 2010, which in 2003 triggered a case filing by the USA and other countries with the World Trade Organization (WTO) against the EU. The ban on GM crops combined with unfavourable and sometimes biased news coverage created fear and insecurity in the EU, resulting in strong opposition against GM technologies by the public. This stalled the corresponding research and caused the industry in the EU to step by step pull out of GM crop research and production, e.g. Bayer Crop Science and BASF closing their GM crop research in Germany between 2004 and 2011.

With the most recent ruling by the ECOJ that also classifies all genome-edited products as GMO, the required checks and controls needed to develop such products for the market would be too expensive for research institutes and small companies. Moreover, the classification of all genome-edited organisms as GMO will probably lead again to strong opposition and general rejection by the public, adding another hurdle for the marketing of genome-edited products. Therefore, in our opinion, decisions like the one by the ECOJ equal a moratorium on genome-edited products in the EU with far-reaching consequences. Again, it will incapacitate a whole biotechnology industry sector that will flourish in countries with less restrictive regulation. It will prevent innovation and the development of new technologies in the EU and will cause biotechnology companies to withdraw their respective departments from the EU market, which in turn will prevent the creation of or destroy jobs. Also, in the international biotechnology sector, there is concern that the EU will continue to lag behind countries such as the USA, where the regulations are more favourable for GMOs and the GM crop market. The ECOJ decision on genome-edited organisms will also influence trade markets, as occurred already during the EU GM moratorium, which experienced in the early 2000s negative impacts on the agricultural export revenue of countries such as the USA, Canada, and Argentina (Disdier and Fontagne 2010).

Regarding pest control programmes intending the area-wide use of genome-edited insects, this could be most problematic for agricultural pests that feed inside fruits/crops like the Mediterranean fruit fly. Marketing of such crops in the EU might be restricted by the control measures for GMO contamination in food, for which the EU tolerance levels are as low as 0.9%. For a polyphagous insect like the Mediterranean fruit fly, which feeds on many different fruits, multiple agricultural products in the treatment area might be affected by the EU import restrictions (Max-Planck-Gesellschaft 2017). Use of genome-edited insects for pest control will therefore require the establishment of a definition of insect contamination in food and a decision on tolerance levels. This general framework will be critical for exporting countries to decide if the trade-off between the possible import restrictions and concomitant loss of markets on one side, and the reduced crop production costs as well as more abundant harvests on the other side, are worth the use of genome-edited insects for pest control.

Besides the import restrictions, however, genome-edited insects might not be regulated by the 2001 EU directive on the release of GMO if the insects are 100% sterile. Sterile insects do not fit the definition of an organism and therefore aren't a GMO either. Thus, 100% sterilized mosquitoes released as part of a SIT programme seem not to be regulated in the EU (HCB 2017). The release of radiation-sterilized *Ae. albopictus* mosquitoes is carried out in Italy and Germany as part of SIT mosquito suppression trials. To our knowledge, in Germany, these releases did not require prior authorization and risk assessment. Thus, one could speculate that scientists might be allowed to use genome editing, for example, to develop sexing strains for a pest species, allowing to remove female insects during rearing and to release the corresponding males after (100%) sterilisation by irradiation.

6. ETHICAL ASPECTS OF GENOME EDITING AND GMO RELEASES

The CRSIPR/Cas system allows genome editing with an ease and effectiveness that appeared to be some way in the future just a few years ago. Now that "everything" seems feasible, the always present question of what is socially, environmentally and also ethically responsible to do is coming into the focus of discussions. As we are no experts on social sciences and bioethics, we want to just briefly touch on some questions that have been raised in different panels and newsgroups.

A major topic evolves around the moral aspect of genome editing. This involves the fundamental questions if we are allowed to edit an organism's genome at all, and in case of area-wide pest management programmes, if we have the right to eliminate invasive or native populations or even a species as a whole. Both questions have been raised before but became more prominent with the discovery of the possibilities that opened up by CRISPR-Cas. While these questions are important to discuss, it is also important to consider the impact that currently applied strategies and technologies have.

The classical mutagenesis and breeding techniques, for example, that have been broadly applied for decades and are widely accepted and mostly haven't been questioned, can randomly change the genome of an organism at multiple positions, and the induced changes on the molecular level are commonly not known. What can be achieved with CRISPR genome editing, depending on the modification, would not be different from what has been done for decades, but can be done now in a less random process.

Similarly, the use of insecticides has the potential to eliminate a pest population in the targeted area, with the side-effect of not only eliminating the target species but also affecting many other beneficial insects, besides the environmental impact. With the recent alarming news on the decline of overall insect numbers and biodiversity (Hallmann et al. 2017), the extensive use of insecticides has come once more into the focus of widespread criticism and public concern.

Another important point for ethical discussion deals with questions concerning constitutional rights, individual expectations, democratic decisions, and also questions about how we can balance the potential elimination of a species against the decreased burden for the human population (e.g. the decrease of infectious diseases, or reduced insecticide use and crop losses due to agricultural pests). The application of GM insects for pest control directly affects the people in the target area by releasing the insects into their air space. Constitutionally everyone has the personal right to decide on things affecting one's own life. Moreover, different groups involved in and affected by the release of genome-edited insects (scientists, companies, authorities, producers, the public) will be guided by a variety of (contradicting) motivations concerning the release. Different perceptions of what is a desirable future and fear of new technological developments due to lack of understanding further complicate the situation.

Thus, it will be essential to identify and involve all the different stakeholders at an early stage of a project. An open and honest discussion of the limits of a technology and of the scientific knowledge, as well as public education will be crucial to build trust in science, in involved organizations, and in the decision-making process. Open discussion forums, where representatives of different stakeholders meet, could help to promote dialogue and the mutual understanding of goals, motivations, expectations and concerns.

Finally, the involvement of the public should not be limited to information and education campaigns. It should also include the collection of concerns of the educated public for discussion and consideration in the decision-making process.

7. POTENTIAL RISKS, CHANCES, AND CHALLENGES OF GENOME EDITING IN INSECT PEST CONTROL

7.1. Are Genome Editing Technologies for Insect Pest Control a Curse?

In general, genome editing could be used to create insect strains for population suppression or replacement approaches. Strains for population suppression could contain a trait that for example allows sexing (female elimination during rearing), and the strain would then be used in a SIT approach (i.e. radiation sterilisation and subsequent repeated mass-releases of the sterile males). On the other hand, they could contain a gene drive construct in combination with a trait allowing population reduction, for example by targeting female fertility genes. Such strains would then be used in a one-time limited release and the trait for population reduction would be driven through the population until a critical population density threshold is reached where effective reproduction is impaired. At this point the population would collapse in the targeted region and the genetic trait would disappear from the environment.

The situation would be different for the release of population replacement gene drives, as these genetic elements are designed to remain in the environment. Such genome-edited insects typically would have the gene drive component in their genome combined with a genetic modification that for example makes a mosquito population refractory to pathogen infection. The goal of such a construct would be to drive the immunity against the pathogen through the mosquito population until all or the majority of the insects are immune, which would interrupt the pathogen transmission cycle between the human host and the mosquito.

7.1.1. Use of Genome Edited Insects for Population Suppression in SIT-like Approaches

From a scientific point of view, the risk of using genome-edited insects for population suppression in SIT-like approaches is comparable to strains developed by classical mutagenesis approaches. The genome-edited trait disappears from the environment with the death of each released generation, as these insects cannot reproduce.

For both genome modifying technologies, genome editing and classical mutagenesis, a random mutation could arise in the generated strains that inactivates the trait. In the example of a genomic modification that kills specifically females for sexing purposes, a revertant mutation could occur at the modification site that allows females to survive again. Studies in *D. melanogaster* showed that such revertant mutations could occur at a frequency of 10^{-7} or less (Chovnick et al. 1971; Handler 2016). Therefore, at a mass-rearing scale of up to 1-3 x 10^9 insects per week, a few revertant insects are expected. Depending on the numbers, this could result in an efficiency concern, the release of females, or the contamination of the wild population with a marker. Nevertheless, all of those insects can be sterilized before release and would therefore not interfere with the overall success of the programme.

Critics might articulate concerns about off-target effects caused by genome editing technologies (for a review on CRISPR off-target effects see Zhang et al. 2015), resulting in additional mutations at other positions in the genome than the intended one. However, classical mutagenesis using chemicals or radiation also causes multiple unknown mutations and chromosome breaks in the genome in addition to the one causing the selected trait. In the case of classical mutagenesis this fact is accepted and not considered as a risk or potential problem, and the respective organisms can be deployed without regulations.

7.1.2. Self-perpetuating Gene Drive Systems Used for Population Suppression

In the case of a self-perpetuating gene drive systems used for population suppression, the success of a programme depends, besides the stability of the population reduction trait, on the stability of the gene drive. Resistance development against the drive would abolish the spread of the population reduction trait in the population, going back to normal Mendelian inheritance. This would not have any harmful consequences except that the suppression approach will not work anymore, and the modified insects will decline until completely gone, unless the mutation not only inactivates the drive but also lends a selective advantage over unmodified insects. However, even then, the non-functional insect is very unlikely to pose a threat to the environment or human health. For further population reduction, a new drive system would have to be developed.

7.1.3. Gene Drive Systems Used for Population Replacement

In the case of population replacement drives, potential resistance development on several levels could impair the success of the approach. First, the mosquito could develop a resistance against the drive, stopping the spread of the immunity trait and resulting in its eventual loss. Second, the pathogen could develop resistance against the immunity trait. In this case, the trait would further spread through the population due to the gene drive component, but it would not have the immunizing effect anymore.

The selection of resistant alleles, in general, is to be expected in each approach that puts a selective or survival pressure on a population of an organism. Such pressure would be applied to the target insect in case of population suppression approaches, and on the pathogen in replacement approaches spreading immunity through a mosquito population. This resistance development mechanism, however, is in no way connected to the use of genome editing technologies. It is already happening globally with the observed pathogen resistance developing against antivirals, antibiotics, or anti-malarial drugs, with the increasing resistance of insects against insecticides, or with behavioural resilience, for example the change in biting behaviour of mosquitoes from night to day to avoid bed nets (Liu 2015; Thomsen et al. 2017). It will always be an arms race, and we have to be aware that every human activity applying survival pressure on an organism will select for an evading reaction.

7.1.4. Risk of Overestimating the Understanding of Gene Functions

Genome editing experiments in different mammals have shown that there is a risk of overestimating our understanding of gene functions. It is often limited to one or two single functions, when instead genes often are part of large regulatory networks or have different functions throughout development. Modification of their expression can lead to unforeseen and unwanted consequences besides the desired effect. A variety of genome editing studies have been performed to knock out the *Myostatin* (*MSTN*) gene in different mammals. *MSTN* controls muscle growth, and existing knockout mutants show higher muscle mass and leaner meat. However, while resulting in the desired higher muscle mass, the *MSTN* knockout also caused unwanted side effects like additional thoracic vertebra in piglets (Qian et al. 2015), or rabbits with enlarged tongues and severe health problems like high rates of stillbirth and early stage death (Guo et al. 2016).

These results show that despite an increasing number of sequenced genomes, we still don't know much about gene functions. Therefore, careful studies will be needed for genome editing approaches aiming at population replacement, which will involve editing of specific genes to produce a certain phenotype – envisioned beneficial – that should stably persist in the target population without side-effects. This is of less concern when genome editing is used for population suppression applications, for example in scenarios where:

- A conditional female-killing mutation like the Mediterranean fruit fly *tsl* is recreated in another species to establish a sexing system
- A sex determination gene is knocked out to produce single-sex offspring for population reduction purposes, or
- A transgene construct is introduced into a specific genomic position previously characterized not to be disadvantageous to the insect.

In these scenarios, the quality of sterilized males in terms of mating performance, reproduction, and life span is the main characteristic to evaluate the usability of any strain.

7.1.5. Other General Risks

There are other general risks associated with area-wide pest or vector management approaches. These are, again, not specific to the use of genome edited insects, but apply to any control approach. In case of vector-borne diseases, the local eradication of a disease maintained over several generations can pose a risk to the human population in case of the reintroduction of the disease. Since the pathogen would not have challenged the human immune system for several generations, a reintroduction of the disease into that population could result in severe outcomes of the infection.

For population suppression approaches targeting endogenous species, the consequences of local eradication for the ecosystem are mostly unknown, as the species' role in the ecosystem is often not well studied. Therefore, possible consequences on the food chain, on competing species, and the possible long-term consequences for the human population, can only be guessed.

One concern in mosquito elimination approaches, for example, is that the niche opened by the (local) elimination of the target species could be filled quickly by another vector species that might transmit the same or even other diseases.

7.2. Are Genome Editing Technologies for Insect Pest Control a Blessing?

Currently the most used strategy to fight agricultural pests and vector insects is the application of insecticides. While they can be very effective in achieving rapid suppression in local applications, insecticides have many disadvantages. A major concern is their lack of species-specificity and the concomitant negative impact on many non-target insect species, or potentially even representing a risk to the environment. Furthermore, increasing resistance to insecticides is being observed in a rapidly growing number of pest insects worldwide, requiring an increase in application doses or the combination of different insecticides to still have an effect. This, however, is a dead end as it will lead to even stronger impacts on the environment and ultimately to complete resistance and loss of function of existing substances. Excessive use of insecticides in combination with other factors has already caused a dramatic decrease in the overall insect mass in Europe (Hallmann et al. 2017) and has led to the drastic decline of beneficial insects like pollinators as described in newspaper articles for some areas of China. An additional limitation of insecticides is that they cannot be applied on an area-wide basis due to public opposition. As a result, remote pest breeding sites are not accessible for treatment, thereby representing a constant, untouchable reservoir for the resurgence of the pest population after or despite of insecticide treatment.

Area-wide control programmes based on the release of modified insects are a promising strategy for the sustainable and species-specific pest control without the adverse side effects of insecticides. 'Modified' in this context can mean anything from sterilized by radiation for pest control using the SIT approach, all the way up to genetically modified insects being, for example, refractory to pathogen infection and carrying a gene drive construct for population replacement. Any method based on the release of modified insects has the substantial advantage of being highly specific to the target species and therefore not negatively impacting other non-target organisms, even those closely related. They also do not have toxic or other adverse side effects for the environment. Methods based on the release of modified insects are therefore environmentally safe and sustainable. Moreover, they allow an area-wide treatment against the whole population of the insect pest, and the mobility of the released insects also allows reaching remote habitats or protected areas that are inaccessible or not open for other approaches like the spraying of insecticides.

Approaches based on the release of modified insects also allow reaching the next generation of pest insects, like the drought-resistant eggs of *Aedes* mosquitoes, that are not affected by insecticide spraying and thus lead to a resurgence of the population in the next rainy season. The presence of modified insects throughout the egg hatching period, in contrast, would intercept the newly emerging generation and thus prevent a resurgence of the wild population.

Currently, the application of the SIT against new pest species is limited by a few bottlenecks that can be overcome. Besides a suitable mass-rearing system, an efficient sexing system that can be applied at large-scale is essential. With genome editing technologies one can envision the generation of such systems. For example, once suitable mutations obtained by classical mutagenesis in one target species are uncovered, genome editing could be used to reproduce these mutations in homologous genes in other insect pests.

One prominent example would be the above-mentioned *tsl* mutation in the Mediterranean fruit fly that specifically kills female embryos upon heat shock, allowing 100% sexing of this pest species at a large scale, which has been the key for Mediterranean fruit fly SIT programmes since more than 25 years and could allow building sexing systems in other pest insects (Robinson 2002; Franz et al. 2021). This mutation is an excellent example of a classically generated mutation that, once identified, can pave the way for similar technologies to be built in other pest insects.

Genome editing technologies are moreover the key to any approach intending to spread, via the use of a gene drive, sterility or pathogen refractoriness in a population without continuous mass-releases of the modified insects. Such strategies can only be pursued with the use of genome editing. The use of a gene drive to spread a lethality or sterility trait into a population for suppression approaches would have the advantage that optimally a one-time release of a seed population would be sufficient to suppress a population compared to continuous mass-releases required for the SIT approach.

Genome editing technologies will therefore be able to support and advance environment-friendly and sustainable pest control methods on various levels, and will thus help protect beneficial insect species, the biological diversity, and healthy ecosystems.

7.3. Challenges and Possible Solutions for Genome Editing Strategies

Genome editing technologies face multiple challenges that have to be understood and evaluated before they will gain the status of being acceptable and safe for use in insect pest control.

7.3.1. Technological Challenges

One of the challenges is the development and selection of resistant alleles observed with different (mostly CRISPR homing) gene drive approaches (Hammond et al. 2017; Reed 2017; Unckless et al. 2017; KaramiNejadRanjbar et al. 2018). While the drives in general work with a satisfying efficiency in laboratory experiments, the evolution of resistance alleles that are not recognized anymore by the gRNA of the CRISPR-Cas system was generally observed with CRISPR homing drives within just a few generations (Champer et al. 2018), resulting in the inactivation of the drive. Much effort is therefore being invested into the development of new drive strategies and improvement of the existing systems. Approaches include the use of multiple gRNAs, the expression of the drive exclusively in the male germline, or the targeting of highly conserved genes (Kyrou et al. 2018).

The rationale of the first approach is that a mutation in one of the gRNA target sites will not be able to inactivate the drive as the other target sites are still functional. A simultaneous mutation of multiple target sites is much less likely (even though it is not impossible), thereby preventing or at least strongly postponing drive inactivation (Prowse et al. 2017). Activating the drive only in the male germline should limit resistance-allele formation post-fertilization, as the sperm should transmit only low amounts of the Cas endonuclease into the embryo, thereby preventing mutagenic events in the embryo. Targeting highly conserved genes like the sex determination gene *doublesex* for homing has been effective in building a functional gene drive system in the laboratory (Kyrou et al. 2018). It can prevent resistance allele formation as mutations in highly conserved sequences would likely be deleterious to the organism, and the resistance alleles would not persist (Esvelt et al. 2014; Champer et al. 2016; Noble et al. 2017; Champer et al. 2018; Nash et al. 2018).

Other proposed systems reduce resistance potential in so-called daisy-chain drives and toxin-antidote systems (Champer et al. 2016; Noble et al. 2019). Current studies and simulations seem to indicate, however, that resistance evolution will remain an issue that can't be completely suppressed, just slowed down, and might require the combination of different strategies to design successful drives (Callaway 2017; Champer et al. 2018). A drive will be successful if it can spread through the whole population before the first resistance allele formation occurs.

Besides the development of resistance to gene drives, any genome editing approach can be affected by the appearance of spontaneous inactivating or revertant mutations due to the natural mutation rate in the genomes of insects. Most of such inactivating mutations would be point mutations, deletions, or changes by recombination events or moving transposons. With a mutation frequency of 10^{-5} to 10^{-7} per base pair, depending on the mutation event (Chovnick et al. 1971; Tobari and Kojima 1972; Bender et al. 1983; Neel 1983; Woodruff et al. 1983; Handler 2016), the occurrence of multiple mutations is to be expected as mass-rearing is scaled up towards 10^9 insects per week.

This phenomenon is not specific to genome editing technologies, however. It is a concern for any genetic modification used in insect mass-rearing approaches, including transgenic technologies or modifications induced by classical mutagenesis. Thus, already now, safeguard technology is used for strain maintenance like the filter rearing system used for ongoing fruit fly SIT programmes, to prevent the occurrence and persistence of inactivating mutations in the release population (Caceres 2005; Franz et al. 2021). Furthermore, besides such filter rearing systems, the safe use of transgenic insects for large-scale programmes will require backup systems. In case of failure of one system due to a random mutation, the other will serve as a safeguard to either preserve the strain function or prevent the strain from surviving in the wild due to the inactivating mutation (Eckermann et al. 2014; Handler 2016).

7.3.2. Ecologic and Economic Challenges

The success of any area-wide control programme involving, but not limited to, genome-edited insects will also depend on understanding the pest insect's population ecology. Population suppression, as well as replacement, strategies will be influenced to a different extent by factors like the dynamics within the population, insect migration behaviour and range, the geographic situation, environmental factors influencing the target population, as well as density threshold levels for successful reproduction or effective disease transmission, but also the adaptive or evasive potential of the target species. For a successful insect pest control programme with regard to environmental and human safety, it will additionally be essential to understand the insect's role in the food chain and its interaction or competition with other species.

Critics of population elimination approaches claim that the target pest could be replaced by a competing pest, causing the same or other problems. However, except for some rare cases, such as a very strong and stable gene drive that in theory should be capable of eliminating a whole population, most strategies for population control will only lead to a reduction in population size. Even if potentially they can lead to elimination in large areas, they won't have the potential for global eradication of a species, and a resurgence of the targeted species is expected as soon as the treatment is stopped.

The commercial applicability of genome-edited insects also depends on the crop and the pest complex threatening this crop. If one major pest species threatens the crop, the use of a species-specific strategy involving genome-edited insects is a practical approach. However, in a situation where a crop is equally affected by two or more pest species, the specific suppression or elimination of one of them could lead to population increases of the remaining pest species. In this case, any species-specific insect release strategy would also need to simultaneously address the other pest(s), but such an approach may be less economically viable.

7.3.3. Regulatory Challenges

The release of genome-edited insects for area-wide pest control programmes will face significant challenges concerning existing diverging regulations for genome-edited organisms. As discussed above in Section 5, there is no uniform international approach to the evaluation, risk assessment and the release of GM and genome-edited organisms, with every country pursuing an individual approach to regulation. Consequently, one country might allow the release of a genome-edited insect after a thorough evaluation of the product for stability and safety, while a neighbouring country might generally prohibit the release of any genome-edited insect. This will pose a problem for releasing countries and their agricultural trade, as the insects will not stop at their country's borders, and they would not be able to guarantee the confinement of the genome-edited insects within their territory, even if the insects are released far from the border (Reeves and Phillipson 2017). While this is already a difficult situation for non-disseminating approaches such as GM crops and insects, it would practically require inter-country regulation of any strategy relying on gene drives which have the potential and the purpose to spread.

Besides the regulations concerning the release of genome-edited insects, some food safety regulations will pose a challenge for the use of these insects in agricultural pest control. This will be most relevant for insects that feed inside of the fruits/crops. Such crops could be contaminated with the genome-edited insects, at least for the time of the releases, which could restrict marketing of these products in other countries, leading to the possible (temporary) loss or shifting of markets (Max-Planck-Gesellschaft 2017). In this case, the producing country would have to decide if the application of the genome-edited insects is economically worthwhile. It will also influence the approval processes for new genome-edited insect strains by the governments and regulatory bodies. How regulatory authorities in different countries will classify genome-edited insect material in agricultural products will, therefore, have a strong impact on international trade.

7.3.4. Identification and Involvement of Stakeholders

Area-wide pest management approaches involving the release of genome-edited insects will affect many different stakeholders. Identifying these stakeholders and their interests, and involving them in the planning and decision-making process, will be crucial for the success of any genome-edited insect deployment; it will also strongly impact the future of pest management projects. We summarize some important points here and refer the interested reader to the more in depths analyses performed by Gould (2008) and Baltzegar et al. (2018) in relation to the deployment of genome-edited insects in agriculture and human health.

Ecological, economic, regulatory, and social contexts need to be understood in depth in a case-by-case assessment to be able to determine all the groups and subgroups that will benefit or be negatively affected by each intervention with genome-edited insects. Regarding the ecological impact of the genome-edited insect