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SECTION 2

ANIMAL AND HUMAN HEALTH

Taylor & Francis

AREA-WIDE MANAGEMENT OF STABLE FLIES

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SUMMARY

Stable flies are highly vagile and their dispersal ability appears to be limited only by the availability of hosts. In addition, stable fly larval developmental substrates are diverse, dispersed and often difficult to locate. This life history necessitates the use of area-wide integrated pest management (AW-IPM) strategies if effective control of stable flies is to be achieved, but complicates the use of the Sterile Insect Technique (SIT) and mating disruption technologies often employed in such programmes against other insect pests. Area-wide management of stable flies will require nationally or regionally coordinated implementation of traditional control methods, including sanitation/cultural, biological, and chemical technologies. An administrative structure will need to be implemented to coordinate, monitor, inspect and enforce compliance, especially if agronomic crop residues are integral to stable fly infestations. Research on stable fly developmental substrates and their management, larval and adult population dynamics, efficient and economical adult suppression systems, including traps and targets, is needed to improve the efficiency and economy of area-wide management of stable flies.

Key Words: Stomoxys calcitrans, control, IPM, livestock, dispersal, crop residues, regional coordination, filth flies

1. INTRODUCTION

Stable flies, *Stomoxys calcitrans* (L.), are important, pests of livestock throughout much of the world. Their painful bites disrupt feeding and other behaviours of livestock (Dougherty et al. 1993, 1994, 1995; Mullens et al. 2006), reducing productivity (Campbell et al. 1993, 2001) and, in extreme infestations, resulting in mortality (Bishopp 1913). In addition to their effects upon cattle, stable flies disrupt human recreational activities (Newson 1977) and molest companion animals (Yeruham and Braverman 1995) and wildlife (Elkan et al. 2009) throughout their range. Landing counts of 80-100 flies per minute on humans have been observed on the beaches of north-western Florida (Hogsette et al. 1987).

Adult stable flies are obligate hematophages, both males and females require blood prior to mating (Anderson 1978). Females need 3-4 blood meals to develop

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their first batch of eggs and 2 more for each additional batch (Bishopp 1913; Anderson and Tempelis 1970). After feeding, stable flies retire to a nearby surface, frequently warmed by the sun, to digest their blood meal. Immature stable flies develop in decaying or fermenting vegetative materials frequently contaminated with animal dung or urine (Simmons and Dove 1941, 1942; Silverly and Schoof 1955; Hafez and Gamal-Eddin 1959; Campbell and McNeal 1979; Hall et al. 1982) where larval densities can exceed 20 000 per square-meter of substrate (Patterson and Morgan 1986; Broce et al. 2005).

Four species of flies, face fly (*Musca autumnalis* De Geer), house fly (*Musca domestica* L.), horn fly (*Haematobia irritans* (L.)), and stable fly are frequently found in association with livestock. Often, these flies are referred to collectively as "filth flies." Although morphologically similar, the behaviour and biology of these flies are distinct (Moon 2002; Zumpt 1973). Stable fly and horn fly are obligate parasites, primarily of livestock, with biting mouth parts. Face fly and house fly are non-biting flies with sponging mouthparts. Face fly and horn fly larvae develop in fresh, undisturbed bovine dung. Stable fly and house fly larvae develop in older or aged manure, frequently mixed with decomposing vegetative material as well as decomposing non-manure substrates. Horn flies are semi-permanent parasites, spending the majority of their adult life on a host, whereas stable flies are temporary parasites, visiting the host only to blood-feed. Face flies are obligate parasites as well, but rather than feeding on blood, they feed on mucus and other fluids around the eyes and mouth of the host. Because of these biological differences, many of the technologies and methods used for their management are species-specific. Proper identification of the offending fly species is essential before initiating a management programme.

2. DEVELOPMENT IN CROP RESIDUES

Many types of decomposing and fermenting organic materials support stable fly larval development (Hogsette et al. 1987), although in North America most practitioners consider residues from livestock production systems and barnyards to be the primary sources. This, however, has not always been the case. In the first half of the 20th century, straw of oats, rice, barley, and wheat were reportedly the most common developmental substrates. Population levels were correlated with grain production (Bishopp 1913) and severe stable fly outbreaks were attributed to development in peanut straw, celery and bay grass (Dove and Simmons 1941, 1942). Recently, agronomic crop residues have re-emerged as important sources of stable flies. Serious outbreaks associated with pineapple production have been reported in Costa Rica (Herrero et al. 1989, 1991), sugarcane in Brazil and Mauritius (Kunz and Monty 1976; Koller et al. 2009; Souza Dominghetti et al. 2015), and vegetable crop residues in Western Australia (Cook et al. 1999). Counts of greater than 2000 stable flies per animal are being associated with development in crop residues (Fig. 1).

This gives rise to one of the primary differences between the stable fly situation in the USA and that in Australia, Brazil, Costa Rica, and potentially other countries. In the USA, stable fly larval developmental sites are typically associated with livestock production. In a sense, livestock producers are responsible for the problem they perceive. Where stable fly developmental sites are being attributed to agronomic production and crop residues (e.g. Australia, Brazil and Costa Rica), another industry, or someone else, is responsible for the problem. Calls for regulation and government action are louder when someone else is to blame.

Figure 1. Stable flies on leg of steer (left, photographer David Cook) and damage from stable fly bites in Costa Rica (right, photographer Jose Solórzano).

3. AREA-WIDE MANAGEMENT

3.1. Need for Area-wide Management

The biology of stable flies necessitates the incorporation of area-wide concepts for their management. Adults are highly vagile, capable of flying up to 30 km in 24 hours on a flight mill (Bailey et al. 1973) and 8 km in less than 2 hours in the field. Mean dispersal distance from a natural larval developmental site was 1.5 km (Taylor et al. 2010), however Hogsette and Ruff (1985) reported individual flies dispersing over 225 km. The dispersal ability of stable flies appears to be limited only by the availability of hosts. They disperse until suitable hosts are located. Because both male and female stable flies require a blood meal prior to mating, most of the dispersing flies appear to be physiologically young. The efficacy of managing stable flies on individual premises, or focusing control efforts to locations where populations exceed the economic threshold, is limited by the ability of flies to disperse from premises and locations with no stable fly control to those attempting to control this pest.

Insects are best targeted for control when they are concentrated, immobile and accessible (Horsfall 1985). For stable flies, as for most pestiferous Diptera, this would be during the immature or larval stage. However, stable fly larval developmental substrates are diverse, dispersed and often difficult to locate. Stable fly larvae have been observed in a broad variety of substrates including flotsam containing decomposing mayfly exuvia (Pickard 1968), aquatic plants (King and Lenert 1936; Simmons and Dove 1941), livestock wastes (Meyer and Petersen 1983; Broce et al. 2005), agronomic wastes (Bishopp 1913; Dove and Simmons 1941; Solórzano et al. 2015; Cook et al. 2011, 2017), grass clippings (Silverly and Schoof 1955; Todd 1964) and sewage sludge (Doud et al. 2012).

Beyond "fermenting organic material", little is known about the biological, chemical and physical factors defining developmental substrates (Gilles et al. 2008; Wienhold and Taylor 2012; Friesen et al. 2016). An active microbial community is necessary for larval development (Lysyk et al. 1999; Romero et al. 2006). As the number of coliform bacteria declines with microbial succession, so does the suitability of the substrate for stable fly development (Talley et al. 2009). Because substrates are suitable for stable fly development only during specific phases of decomposition, developmental sites are most often ephemeral, supporting only one generation of flies (Talley et al. 2009; Taylor and Berkebile 2011). These sites are broadly dispersed throughout rural and urban landscapes. Even relatively small sites can produce large numbers of flies (Todd 1964; Patterson and Morgan 1986). These characteristics complicate our ability to locate larval developmental sites for management prior to adult emergence.

Because of their painful bites and persistent feeding behaviour, just a few stable flies can reduce the productivity of livestock, harass companion animals and disrupt human recreational activities. The economic threshold for stable flies on feeder cattle in feedlots has been established at five flies per front leg (≈15 per animal as stable flies preferentially bite the front legs (Campbell and Berry 1989; Berry et al. 1983)), although cattle often exhibit defensive behaviours with fewer flies (Mullens et al. 2006). When infestations reach very high levels, cattle may no longer resist, and mortality may follow (Bishopp 1913).

Classic integrated pest management (IPM) programmes are based upon the concept of initiating control measures only after the pest population reaches an economic threshold (Metcalf and Luckman 1975). Because stable fly larval developmental sites are difficult to locate prior to adult emergence and larvae are intrinsically innocuous, economic thresholds are based upon counts of adult flies biting animals (Campbell and Berry 1989). By the time adult counts exceed the economic threshold, most have already emerged, and it is too late to initiate larval control procedures. Because of the ability of stable flies to move from property to property, the broad range of development sites and substrates, and the relatively low numbers of flies needed to inflict economic damage, effective management must be approached from a preventive, area-wide perspective.

3.2. Challenges for Area-wide Management

Area-wide management programmes often involve the use of mating disruption with pheromones or mass-production and release of insects with reduced reproductive potential. Unfortunately, aspects of stable fly biology are not conducive to the use of mating disruption. Unlike many Lepidoptera, muscoid Diptera such as stable flies lack volatile pheromones (Blomquist et al. 1987) suitable for mating disruption. Rather, their mate recognition pheromones are non-volatile cuticular hydrocarbons which act on contact, or at very close range (Muhammed et al. 1975; Uebel et al. 1975; Carlson and Mackley 1985).

Release of flies with genetic changes, whether induced by irradiation or transgenesis (Box 1), is complicated by three aspects of stable fly biology:

First, both male and female stable flies are obligate hematophages, blood-feeding 1-3 times per day for their entire life (Harris et al. 1974). Releasing large numbers of biting flies will increase the burden on livestock significantly.

Secondly, stable fly populations can be very large. Huge numbers of flies with non-persistent or threshold dependent genetic modifications must be released to attain the ratios necessary for control. Releasing such numbers of painful biters will meet with public protests. Added to this, their ability to disperse requires that even greater numbers be released over wider areas.

Thirdly, because of their high reproductive rate, short of eradication, small populations can recover to outbreak proportions quickly, precluding the concept of releasing flies with genetic changes for a limited period of time when natural populations are low with the hope of retarding later population growth.

Box 1. Non-Persistent and Persistent Genetic Changes

Genetic changes caused for example by irradiation, or the insertion of external genetic constructs (*transgenesis*) through modern biotechnology, can reduce the reproductive fitness of an insect. These genetic changes can be non-persistent or persistent in the target pest population (Carter and Friedman 2016). For example, the genetic changes of released sterile insects are non-persistent because they are not expected to persist in the environment. The released insects mate with wild insects reducing their fitness, but their genetic changes are not passed to their progeny. Therefore, programmes releasing insects with non-persistent genetic changes such as the Sterile Insect Technique (SIT) must release them continuously in numbers that greatly exceed the target population, often in the range of 10:1 to 200:1 (Knipling 1955). Because the random dominant mutations induced by irradiation render flies sterile, genetic changes do not persist in the environment, thus these SIT programmes have met broad public acceptance, and several are currently active (Klassen et al. 2021).

Persistent genetic modifications are designed to be, at least temporarily, incorporated into the gene pool of the target reducing either fitness or pathogenicity (Champer et al. 2016). Persistent modifications are often linked with a genetic drive mechanism to allow them to increase their frequency in the pest population. Genetic drive constructs can be subdivided into threshold dependent and threshold independent (Carter and Friedman 2016). The frequency of threshold dependent constructs must exceed a given level, the threshold, before increasing in frequency. Threshold independent constructs can theoretically be introduced into a population at a very low level and they will increase their frequency to fixation, replacing the original or natural population. Because these constructs can persist in nature and even replace the natural population, they are receiving a great deal more regulatory and ethical scrutiny than non-persistent technologies.

Given these constraints, the most viable option for genetic control of stable flies would be to release small numbers of persistent genetically modified flies with a threshold independent gene drive construct. Pending the development of such constructs and public acceptance of the release of such genetically modified organisms that are expected to become established and spread in the pest population (Box 1), our area-wide options for stable fly control are limited to the integrated implementation of traditional management technologies including cultural, biological and chemical methods.

3.3. Prerequisites for Area-Wide Management

3.3.1. Public Support / Consensus / Demand

Area-wide management programmes are administratively complex and require longer-term commitment (Hendrichs et al. 2007; Vreysen et al. 2007). A primary prerequisite for establishing such a programme is stakeholder collaboration and public recognition of the costs and benefits. This requires effective outreach to ensure that the public is aware of the damages and knowledgeable of the etiological agent.

Outreach is especially important for a pest such as stable flies. Producers often fail to differentiate among the species of muscoid flies associated with livestock. These flies are morphologically similar to the untrained eye. When querying producers about stable fly problems, one frequently hears "no, I don't have a stable fly problem, I have a fly problem." Similarly, when fielding calls from producers seeking assistance with flies, they are rarely able to identify the species of fly with which they are dealing. Smaller species, such as horn flies, are frequently mischaracterized as young flies that will "grow up" into larger flies (flies do not grow after metamorphosis to the adult stage). The biology and management methods for these species differ significantly, making proper identification essential prior to developing management strategies.

In Costa Rica, livestock producers refer to stable flies developing in pineapple fields as "mosca de la piña" and are insistent that they are a different species from the stable fly, "mosca del establo" that they observed prior to the large-scale pineapple production in the country. The importance of education and outreach to gain public support for an area-wide management programme cannot be over-emphasized. Economic assessments of the damage are also essential. Annual production losses to the cattle industry from stable flies are estimated to be USD 2.2 billion in the USA (Taylor et al. 2012a), USD 340 million in Brazil (Grisi et al. 2014), and USD 6.8 million in Mexico (Rodríguez-Vivas et al. 2017).

3.3.2. Regulatory Authority

Common concerns for area-wide management programmes are "free-riders", individuals who take advantage of the programme, but fail to contribute. This problem is exacerbated when the "problem", in this case stable flies, does not affect source producers, for example crop producers. Stable flies have no negative effects on crop production. Without regulatory authority, it will be very difficult to convince those producers to control the flies developing on their farms.

In Australia, Brazil, and Costa Rica, there have been public calls and demands for regulatory actions by the governments to address stable flies. The governments of those countries have enacted policies requiring agronomic producers to manage stable flies developing on their properties. In the USA, no such public demands have been made and regulatory policies have not been enacted. Public demand and pressure may ultimately lead to the development of regulation of stable fly source industries.

3.3.3. Funding

Regional differences in the sources and nature of stable fly infestations make detailed discussion of funding for area-wide management programmes beyond the scope of this discussion. In most cases, some degree of public funding will be needed to support the administrative and regulatory framework. Where an industry or agronomic system is deemed responsible for economically significant outbreaks, stable fly management should be considered a production expense. Sources of funding for management of non-commercial sources and research will need to be identified by the regionally interested parties.

3.4. Management Options

3.4.1. Cultural / Sanitation Methods

Elimination of larval development substrates has always been the primary recommendation for stable fly control (Greene 1993). In the USA, where substrates associated with livestock production are considered primary developmental sites, this largely involves manure management. Piling manure reduces the surface area suitable for stable fly development and allows metabolic heat to raise the substrate temperature to a level where stable fly larvae cannot survive. Covering manure and silage excludes ovipositing females. Spreading manure thinly on fields permits it to dry before stable fly larvae can complete development. Avoiding and removing spilled feed reduces the amount of substrate available for larval development.

Cultural methods can be applied to stable flies developing in agronomic wastes as well. Burying post-harvest vegetable residues with several different types of agricultural machinery and then compacting the soil with a landroller has proven effective for reducing stable fly development in Western Australia (David Cook, personal communication). Burial of waste is less effective for pineapple because of the quantity, 230 tons per hectare (Solórzano et al. 2015). Removal of pineapple waste would rapidly deplete soil fertility and is technically not feasible due to the quantity.

Some cultural methods for reducing stable flies have negative environmental ramifications. For example, burning sugarcane prior to harvest reduces the amount of substrate available for stable fly larval development, but also has serious consequences for air quality. Likewise, disposal of vinasse (a byproduct of ethanol distillation) in bodies of water renders it unsuitable for stable fly development, but it pollutes aquatic ecosystems (Souza Dominghetti et al. 2015).

3.4.2. Biological Methods

Biological control agents for stable flies can be divided into three categories, parasitoids, predators, and pathogens. Under natural conditions, egg to adult mortality of stable flies is estimated to exceed 95%, about half of which can be attributed to parasitoids and predators (Smith et al. 1985). The remainder is the result of pathogens and environmental stressors.

Pupal parasitoids are the most commonly used biological control agent for filth flies (Rueda and Axtell 1985; Machtinger et al. 2015). Two genera of pteromalid wasps, *Muscidifurax* and *Spalangia,* are frequently observed parasitizing stable flies in North America with 2 and 4 relatively common species, respectively. Several species, including both genera, can be seen in individual collections. How these parasitoids partition their resources is not clear.

The efficacy of augmentative releases of parasitoids is equivocal. Several studies indicated released parasitoids decreased fly populations (Weinzier and Jones 1998; Skovgård 2004; Geden and Hogsette 2006), while others failed to show a significant effect (Meyer et al. 1990; Andress, and Campbell 1994; Skovgård and Nachman 2004). In Costa Rica, two species of parasitoids have been collected from stable fly pupae in pineapple residues, *Muscidifurax raptoroides* Kogan & Legner and *Spalangia gemina* Boucek (unpublished observations), and a pilot programme using inundative releases of *Spalangia endius* Walker is showing promising results (Solórzano et al. 2017).

Several predators have been observed feeding on immature stable flies including macrochelid mites and staphylinid beetles (Smith et al. 1987; Seymour and Campbell 1993). Augmenting predator populations has not been evaluated for stable fly control.

Pathogens of stable fly were reviewed by Greenberg (1977). Entomopathogenic fungi have been evaluated for control of immature (Moraes et al. 2008, 2010; Alves et al. 2012; Machtinger et al. 2016) and adult (López-Sánchez et al. 2012; Cruz-Vázquez et al. 2015; Weeks et al. 2017) stable flies. Various formulations are commercially available. Several studies have evaluated entomopathogenic nematodes in the genera *Heterorhabditis* and *Steinernema* for filth fly control. In laboratory assays, results have been very promising (Taylor et al. 1998; Mahmoud et al. 2007). However, field trials have been disappointing (unpublished data). Although we observed slightly reduced numbers of flies emerging from sites treated with nematodes, we were unable to find infected fly larvae or detect infective juvenile nematodes more than 24 hours after treatment using sentinel greater wax moth larvae *Galleria mellonella* L.

3.4.3. Traps and Targets

The majority of stable fly traps are based upon visual attractants with a sticky surface to catch the flies. Williams (1973) recognized that Alsynite® fiberglass panels selectively attracted stable flies and Broce (1988) modified the trap making it more efficient and resistant to windy conditions. The next generation of traps was derived from blue and black fabric traps designed for tsetse fly (*Glossina* spp.) control (Mihok et al. 1995). The blue and black fabric traps are of limited utility in temperate parts of North America where sticky traps outperform them and they are susceptible to damage from gnawing insects such as grasshoppers (Orthoptera: Acrididae)

(Taylor and Berkebile 2006). However, in tropical regions such as La Réunion Island, they have proven to be very effective, especially the Vavoua trap (Laveissière and Grébaut 1990; Gilles et al. 2007).

In Costa Rica, improvised traps constructed from white plastic bags coated with an adhesive (Fig. 2) have been employed by the thousands for control of stable flies around pineapple plantations (Solórzano et al. 2015). These traps must be replaced every 1-2 days because they become saturated with insects and lose their effectiveness (Beresford and Sutcliffe 2017). Because of the environmental impact of disposing of such large numbers of plastic bags, research is currently underway to replace the white traps with insecticide-treated Vavoua traps.

Figure 2. Sticky traps used for stable fly control in Costa Rica.

Targets are like traps, but they intoxicate the attracted insects rather than catch them. Therefore, they do not need to be emptied or replaced routinely. Meifert et al. (1978) developed an early target system for stable flies by applying permethrin to the fiberglass panels of the William's trap. They indicated that the system was able to reduce the stable fly population by 30% per day when employed at a density of one target for every five animals. Blue and black targets are a modification of the blue and black traps (Foil and Younger 2006). When impregnated with 0.1% λ-cyhalothrin or 0.1% ζ-cypermethrin targets remain effective for ≈4 months (Hogsette et al. 2008). In a study in Louisiana, an average of 220 stable flies landed per hour on targets long enough to be intoxicated (Hogsette and Foil 2018).

A disadvantage of the targets relative to traps is that they cannot be used to quantify the number of flies in the population nor the number of flies killed. In addition, targets provide less psychological satisfaction because dead or trapped flies are not apparent. However, both of these concerns can be mitigated by placement of sticky traps adjacent to selected targets (Foil and Younger 2006).

3.4.4. Chemical Control

- *Immatures*. Because substrates for stable fly larval development tend to be microbially very active (Romero et al. 2006; Talley et al. 2009; Scully et al. 2017), most insecticides applied to substrates tend to degrade quickly and have little residual activity. Two classes of insect growth regulators (IGRs), cyromazine and benzoylureas have proven to be the most effective for controlling stable fly larvae (Taylor et al. 2012b, 2014; Solórzano et al. 2015). A single application of these compounds can provide 12 or more weeks of control and they have relatively low vertebrate toxicity (Tunaz and Uygun 2004). Cyromazine and benzoylureas belong to different insecticide mode of action classes with distinct resistance mechanisms (Keiding et al. 1991; IRAC 2017). Therefore, they are suitable for rotation to reduce the development of insecticide resistance. In addition, cyromazine and benzoylureas are compatible with biological control using parasitoids (Ables et al. 1975; Morgan and Patterson 1990).
- *Adults*. Chemical options for controlling adult stable flies associated with food animals such as cattle are limited. Premise or area sprays should be reserved as a last resort for outbreaks where other control measures have failed. Pyrethroids remain effective, although resistance has been detected (Cilek and Greene 1994; Olafson et al. 2011). Their continued effectiveness is probably a reflection of the low efficiency of treatments (Greene 1993). Insecticide-impregnated netting provided as resting sites near livestock are showing promise, especially in the dairy environment. In a study in Nebraska, ≈1000 meters of netting were installed on the periphery of two dairy barns. Up to 60 stable flies per linear meter per day were collected dead beneath the netting. Based upon observations, we estimate that the collections represented less than 10% of the flies that were lethally intoxicated (unpublished data). As methods for targeting stable flies with insecticides improve, resistance will become a greater problem.

3.4.5. On-Animal

On-animal strategies include physical protection or barriers such as boots, masks, sheets, etc., and chemical agents such as repellents and insecticides. Physical protection is frequently used for high value animals such as horses, but it is not practical for livestock such as cattle.

On-animal chemical technologies such as ear tags and pour-on insecticides are commonly used to protect livestock from horn flies. However, because stable flies spend little time on the host and bite primarily on the lower legs, these technologies are less effective against them (Foil and Hogsette 1994; Broce et al. 2005). The primary disadvantage of on-animal chemical treatments is that they have short residual activity against stable flies, less than 3-4 days for most and less than 6-8 hours for many (Foil and Hogsette 1994; Mullens et al. 2009; Benelli and Pavela 2018). A combination of fipronil and permethrin provided 5 weeks of repellence when applied to dogs in the laboratory (Fankhauser et al. 2015); however, this formulation has not been tested on livestock in pastures.

3.5. Area-Wide Strategy for Stable Flies

With the current state of technology, management strategies incorporating the release of large numbers of biting, sterile or genetically modified, stable flies are unlikely to be accepted by livestock producers or the public. Pending the development, and public acceptance, of threshold independent genetic drive mechanisms for stable flies, management options are limited to the area-wide application and integration of traditional methods such as cultural, biological and chemical.

Cultural management of animal and vegetative wastes should be the first priority. In an area-wide programme, especially if agronomic systems are contributing significant numbers of flies, such control will need to be mandated along with inspection and enforcement systems. Most of the currently recognized larval developmental substrates originate from human activities, and therefore are more manageable. Those developmental substrates that cannot be rendered unsuitable for stable fly development by cultural methods will need to be treated with biological and/or chemical control agents. Although biological control programmes on stable flies have had inconsistent results, pteromalid parasitoids are the most developed option. IGRs are the most effective and environmentally sound chemical alternatives available. Insecticide resistance management including rotation of insecticides with distinct modes of action must be included for a sustainable management plan.

A concerted effort must be made to identify and remediate all larval developmental sites within the control region. Management of larval developmental sites must be the primary emphasis of an area-wide stable fly management programme. However, outbreaks of adult flies due to control failure or unanticipated developmental sites are still likely to occur. Adult stable flies need to be managed in the vicinity of the developmental sites and susceptible hosts including humans and livestock. Traps, targets and insecticide impregnated artificial resting sites are the best options for managing adult stable flies. On-animal insecticides and repellents may be necessary for short-term remediation in cases where other control measures failed, but these are best applied on a premise by premise basis and in pest hot spots, rather than an area-wide basis.

Depending upon the situation, one cultural method such as burying vegetable residues may be adequate to control a stable fly problem. Alternatively, multiple strategies including both larval and adult control may be required if no single technology is adequately effective. Reliance upon chemical control alone is short-sighted and will lead to insecticide resistance and eventual loss of control. Cultural, and often biological, control efforts should accompany chemical control.

3.6. Research Needs

Because area-wide management of stable flies is dependent upon reducing and eliminating larval developmental sites, it is imperative that we develop a better understanding of the biological, environmental and physical characteristics of developmental substrates. In addition to the developmental substrates discussed in Section 2, developmental sites which do not fit into the current paradigm appear to be contributing to the adult stable fly populations (unpublished data). Recognized larval developmental sites tend to have high densities of larvae restricted to small areas. Is it possible that we are overlooking a second type of developmental sites, those with low densities of larvae, possibly one or two per square meter, but distributed over many hectares of land? Possibilities include crop residues in agronomic fields and grass and other plant residues (thatch) in grasslands. If such "low-density, large-area" developmental sites are widespread, then a very different approach to stable fly management will be needed. A better understanding of developmental substrates will help with the development of cultural and mechanical methods to render substrates unsuitable for stable flies as well.

A second research priority is a better understanding of the population dynamics of both larval and adult stable flies. How are females locating oviposition sites and how are larvae utilizing the substrates? What environmental factors are driving dispersal and population fluctuations? How far are adults dispersing? Incorporation of this information into area-wide management projects will improve their efficiency greatly.

Lastly, improved adult suppression systems are needed; more efficient traps and targets requiring less maintenance and novel adult suppression methods will add greatly to management programmes. It is unlikely we will ever be able to locate and remediate all larval developmental sites within the potential dispersal distance of stable flies. Therefore, adult suppression will remain an important component of any management programme.

3.7. Education and Outreach

An area-wide management programme for stable flies must include an educational component. Primary to this effort is information on the types of flies associated with livestock, their biology and effects on the productivity and comfort of the animals. Education will improve public support from both political and applied perspectives. Without such education, a successful area-wide programme may be perceived by the public as a failure if infestations of other species of muscoid flies continue and cannot be differentiated from stable flies. Livestock producers and landowners should also be aware of the natural enemies of flies and methods to preserve and augment their populations.

All levels of the distribution chain for chemical control agents from producers and suppliers to cattlemen must know their proper use for the species of flies affecting livestock production systems.

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ADVANCES IN INTEGRATED TICK MANAGEMENT RESEARCH FOR AREA-WIDE MITIGATION OF TICK-BORNE DISEASE BURDEN

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SUMMARY

In some parts of the world, ticks are the most dangerous animals followed by mosquitoes as ectoparasites and vectors of infectious agents, causing morbidity and mortality in domestic animals including wildlife and humans. The majority of tick-borne diseases are zoonotic. The global importance of ticks and tick-borne diseases in veterinary medicine and public health keeps growing. Some ticks are invasive and transmit pathogens causing transboundary diseases of high consequence for populations of domestic animals and humans. Integrated management pursues the optimized use of compatible methods to manage pests in a way that is safe, economically viable, and environmentally sustainable. The area-wide approach augments and expands the benefits of integrated pest management strategies. Issues challenging the implementation, adoption, and viability of area-wide tick management programmes include funding and socio-political aspects, the availability of support systems related to extension and veterinary services, and stakeholder involvement. Management strategies need to adapt and integrate novel technologies to decrease significantly the use of pesticide and address the complex problem of ticks and tick-borne diseases effectively. Applying the *One Health* concept, the strategy to optimize health outcomes for humans, animals, and the environment, facilitates research on the interplay between climate, habitat, and hosts driving tick population dynamics. It enhances our understanding of the epidemiology of tick-borne diseases and advances their management. This overview of research for adaptive area-wide integrated management concentrates on ticks affecting livestock. Examples focus on *Rhipicephalus microplus* (Canestrini) as one of the tick disease vectors most studied worldwide. Highlights of integrated management research for ticks

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of public health importance transmitting zoonotic diseases are reviewed to document opportunities for integrated control that mitigate the health burden of tick-borne diseases on humans, domestic animals, and wildlife. Implementation of the research conducted so far is needed to accelerate advancements in area-wide management of tick populations that can be applied to improve prevention across tick-borne diseases, while decreasing pesticide application and contributing to vector control globally.

Key Words: Acari, *Rhipicephalus annulatus*, *Rhipicephalus microplus,* babesiosis, ectoparasites, disease vectors, tick-borne pathogens, acaricides, resistance, cattle fever tick reservoirs, livestock vaccination, area-wide tick management, integrated tick-borne disease prevention, One Health, global change, invasive

1. INTRODUCTION

In some parts of the world, ticks (Acari) are the most dangerous animals followed by mosquitoes as ectoparasites and vectors of infectious agents causing morbidity and mortality in domestic animals, wildlife and humans (Ahmed et al. 2007; Socolovschi et al. 2008; Heyman et al. 2010; Barker et al. 2014; Paddock et al. 2016). Approximately 80% of the cattle in tropical and subtropical regions of the world are affected by economically important ticks and tick-borne pathogens (McCosker 1979; de Castro 1997). In addition, estimates indicate that Lyme disease and other diseases caused by tick-borne pathogens could burden over 30% of the global human population by 2050 (Davidsson 2018; Sakamoto 2018). Most tick-borne diseases affecting people are zoonotic because they can be transmitted from wild and domestic animals to humans through the bite of an infected tick (Lorusso et al. 2016; Ojeda-Chi et al. 2019).

Life history traits afford ticks considerable importance as pests and vectors of pathogens. Ticks are ancient arthropods that parasitize vertebrate hosts by feeding on blood to be able to complete their life cycle (Mans et al. 2011; Peñalver et al. 2018). Tick-borne pathogens include protozoa, bacteria, and viruses that co-infect their vectors and hosts (Brites-Neto et al. 2015; Talactac et al. 2018; Wikel 2018). Being local specialists and global generalists in their host associations underlie the global distribution of ticks and their ability to adapt to diverse environmental niches (McCoy et al. 2013; de la Fuente et al. 2015b; Beati and Klompen 2019).

There are ca. 920 described tick species in the world, but the diversity of ticks remains to be fully established (Dantas-Torres 2018; Mans et al. 2019). The so-called hard ticks belong to the Ixodidae family that have a sclerotized scutal plate in their dorsum (Sonenshine and Roe 2014). By comparison, soft ticks in the family Argasidae lack the scutum and have a flexible leathery cuticle (Uspensky 2008). Depending on the tick species, the parasitic larva, nymph, and adult stages are completed in one, two, or three hosts (Estrada-Peña 2015). After blood-engorged, females that mated on the host, then drop off and lay their eggs in the environment (Needham and Teel 1991).

Some ticks are invasive and transmit pathogens causing transboundary diseases of high consequence for populations of domestic animals and humans (Minjauw and McLeod 2003; Burridge 2011; Fernández and White 2016; Higgs 2018; Robles et al. 2018; Spengler et al. 2018).

Non-anthropogenic and anthropogenic factors associated with global change, including environmental disturbance and climate variability (Benavides Ortiz et al. 2016; Ogden and Lindsay 2016; Singer and Bulled 2016), increased international trade and travel (Abdullah et al. 2018; Hansford et al. 2018), and the wildlife-livestock-human interface (Gortazar et al. 2015), have increased tick densities resulting in a greater prevalence of tick-borne disease cases (Gasmi et al. 2018; Rasi et al. 2018; Sonenshine 2018). Furthermore, several of the newly discovered tick-borne microbes are pathogenic to humans and domestic animals (Mansfield et al. 2017; Harvey et al. 2019).

Discoveries by Smith and Kilborne (1893), documenting that *Rhipicephalus annulatus* Say was a vector of *Babesia bigemina* (Smith et Kilborne 1893), were important in the history of science by showing for the first time that arthropods can transmit pathogens to their hosts (Smith and Kilborne 1893; McCosker 1993; Egerton 2013).

Smith and Kilborne (1893) suggested the destruction of all *R. annulatus* infesting cattle to treat the disease after noting that outbreaks of bovine babesiosis, caused by *B. bigemina*, also known as redwater or cattle tick fever, and considered to be the most economically important arthropod-borne disease of cattle worldwide (Bock et al. 2008), could not happen without tick parasitism. In retrospect, this research association is an example of the One Health concept described below because T. Smith was a physician and F. L. Kilborne a veterinarian (Schultz 2008).

By 1893, cattle in the USA, Australia, and parts of Africa were already immersed in dipping vats containing various chemical pesticides active against ticks commonly referred to through time as tickicides, ixodicides, or acaricides, to manage infestations associated with what we now know are tick-borne diseases (Angus 1996; George 2000; Alonso-Díaz et al. 2006). The term acaricide used here refers to pesticides used to kill ticks of veterinary and public health importance following the conventions of most literature published on the topic. Vaccination against the pathogen is another approach to prevent and control tick-borne diseases. Attempts by Connaway and Francis (1899) to protect cattle from bovine babesiosis were among the first ones to vaccinate against a tick-borne disease. Several vaccines are commercially available in Europe to prevent tick-borne encephalitis (Riccardi et al. 2019). Nevertheless, the need remains for improved and cost-effective vaccines to prevent tick-borne diseases affecting humans (Šmit and Postma 2016; Reece et al. 2018), as well as domestic animals (Perry 2016; Pruneau et al. 2018; Suarez et al. 2019).

Effective and safe tick and tick-borne disease management requires integration of rational tactics involving multiple biological, chemical, physical and vaccine technologies on and off hosts. They can include the judicious application of safer acaricides to address the concerns with chemical treatments (de Meneghi et al. 2016; Pfister and Armstrong 2016; Ginsberg et al. 2017).

Here we review highlights of integrated management research for ticks of public health importance transmitting zoonotic diseases to document opportunities for combined interventions that mitigate the health burden of tick-borne diseases, benefitting humans, domestic animals, and wildlife (Drexler et al. 2014; Khamesipour et al. 2018; Wang et al. 2018).

2. AREA-WIDE TICK MANAGEMENT AND RESEARCH

2.1. Research Needs for Integrated Area-wide Tick Management

This overview concentrates on research to enable the area-wide integrated management of livestock ticks. Examples focus on *Rhipicephalus microplus* (Canestrini), a one-host tick commonly known as the Asian blue tick or southern cattle fever tick, originally described as *Haemaphysalis micropla* by Canestrini (1887). It is one of the ticks most studied worldwide as it is a vector of *B. bigemina* and *B. bovis* Babes causing bovine babesiosis (Pérez de León et al. 2014b; Gray et al. 2019), and *Anaplasma marginale* Theiler causing anaplasmosis (Atif 2015). *R. microplus* is an invasive species considered the most economically important ectoparasite of livestock globally (Rodríguez-Vivas et al. 2017a; Betancur-Hurtado and Giraldo-Ríos 2018; Sungirai et al. 2018).

The synonym concepts of area-wide integrated pest management, system-, or area-wide pest management, convey the need for research that can be applied to address the complex problem with ticks and tick-borne diseases (Brévault and Bouyer 2014; Pérez de León et al. 2014a; Bourtzis et al. 2016). Efficiency and cost-effectiveness are fundamental to area-wide approaches dealing with societal problems for centuries, including those related to tick disease vectors (Hendrichs et al. 2007; Koul et al. 2008; Shepard et al. 2014).

The goal of integrated pest management is to optimize the use of compatible methods in a way that is safe, economically viable, and ecologically sustainable (Jørs et al. 2017; Mullens et al. 2018). The area-wide approach augments and expands to the population level the benefits of integrated pest management strategies. Tick suppression and eradication can be considered as a continuum in the spectrum of area-wide strategies to manage tick-borne diseases. Approaches for sustainable area-wide control of tick populations recognize the need for translational research to develop new and improved technologies before eradication can be contemplated (Bram and Gray 1979; Pegram et al. 2007; Pluess et al. 2012; Suckling et al. 2014). A common theme for these strategies is the continued need to re-evaluate our understanding of tick biology and ecology (Tatchell 1992; Schmidtmann 1994; Esteve-Gassent et al. 2016; Canevari et al. 2017).

2.2. Unifying Area-wide Tick-borne Disease Mitigation and One Health through Integrated Tick Management Research

Applying the One Health concept, i.e. a strategy to optimize health outcomes for humans, animals, and the environment, facilitates research on the interplay between climate, habitat, and hosts driving tick population dynamics. It enhances our understanding of the epidemiology of tick-borne diseases and advances their management (Dantas-Torres et al. 2012; Vayssier-Taussat et al. 2015; Laing et al. 2018; World Bank 2018) (Fig. 1).

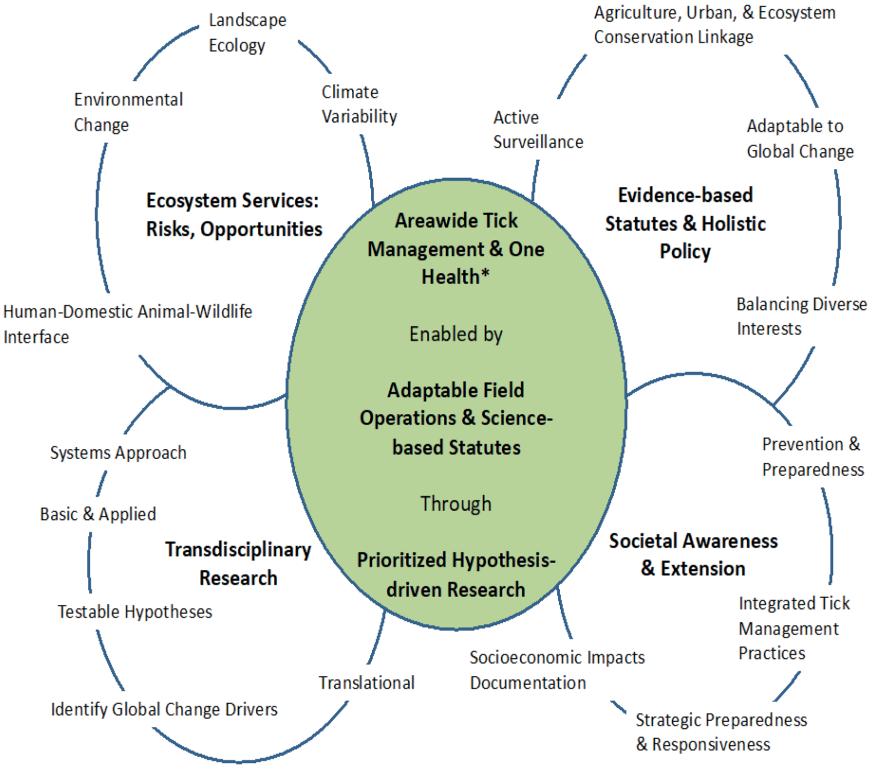

*Figure 1. Suggested research and implementation framework toward sustainable area-wide integrated tick management to prevent tick-borne diseases in the context of global change and the One Health approach (*adapted from Pérez de León et al. 2012).*

Previous efforts indicate socio-economic and cultural aspects must be considered in the planning and evaluation of area-wide tick management programmes (Pegram et al. 2000; Hendrichs et al. 2007; Rushton 2009; Mutavi et al. 2018). This can be done using algorithms to select area-wide tick management interventions where the evidence thus generated is used to enhance model predictions that improve area-wide tick management practices (Sutherst et al. 2007; Wang et al. 2017).

Ideal characteristics of technologies for broad acceptability and integrated use against ticks include low cost, minimal effort required for their application, spectrum of efficacy covering as many tick species as possible, and residual activity (Graf et al. 2004; Playford et al. 2005; Eisen and Eisen 2018). Control technologies can target ticks on or off the host.

In addition to acaricides, parasitoids and predators, alteration of the environment, and physical methods were identified for off-host tick control (FAO 1961). The Sterile Insect Technique, botanical repellents and acaricides, host resistance, pheromone-based approaches, and anti-tick vaccines are potential approaches to be integrated for the control of ticks infesting hosts (IAEA 1968; Ghosh and Nagar 2014; de Oliveira Filho et al. 2017). However, reducing to practice the integration of acaricides with other technologies in area-wide tick management remains to be fully accomplished (Jonsson 2004; de la Fuente et al. 2015a).

Adequate protocols and implementation research to evaluate technologies under field conditions are needed to generate the scientific evidence required to justify the investment of resources for area-wide tick management programmes (Piesman and Eisen 2008; Bautista-Garfias and Martínez-Ibañez 2012).

The adoption and viability of these programmes require attention to resource allocation and socio-political aspects, the availability of support systems related to extension and veterinary services, and the engagement of stakeholders (Walker 2011; Estrada-Peña and Salman 2013; Bugeza et al. 2017; Kerario et al. 2018; Mihajlović et al. 2019).

2.3. Alternatives to Acaricide Use and Strategies to Solve Resistance to Chemical Treatments

Chemical treatment practices in livestock production systems are under scrutiny because of the impact acaricides and endectocides like ivermectin have on public health, the environment, and the international trade of livestock and animal products (González and Hernández 2012; Arisseto-Bragotto et al. 2017; Miraballes and Riet-Correa 2018).

Intense chemical treatment of infested hosts exerts strong selection pressure for acaricide resistance among tick populations (Guerrero et al. 2014b; de Miranda Santos et al. 2018; Rodríguez-Vivas et al. 2018). Resistance to multiple classes of acaricides keeps spreading among tick populations due to intensive application (Miller et al. 2013; Cuore et al. 2015; Klafke et al. 2017b; Vudriko et al. 2018).

Acaricide resistance resulting from chemical treatment intended to control other parasites can exacerbate the problem with ticks and tick-borne diseases (Foil et al. 2004). Ivermectin used to treat gastrointestinal parasitic infections in cattle simultaneously infested with *R. microplus* selected for resistance due to exposure of the ticks to sublethal doses of that endectocidal drug (Alegría-López et al. 2015). *R. microplus* ranks sixth among the arthropods most resistant to pesticides in the world (Whalon et al. 2008).

Resistance to organophosphates, pyrethroids, amitraz, and ivermectin was reported in the brown dog tick, *Rhipicephalus sanguineus* sensu lato (Latreille) (Klafke et al. 2017a, Rodríguez-Vivas et al. 2017b).

Amblyomma cajennense s.l. (Fabricius) is another three-host tick that was found to be resistant to organophosphates and amitraz in Mexico (Alonso-Díaz et al. 2013). Different acaricide resistance profiles have been reported for two- and three-host tick species parasitizing cattle in South Africa (Ntondini et al. 2008). Widespread resistance to commonly used acaricides has not been reported for other important vectors of zoonotic tick-borne diseases parasitizing humans in the USA and Europe (Coles and Dryden 2014; EMA 2018).

Strategies to diminish acaricide use in domestic animals need to consider the concept of integrated parasite/vector management to maximize the contributions of veterinary public health towards sustainable development (Henrioud 2011; Scasta 2015; Narladkar 2018).

The commercial availability of a technology based on the recombinant protein Bm86 in the 1990s to vaccinate cattle against *R. microplus* represented a significant research achievement towards sustainable area-wide tick management (de la Fuente et al. 2007; Willadsen 2008). Integrating the use of a Bm86-based anti-*R. microplus* vaccine in an area-wide management programme confirmed that this approach decreases the frequency of acaricide treatments and diminishes the amount of chemicals used to control infestations, while reducing tick-borne cases in a cost-effective manner (de la Fuente et al. 1998; Redondo et al. 1999; Valle et al. 2004; Suarez et al. 2016). This is a rational and environment-friendly approach to manage *R. microplus* populations that are resistant to multiple classes of acaricides. Various research efforts to develop vaccines against other hard and soft ticks and the application of an anti-tick vaccine to protect humans, domestic animals, and wildlife from tick-borne diseases are ongoing (Évora et al. 2017; Almazán et al. 2018; de la Fuente et al. 2018).

Collaborative partnerships established to improve efficiencies in the research and development process of those anti-tick vaccines are examples of how global efforts could fully realize the benefits of international cooperation to enable breakthroughs allowing the adaptation of area-wide tick management practices to protect livestock and humans from tick-borne diseases (Sprong et al. 2014; Schetters et al. 2016; Rodríguez-Mallon et al. 2018; Ybañez et al. 2018). These joint international efforts have also resulted in the sequencing the genome of *R. microplus* to mine the information therein for the innovation of management technologies (Barrero et al. 2017).

Integrative taxonomy studies reinstated *Rhipicephalus australis* as a species and revealed that *R. microplus* consists of 3 clades (Estrada-Peña et al. 2012; Roy et al. 2018). Furthermore, some of the new microbes found to be associated with *R. microplus* are known livestock pathogens, while the pathogenicity of others remains unknown (Andreotti et al. 2011; Biguezoton et al. 2016; de Souza et al. 2018; de Oliveira Pascoal et al. 2019).

3. RESEARCH FOR ADAPTIVE AREA-WIDE TICK MANAGEMENT

3.1 Eradication Efforts Exemplify Challenges with Tick Management in the Context of Global Change

In 1906 the USA established the Cattle Fever Tick Eradication Program (CFTEP) to eliminate bovine babesiosis by exterminating the tick vectors of the disease based on the scientific evidence generated by the research of Curtice (1891) and Smith and Kilborne (1893) (Curtice 1910). In the context of complex socio-economic dynamics (Strom 2010), the CFTEP efforts has involved cooperation between federal, state governments, and the livestock industry. In 1943, with the exception of a Permanent Quarantine Zone along the Rio Grande in south Texas at the border with Mexico, the USA was declared free of the tick vectors (Graham and Hourrigan 1977).

The cattle fever ticks *R. annulatus* and *R. microplus* remain widespread in Mexico (Bautista-Garfias and Martínez-Ibañez 2012), and incursions from Mexico into the free zone, which comprises the rest of the continental USA, are buffered by the Permanent Quarantine Zone (George 1989; Klassen 1989). After 112 years of operations, events related to global change, such as land use changes, livestock-wildlife interface intricacies, and climate variability, complicate efforts by the CFTEP to keep the USA cattle fever tick-free (George 2008; Esteve-Gassent et al. 2014; Rutherford 2019).

A surge of cattle fever tick outbreaks in the free zone during the first decade of this century prompted a re-evaluation of the research agenda in support of the CFTEP (Pérez de León et al. 2010; Lohmeyer et al. 2011). Action was taken based on research needs in consultation with stakeholders to address the main concerns with acaricide resistance (Pérez de León et al. 2013), the role of native and exotic ungulates as cattle fever tick reservoirs (Pound et al. 2010), climate variability as a driver for the reintroduction of cattle fever ticks into the free zone (Giles et al. 2014), and a re-evaluation of financial losses associated with these concerns and events (Anderson et al. 2010).

Research in support of integrated cattle fever tick eradication provides a pathway to generate scientific evidence that could be used to adapt CFTEP operations that minimize the impact of global change (Pérez de León et al. 2012). Aspects related to the mechanism of action of organophosphate acaricides allowed charging the dipping vats at 0.3% coumaphos to mitigate concerns by the CFTEP with organophosphate-resistant cattle fever tick outbreak populations (Miller et al. 2005). The detection of pyrethroid resistance in cattle fever ticks infesting cattle and white-tailed deer, *Odocoileus virginianus* (Zimmermann), a wild ungulate species native to the Americas and a host of cattle fever ticks that is abundant in south Texas, limits treatments with permethrin by the CFTEP (Busch et al. 2014). Use of an injectable formulation of 1% doramectin was adapted by the CFTEP as an alternative eradication procedure (Davey et al. 2012). Macrocyclic lactone resistance among cattle fever tick outbreak populations has so far not been reported.

Further studies are required to determine if research can be translated into protocols involving the use by the CFTEP of safer acaricides to treat cattle and wild ungulates (Costa-Júnior et al. 2016; Gross et al. 2017).

A device using corn as bait to attract white-tailed deer for self-treatment was developed for use by the CFTEP because white-tailed deer cannot be gathered for treatment as it is done with cattle (Pound et al. 2012). The white-tailed deer consuming corn rub against acaricide-impregnated rollers placed on the device during the hunting season and are thus treated topically, whereas corn medicated with ivermectin acting systemically to control cattle fever ticks is used to bait white-tailed deer during the off-hunting season (Lohmeyer et al. 2013). However, complex white-tailed deer behaviours and social interactions to access the bait stations and other logistical aspects limit the use of this technology in the Permanent Quarantine Zone (Currie 2013).

Impediments for cattle fever tick eradication associated with the presence of nilgai (*Boselaphus tragocamelus* (Pallas) in parts of south Texas, where they can coexist with cattle and white-tailed deer, further exemplify the challenges presented by the livestock-wildlife interface for area-wide tick management (Wang et al. 2016; Singh et al. 2017; Lohmeyer et al. 2018). Nilgai are introduced bovid hosts of cattle fever ticks and suspected reservoirs of *B. bovis* and *B. bigemina* with home ranges larger than white-tailed deer (Foley et al. 2017; Olafson et al. 2018). Research is underway to determine if nilgai can be attracted to sites where they would be treated against cattle fever tick infestation (Goolsby et al. 2017).

The high efficacy of the Bm86 antigen against *R. annulatus* prompted efforts to research the use of an anti-tick vaccine as part of integrated cattle fever tick eradication procedures (Miller et al. 2012). Research involved reverse vaccinology to pursue the discovery of antigens that could be formulated for use by the CFTEP with efficacy against *R. microplus* equivalent to that of Bm86-based vaccines against *R. annulatus* (Guerrero et al. 2014a). In the interim, a public-private partnership enabled the use of a Bm86-based vaccine by the CFTEP (Pérez de León et al. 2018). This was a significant event in the history of cattle fever tick eradication in the USA because federal and state statutes, more than a century old governing the CFTEP, were adapted to use the anti-tick vaccine technology. This Bm86-based vaccine was used in a research project for integrated *R. microplus* management in Puerto Rico (Wang et al. 2019).

Integrating vaccination of white-tailed deer against cattle fever ticks would complement the effects of the self-treatment bait stations described above (Carreón et al. 2012; Estrada-Peña et al. 2014). However, delivery systems remain to be refined to vaccinate free-ranging white-tailed deer against cattle fever ticks in the Permanent Quarantine Zone.

3.2. Research Perspectives to Mitigate Tick-borne Disease Burden Focused on Integrated Tick Management

Applying the concept of precision agriculture and making use of newly available technologies, provides the opportunity to establish exact and targeted interventions to realize substantial savings in inputs for area-wide tick management (Urdaz-Rodríguez et al. 2015; Pérez de León 2017).

Experiments with unmanned aerial vehicles or drones showed this technology can support surveillance by the CFTEP (Goolsby et al. 2016), but it could also be integrated with remote sensing using ground-truth data for strategic cattle fever tick suppression (Phillips et al. 2014; Leal et al. 2018). Robotic technology is being adapted for tick control as well, showing potential in reducing tick densities (Gaff et al. 2015).

Precision tick management could facilitate the adoption of safer control technologies for effective area-wide campaigns. These include commercially available alternatives to the conventional use of acaricides such as acaropathogenic fungi or nematodes, and botanical acaricides, although they require further testing for adoption by the CFTEP (Thomas et al. 2017; Goolsby et al. 2018; Singh et al. 2018). Additionally, big data strategies facilitate the translation of genomic information into knowledge that can be applied to develop technologies which specifically target cattle fever ticks (Munoz et al. 2017; Brock et al. 2019).

Tick-borne diseases threaten public health in the USA. Around twenty human diseases or clinical conditions are associated with tick bites (USHHS 2018). Current trends indicate that >75% of the vector-borne disease cases reported are tick-borne (Rosenberg et al. 2018). Among the ticks commonly found biting humans (Eisen et al. 2017), the black-legged tick *Ixodes scapularis* Say is known to transmit seven pathogens of human diseases (Eisen and Eisen 2018). Controlling tick populations, together with personal protection measures, reduce exposure of the public to infected ticks, which prevents tick-borne diseases (Stafford III et al. 2017; White and Gaff 2018).

A higher level of public acceptability is associated with area-wide interventions employing technologies that are safe for people, pets, and the environment (Aenishaenslin et al. 2016; Keesing and Ostfeld 2018). These include the integrated use of host-targeted devices delivering minimal acaricide quantities with broadcast application of acaropathogenic fungus, as well as white-tailed deer reduction to decrease the risk of human exposure to *I. scapularis* infected with *Borrelia burgdorferi* Johnson et al. (Telford 2017; Williams et al. 2018). Additionally, internet-based surveillance tools and citizen science participation may enhance area-wide integrated tick management practices (Pollett et al. 2017; Nieto et al. 2018; Jongejan et al. 2019).

The detection in 2017 of *Haemaphysalis longicornis* Neumann, commonly known as the Asian longhorned tick, and subsequent reports of infestations in humans, domestic animals, and wildlife in the USA is a reminder of the threat posed by invasive ticks to the health of humans and other animals (Rainey et al. 2018). *H. longicornis* is a known vector of pathogens affecting humans, domestic animals, and wildlife in its native range and previously invaded areas, but it remains to be determined if it is transmitting pathogens in the USA (Beard et al. 2018).

Habitat suitability analyses indicate that *H. longicornis* could become established also in other parts of North America (Magori 2018; Hutcheson et al. 2019; Rochlin 2019). Challenges managing the spread of this Asian longhorned tick in the USA present an opportunity to apply the One Health concept where governmental agencies, academic institutions, public organizations, and private industry representing the agricultural, public health, medical, and veterinary sectors operate under a national strategy to prevent cases of *H. longicornis*-borne diseases in humans and other animal species.

Implementation research is needed to accelerate advancements in area-wide tick management. Achieving this goal will facilitate the adaptation and adoption of those advancements to improve prevention across tick-borne diseases while contributing to vector control globally (WHO 2017; Theobald et al. 2018; Fouet and Kamdem 2019; Petersen et al. 2019).

4. CONCLUSIONS

Ticks and tick-borne diseases continue to present new and emerging threats to humans, domestic animals, and wildlife. Constraints faced by the CFTEP to continue maintaining the USA cattle fever tick-free, a successful area-wide programme that has been operating in the USA since its establishment in 1906, illustrate how global change impacts area-wide tick management efforts.

Current issues are complex and need to be addressed by veterinary and public health programmes dealing with ticks and tick-borne diseases. This grand challenge requires a reassessment of strategies to manage tick populations. The One Health approach provides a framework to mitigate the health burden of tick-borne diseases on humans, domestic animals, and wildlife.

Advances in transdisciplinary scientific research present opportunities to adapt the strategy for area-wide tick management. The integration of novel technologies can decrease the use of acaricides significantly. Pilot field studies help determine the utility of integrated tick management strategies under real-life conditions. Outcomes from those pilot field studies inform decisions on the extent of interventions to prevent tick-borne diseases through improved tick population management. Progressive tick control affords flexibility to fine-tune the integration of technologies through the exchange of scientific information between stakeholders engaged in the adaptation process and provides feedback to revise the research agenda.

Implementation research can accelerate the translation of earlier research efforts to area-wide tick management practice. It is important for scientists to also understand the socio-economic context of research. Grasping the expectations of end-users of technology is paramount to realize the common vision of improving the outcomes of tick control interventions. This process will enhance the quality of evidence delivered by scientific research. Such scientific evidence can be used to generate the support for resources to establish the capacities required for the effective management of ticks to mitigate the burden of tick-borne diseases.

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AREA-WIDE INTEGRATED MANAGEMENT OF A *Glossina palpalis gambiensis* POPULATION FROM THE NIAYES AREA OF SENEGAL: A REVIEW OF OPERATIONAL RESEARCH IN SUPPORT OF A PHASED CONDITIONAL APPROACH

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SUMMARY

In 2005, the Government of Senegal initiated a project entitled "Projet de lutte contre les glossines dans les Niayes" (Tsetse control project in the Niayes) with the aim of creating a zone free of *Glossina palpalis gambiensis* in that area. The project received technical and financial support from the International Atomic Energy Agency (IAEA), the Food and Agriculture Organization of the United Nations (FAO), the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) and the US Department of State through the Peaceful Uses Initiative (PUI). It was implemented in the context of the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC) following a phased conditional approach (PCA) that entails implementation in distinct phases, in which support to the next phase is conditional upon completion of all (or at least the majority of) activities in the previous phase. In the case of the tsetse project in Senegal, the PCA consisted of 4 phases: (1) commitment of all stakeholders and training, (2) baseline data collection, feasibility studies and strategy development, (3) preparatory pre-operational activities and (4) operational activities. This paper provides an overview of the main activities that were carried out within each phase, with emphasis on the operational research carried out in phases 2

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and 3, that was instrumental in guiding the project's decision-making. Activities of phase 2 focused on the collection of entomological, veterinary, socio-economic and environmental baseline data, and a population genetics study that proved the isolated character of the *G. p. gambiensis* population of the Niayes. These data enabled the tsetse-infested area to be delimited to 1000 km^2, the impact of animal trypanosomosis on the farmers' welfare to be quantified (annual benefits of 2 million Euro in the tsetse-infested zone), and the formulation of an area-wide integrated pest management (AW-IPM) strategy that included a sterile insect (SIT) component to eradicate the isolated tsetse populations from the Niayes. In view of the extreme fragmentation of the remaining favourable habitat of the Niayes and the high human population density (peri-urban area), which excluded the possibility of using the Sequential Aerosol Technique, the IPM strategy that was selected comprised the suppression of the tsetse population with insecticide-impregnated traps/targets and the use of "pour-on" for cattle, followed by the release of sterile males to eliminate the remaining relic pockets. During phase 3, the pre-operational phase, a series of activities were carried out that were needed to implement the operational phase. These included the establishment of a colony of tsetse originating from the target area in Senegal, competitiveness studies between the sterile flies and those from the target area, development of transport methods for long-distance shipments of sterile male pupae, competitiveness of the sterile male flies after release in the target area, development of aerial release methods (including a new chilled adult release system) and development of a Maxent-based distribution model to guide the suppression, sterile male releases and monitoring of the eradication campaign. To be able to properly manage the eradication campaign in different phases, the entire target area was divided into 3 operational blocks. This paper demonstrates how, during the operational phase, scientific principles continued to guide the implementation process. The results to date are encouraging, i.e. the deployment of 269 insecticide-impregnated Vavoua traps in favourable habitat of Block 1 reduced the apparent density of the *G. p. gambiensis* population significantly (from 0.42 (SD 0.39) to 0.04 (SD 0.11) flies/trap/day). This was followed by the aerial release of sterile males that reduced the apparent density to zero after six months of releases. The last wild fly was trapped on August 9, 2012 in Block 1. In Block 2, during the suppression, the apparent fly density dropped from 1.24 (SD 1.23) to 0.005 (SD 0.017) flies/trap/day. Sterile male releases were initiated in February 2014 and expanded to cover the entire Block 2 in January 2015. The apparent fly density has so far been reduced to < 0.001 fly per trap per day until the end of 2018 and releases are still ongoing. The results of the campaign are discussed with respect to the "adaptive management approach" used, which was deemed critical for the success of the campaign.

Key Words: African animal trypanosomosis, *Trypanosoma vivax*, *Trypanosoma congolense*, *Trypanosoma brucei*, nagana, livestock, Sterile Insect Technique, SIT, vector control, elimination, tsetse flies, integrated vector management, adaptive management

1. THE TSETSE AND TRYPANOSOMOSIS PROBLEM IN THE NIAYES AND THE POLITICAL WILL TO FIND A SUSTAINABLE SOLUTION

In the sub-humid savannah of West Africa, riverine tsetse species such as *Glossina palpalis gambiensis* (Vanderplank 1949) inhabit riparian forests where they are major vectors of African animal trypanosomosis (AAT) or nagana (Bouyer et al. 2006; Guerrini et al. 2008) and human African trypanosomosis (HAT) or sleeping sickness (Camara et al. 2006). In Senegal, as in other parts of West Africa, AAT is a major obstacle to the development of more efficient and sustainable livestock production (Itard et al. 2003) and the presence of tsetse flies is considered a major cause of hunger and poverty (Feldmann et al. 2021).*Glossina p. gambiensis* normally thrives in areas that receive a minimum of 600 mm annual rainfall (Brunhes et al. 1998), but in western Senegal, annual precipitation is limited to 400-500 mm. Here, *G. p. gambiensis* populations are mainly confined to a specific ecosystem called the "Niayes" (Morel and Touré 1967; Touré 1971, 1973, 1974) that are situated around Dakar. These habitats are characterized by remnants of Guinean forests that are located in low-lying inter-dune depressions that are periodically or permanently flooded. However, in the last decades, these habitats have been drastically changed due to human intrusion. The second similar but drier ecosystem, “La Petite Côte”, is situated south of Dakar and extends along the Atlantic coast towards Joal and the Sine Saloum River (Fig. 1).

In the Niayes, temperature is lower and rainfall higher as compared with the interior of the country, and these conditions facilitate intensive cropping and cattle production even during the dry season. Horses are present in high numbers and are mainly used for the transport of food crops. The bites from tsetse flies pose a continuous nuisance for human populations, especially in Sebikotane and Pout. In addition, the flies seem to have adapted to peri-urban, densely populated areas such as the “Parc de Hann”, located in the city centre of Dakar.

The *G. p. gambiensis* populations that inhabited the Niayes and La Petite Côte belonged to one of the most north-western distributions of the tsetse belt in West Africa (Fig. 1). In 2007, a parasitological and serological survey of resident cattle revealed the seriousness of the tsetse and trypanosomosis problems in the area with AAT herd prevalence rates of 10–90% (Baba Sall, unpublished data). This survey showed that *Trypanosoma vivax* Ziemann was the most prevalent species, followed by *T. congolense* Broden. However, the parasitological prevalence may be grossly underestimated, due to the poor sensitivity of the buffy coat technique that was used (Pinchbeck et al. 2008).

In the 1970s, the first attempt was made to eliminate *G. p. gambiensis* populations from more than 150 km of linear habitat in the Niayes, using selective bush clearing and residual ground spraying with dieldrin. Although no tsetse flies were detected after the campaign (Touré 1973), they reappeared in the 1980s, necessitating a second campaign combining insecticide spraying with the deployment of traps and insecticide-impregnated screens. The tsetse problem seemed to have disappeared until in 1998 flies were again detected (Baba Sall, unpublished data).

Staff of the Direction de l’Elevage (DIREL) (now called Direction des Services Vétérinaires (DSV)) and the Institut Sénégalais de Recherches Agricoles (ISRA), in collaboration with the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture (the Food and Agriculture Organization of the United Nations (FAO), the International Atomic Energy Agency (IAEA)) and the IAEA’s Department of Technical Cooperation, carried out more extensive surveys in 2002–2003. These surveys confirmed the presence of *G. p. gambiensis* and in view of the isolated nature of the Niayes population (Solano et al. 2010) it is highly likely that the resurgence of the tsetse fly population can be attributed to a population build-up from small residual pockets inside the Niayes, rather than to reinvasion from the main tsetse belt of the Sine Saloum region that is located more than 100 km southeast of Dakar (S. Leak, unpublished reports to the IAEA; Baba Sall, unpublished data).

Following confirmation of *G. p. gambiensis* presence in the Niayes, the DSV and FAO/IAEA initiated a tsetse control campaign that officially started in 2005. Entitled “Projet de lutte contre les glossines dans les Niayes” (Tsetse control project in the Niayes), it was mainly funded and implemented by the DSV of the Ministry of Livestock and Animal Production and ISRA of the Ministry of Agriculture and Rural Equipment.

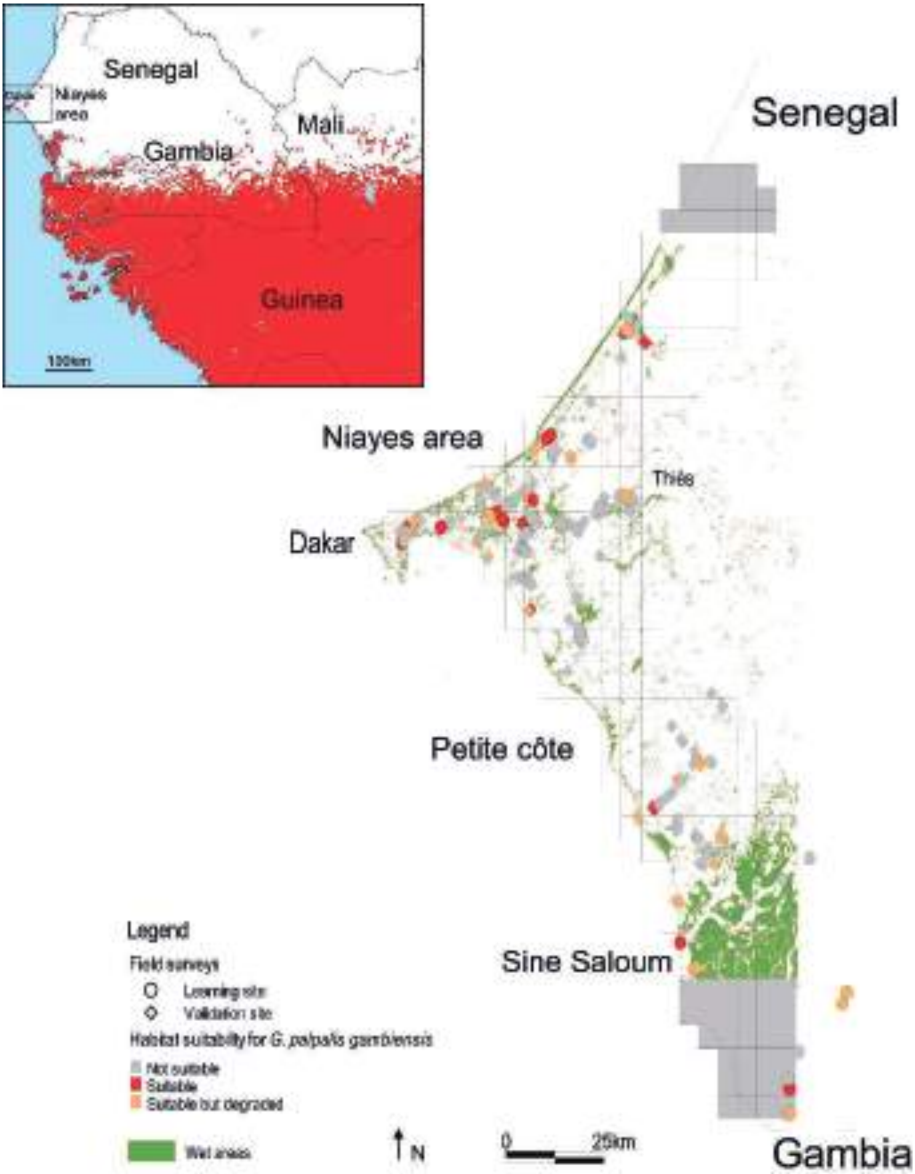

Figure 1. Map top left: Distribution of Glossina palpalis gambiensis *in West Africa and location of the Niayes area around Dakar in Senegal. The red area represents suitable habitats predicted from a Maxent model. Map right: The project area indicating the suitability of the vegetation for harbouring* G. p. gambiensis *after a phytosociological study, and the "wet areas" as obtained from a supervised classification (modified after Bouyer et al. 2010, 2015b).*

The project received technical and financial support from the IAEA, the FAO, the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), and the US Department of State through the Peaceful Uses Initiative (PUI). The Centre International de Recherche-Développement sur l'Elevage en zone Sub-humide (CIRDES), Burkina Faso, the Slovak Academy of Sciences (SAS), Slovakia, and l'Institut de Recherche pour le Développement (IRD), France, were other full- or part-time partners in the project. The project was implemented in the context of the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), a political initiative of the African Heads of State that called for increased efforts to better manage the tsetse and trypanosomosis problem on the African continent (PATTEC 2019).

2. PHASED CONDITIONAL APPROACH

From the onset, it was decided that the project would be implemented following a phased conditional approach (PCA), whereby project implementation follows distinct phases and in which support to the next phase is conditional upon completion of all (or at least the majority of) activities in the previous phase (Feldmann et al. 2018). Whereas the diverse phases of the PCA might differ with the target pest species, or if a suppression rather than an eradication strategy is selected (Hendrichs et al. 2021), the PCA consisted of 4 phases for the tsetse project in Senegal, i.e. (1) commitment of all stakeholders and training, (2) baseline data collection and feasibility studies, (3) pre-operational activities and (4) operational activities.

2.1. Phase 1: Stakeholder Commitment and Training

After the FAO/IAEA-supported surveys of 2002–2003, discussions within and between the Government of Senegal and the FAO/IAEA culminated in the submission of an official request by the Government of Senegal to the IAEA for technical and financial support. A technical cooperation project entitled "Feasibility Study to Create a Tsetse-free Zone Using the Sterile Insect Technique" was approved in 2005, which provided substantial support to phase 1 of the PCA.

The commitment of the Government was evidenced by the involvement of various Ministries in the project, i.e. the DSV of the Ministry of Livestock and Animal Production took responsibility for coordinating and implementing the project, the ISRA of the Ministry of Agriculture and Rural Equipment was given responsibility for operating the insectary/sterile male emergence and dispersal centre in Dakar and to guide the operational research that accompanied the project, and the Ministry of Environment and Sustainable Development provided the license to operate the project as it was considered environment-friendly.

Initially, training of essential project staff was emphasized, and a total of 16 veterinary field staff received training in tsetse biology, baseline data collection and control. This was a crucial step for the smooth implementation of the project in view of the limited experience of the field and insectary staff with tsetse flies, due to its absence from the Niayes for almost 20 years.

2.2. Phase 2: Collection of Baseline Data, Feasibility Studies and Strategy Development (2007-2010)

The importance of the availability of relevant baseline data (phase 2) cannot be overemphasized, as an appropriate control strategy cannot be developed without such detailed and accurate data. Data were required on the geographic distribution of the target tsetse population, their spatial and temporal dynamics, their spatial occupation of the habitat, their genetic profile, the correlation between tsetse presence/density and the parasitological and serological disease prevalence, the socio-economic impact of AAT on the farming community and the potential impact of the selected strategy on the environment (Fig. 2).

At the onset of the project, only limited data was available; therefore, during the first four years, all efforts were focused on collecting these data as part of a feasibility study. The data collected greatly assisted the decision-making process for selecting an appropriate strategy to sustainably manage the tsetse and trypanosomosis problem in the Niayes (Vreysen et al. 2007). The baseline data also enabled accurate monitoring of the operational eradication phase and continuous assessment of the progress made (Leak et al. 2008; Vreysen 2021).

The feasibility study was initiated with the development of a specific entomological sampling protocol aimed at accurately defining the distribution of the *G. p. gambiensis* populations in the Niayes and La Petite Côte. To enable the practical implementation of the protocol, a 5 x 5 km grid (286 cells) was superimposed over the entire initially defined project area of 7150 km^2 to facilitate the field sampling procedures (Leak et al. 2008) (Figs. 1 and 2). Spatial analytical tools were used to facilitate a preliminary phytosociological census that identified eight different types of habitat suitable to harbour *G. p. gambiensis*, which were denominated "wet areas" (Fig. 1).

In early 2009, 683 unbaited Vavoua traps Laveissière and Grébaut 1990) were strategically deployed in the area and the trapping data indicated that tsetse flies were present in 21 grid cells representing an area of 525 km^2. In the area of zero catches adjacent to the infested area (84 grid cells or 2100 km^2), a mathematical model was used to assess the risk that flies were present despite a sequence of zero catches (Barclay and Hargrove 2005; Bouyer et al. 2010).

The analysis showed a risk of tsetse presence > 0.05 in 16 grid cells or 400 km^2 which represented 19% of the area, which was therefore considered potentially infested and included in the target area. The remote sensing analysis identified 285 km^2 as wet areas, which comprised only 4% of the total project area of 7150 km^2, whereas the mathematical model provided an efficient method to improve the accuracy and the robustness of the sampling protocol (Bouyer et al. 2010). Thus, the total area that could be considered as potentially infested with tsetse flies and that could be subjected to the control effort was estimated at approximately 1000 km^2.

The entomological baseline data survey already indicated a high probability that the *G. p. gambiensis* populations of the Niayes were isolated from the remainder of the tsetse belt in the south-eastern part of Senegal. This assumption was mainly based on the absence of tsetse fly captures in La Petite Côte and the lack of any suitable tsetse habitat between the Niayes and the Sine Saloum, the nearest tsetse-infested area in the southeast.

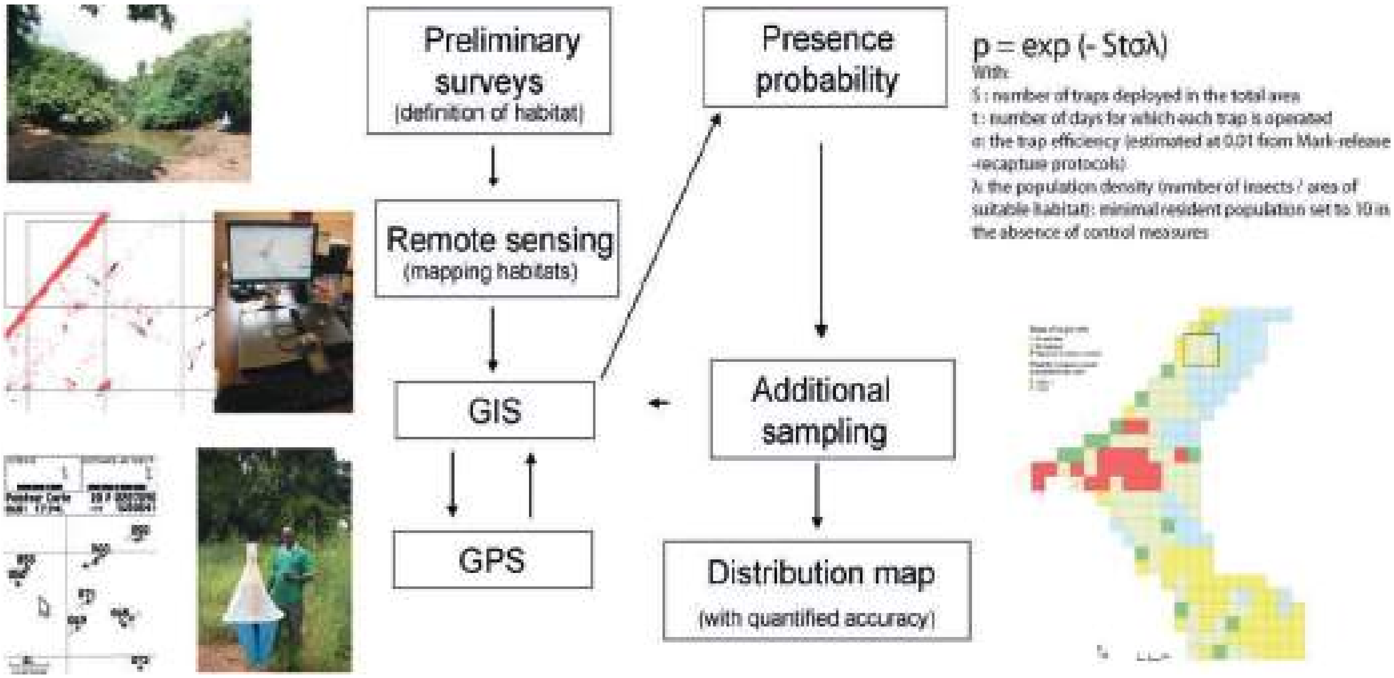

Figure 2. Sampling strategy used in the Niayes of Senegal to delimit the target population of G. p. gambiensis *(modified from Bouyer et al. 2010).*

To confirm this assumption, the genetic differentiation between the *G. p. gambiensis* populations from the Niayes and those from the south-eastern tsetse belt (Missira) was assessed. Using microsatellite DNA, mitochondrial COI DNA and geometric morphometrics of the wings of 153 individuals, complete genetic isolation of the *G. p. gambiensis* populations of the Niayes was confirmed. In addition, the *G. p. gambiensis* tsetse population from the Parc de Hann in Dakar proved to be isolated from other populations in the Niayes (Solano et al. 2010).

A third study focused on the parasitological and serological prevalence of AAT in cattle residing inside and outside the tsetse-infested areas of the Niayes. Before any control efforts were implemented, a mean parasitological prevalence of 2.4% was detected at the herd level in the tsetse-infested area, whereas serological prevalences of 28.7, 4.4, and 0.3% were obtained for *T. vivax*, *T. congolense* and *T. brucei brucei* Plimmer and Bradford, respectively (Seck et al. 2010). Moreover, the observed risk of cattle becoming infected with *T. congolense* and *T. vivax* was 3 times higher in the tsetse-infested as compared with the assumed tsetse-free areas. Furthermore, AAT prevalence decreased significantly with distance from the nearest tsetse sampled, indicating that cyclical transmission of trypanosomes by tsetse flies predominated over any potential mechanical transmission by other biting flies present in the area (Seck et al. 2010).

In addition to these studies, a socio-economic study was carried out to assess potential benefits from the sustainable removal of *G. p. gambiensis* from the Niayes. The study identified three main cattle farming systems, i.e. (1) a traditional system using trypano-tolerant cattle, and (2) two "improved" systems using more productive cattle breeds for milk and meat production. Herd size in improved farming systems was 45% lower and annual cattle sales amounted to €250 per head as compared with €74 per head in the traditional farming system. Tsetse distribution significantly impacted the frequency of occurrence of these farming systems with 34% and 6% of farmers owning improved breeds in the tsetse-free and tsetse-infested areas, respectively.

Two scenarios were considered with respect to potential increases of cattle sales as a result of the sustainable removal of the *G. p. gambiensis* population from the Niayes, i.e. a conservative scenario with a 2% annual replacement rate of the traditional system with improved ones, which was the rate observed just after tsetse eradication in Zanzibar (Vreysen et al. 2014), and a scenario with an increased replacement rate of 10% five years after the removal of the tsetse fly population. The final increase of cattle sales was estimated at ~€2800/km^2/year as compared with the total cost of the eradication campaign of ~€6400/km^2. The benefit-cost analysis indicated that the project was highly cost-effective, with internal rates of return of 9.8% and 19.1% and payback periods of 18 and 13 years for the two scenarios, respectively. In addition to an increase in farmer's income, the benefits of the eradication project included a reduction of grazing pressure on the already fragile ecosystem (Bouyer et al. 2014) (Fig. 3).

The project was considered an ecologically sound approach to achieving intensified cattle production without having a significant negative impact on the environment. Although the strategy included an initial insecticide (deltamethrin) component to suppress the tsetse fly population, the insecticide use was limited to impregnation of cloth traps, targets (Laveissière et al. 1985) and nets (around pig pens), and direct application to cattle (Bauer et al. 1995). The Sterile Insect Technique (SIT) (Knipling 1955) used as the final eradication component in the operational phase is a non-polluting control tactic that is very environment-friendly.

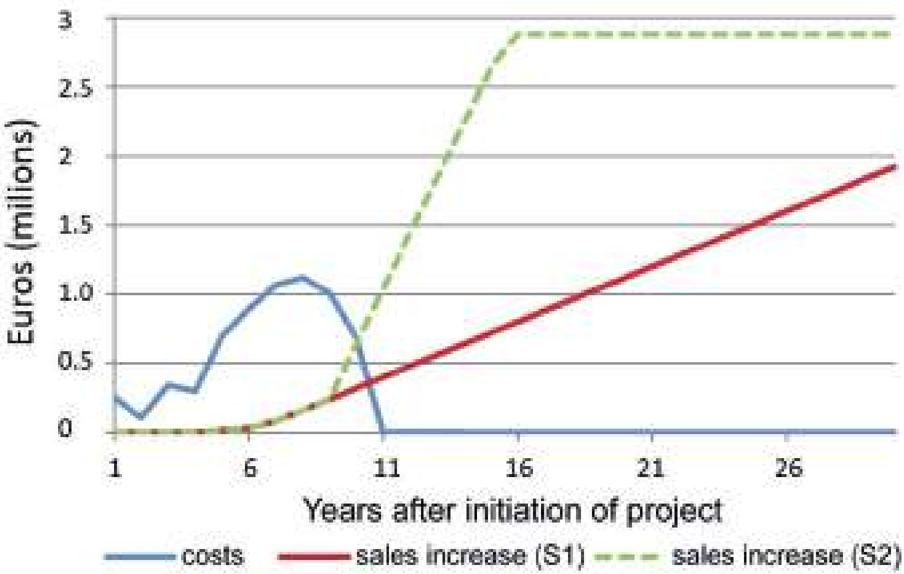

Figure 3. Comparison of the total costs of the eradication project and increase in global cattle sales per year (year 1 = 2007) taking into account two scenarios: a 2% annual replacement rate (S1) of local cattle with improved breeds, and an increased replacement rate of 10% five years after tsetse eradication (S2) (from Bouyer et al. 2014).

The SIT requires the production of large numbers of the target insect in mass-rearing centres, the sterilisation of the male insects using ionizing radiation (gamma rays or X-rays) and the sustained and sequential dispersal of the sterile insects over the target area in numbers large enough to outcompete the wild males for mating with wild females (Vreysen et al. 2013). The transfer of sterile sperm to wild virgin females results in embryonic arrest and hence the absence of offspring (Dyck et al. 2021). With each generation, the ratio of sterile to wild males will increase and as a result, the SIT becomes more efficient as population densities decline (inverse density dependent action of the SIT) (Vreysen and Robinson 2011).

In order to assess the potential impact of the eradication project and of the control tactics on the non-target fauna, an environmental monitoring project was implemented in five sites, one outside the tsetse-infested area (Mbour-centre IRD, a private protected area) and four within the targeted tsetse-infested areas (Dakar-Hann, Kayar, Thiès and Pout). Two fruit-feeding insect families (Coleoptera: Scarabaeidae (Cetoniinae) and Lepidoptera: Nymphalidae) were selected as indicator species as they have been shown to be highly appropriate for measuring the impact of various management practices on general ecosystem health in similar savannah areas in West Africa (Bouyer et al. 2007).

Monitoring with banana-baited traps indicated that of the ten most abundant Cetoniinae species, only one (*Pachnoda interrupta* Olivier) showed a significant reduction in apparent density in Block 1 (Kayar) during the operational phase (when insecticide-impregnated traps were deployed and cattle were treated with "pour-on"), but the population recovered to pre-suppression levels one year later. Similar observations were made with another Cetoniinae species, *Pachnoda marginata* spp. (predominantly *Pachnoda marginata aurantia* (Herbst) in Block 2. No significant impact was observed with the four most abundant Nymphalidae species (*Charaxes* butterflies). These data indicate that the overall impact of the project, as assessed using these sensitive non-target indicator species, was transient and very limited (Bouyer, unpublished data).

In addition to the above-mentioned studies, data were collected on population dynamics of the wild *G. p. gambiensis* populations in four different ecological sites. Apparent densities were shown to fluctuate both in space and time. Natural abortion rates were also highly variable in space and time and were modelled using MODIS satellite data, allowing the correction of apparent abortion rates during the sterile male releases (Bouyer, unpublished data). This in turn allowed the Fried competitiveness index to be estimated (Fried 1971), considering the observed abortion rate under a given sterile to wild ratio during the pre-operational phase. These data were crucial to make correct interpretations of the monitoring data during the control operations.

In conclusion, the data that emanated from these studies contributed to the strategic decision-making and the development of a control strategy. It prompted the Government of Senegal to adopt once more an eradication strategy (Hendrichs et al. 2021), as the isolated character of the *G. p. gambiensis* population of the Niayes and the integration of the SIT in this third attempt offered an opportunity to create a sustainable zone free of tsetse flies and trypanosomosis. In addition, it was decided to implement a project following area-wide integrated pest management (AW-IPM) principles that aimed to integrate the various control tactics (Vreysen et al. 2007) against the entire tsetse population within the circumscribed area to ensure that no population remnants would be left after the campaign.

Moreover, there were several aspects that made the inclusion of the SIT as a component of the AW-IPM strategy a prerequisite, these were: the fragmented nature of the preferred tsetse habitat, the two earlier failures to eradicate the target population in the 1970s and 1980s (Touré 1973) and the low impact/efficiency of insecticide-based bait methods on low-density populations of the targeted species (Bouyer, unpublished data).

It needs to be emphasised that most AW-IPM projects, especially those that incorporate a SIT component, are management-intensive and technically complex. In addition to a complete set of relevant baseline data, AW-IPM projects need to be implemented following sound scientific principles (Vreysen et al. 2007), and embarking on such a project without sound baseline data and a resulting comprehensive control strategy will have a high probability of failure. The probability of success will increase significantly when the project is accompanied by an all-inclusive operational research component to solve emerging problems during its implementation.

2.3. Phase 3: Pre-operational Phase (2009-2011)

2.3.1. Successful Suppression Trial in a Suitable Area of Kayar

A pre-release suppression trial, using insecticide-impregnated Vavoua traps, was carried out in the most northern part of the target area (Kayar) between November 2009 and December 2010, to assess the efficiency of this suppression tactic. Geographic information systems (GIS) and remote sensing were used to select favourable habitat sites at which to deploy the traps at a density of 40 traps per km^2 of suitable habitat, corresponding to 3.2 traps per km^2 in the test area (8% of suitable habitat) (Bouyer, unpublished data). Monitoring data indicated that the *G. p. gambiensis* fly populations were reduced to very low numbers, which confirmed the suitability of the suppression tactic selected for this ecological zone.

2.3.2. Establishment of an Insectary/Dispersal Centre at Dakar

In preparation for the development of colonies (see next Section), a building at the ISRA was refurbished and modified into an insectary/dispersal centre. Essential rearing and release equipment was provided through the IAEA's Department of Technical Cooperation to enable the rearing and maintenance of the tsetse flies.

2.3.3. G. p. gambiensis *Strains and Colony Establishment*

Since the 1970s, a colony of the target species, *G. p. gambiensis,* has been maintained at the CIRDES, Burkina Faso (denoted BKF strain), and was used for the successful eradication of a target population from 1500 km^2 of agro-pastoral land in Sidéradougou (Cuisance et al. 1984; Politzar and Cuisance 1984). From the onset of the project in Senegal, the Government decided not to develop its own mass-rearing facility to produce and sterilize the insects required for the SIT component, as the project area was judged too small to justify the expense of constructing and operating a tsetse mass-rearing facility.

Instead, it was proposed to procure the sterile male flies from the CIRDES. Although a recent study indicated that sterile males from this BKF strain were still competitive in riparian forests in Burkina Faso (Sow et al. 2012), relatively poor survival rates were obtained when released in the Parc de Hann of Dakar (B. Sall and M. Seck, unpublished data). It was speculated that this poor performance could be related to the extreme environmental conditions of this special micro-habitat in an urban setting.

To mitigate the risk that sterile males from the BKF strain would not perform in certain ecosystems in the Niayes, a decision was taken early on in the project to establish a *G. p. gambiensis* colony with pupae originating from Senegal (denoted SEN strain). Between October 2009 and September 2010, a total of 2185 pupae produced by wild-collected females were received at the FAO/IAEA Insect Pest Control Laboratory (IPCL), Seibersdorf, Austria, to develop a SEN colony. By the end of December 2010, the SEN colony had increased to about 450 producing female flies, and by mid-2012 the colony reached a maximum size of 4500 females. Thereafter the colony was maintained with around 1500 females (M. Vreysen, unpublished data).

In addition, a colony was established at the IPCL with pupae derived from the BKF colony in CIRDES to develop a back-up colony for the eradication project and to provide material for experimental work, such as mating compatibility studies between the target strain (SEN) and the strain used for release (BKF), development of transport protocols of the sterile male pupae under low temperatures and the development of an introgressed strain (BKF-SEN).

In view of the fact that colonization of a wild tsetse strain is a labour-intensive and lengthy process, an introgressed strain with a genetic background of 99% from SEN, that would also retain the adaptation to an artificial rearing environment (BKF strain) was developed. However, the strain proved to have a very low fecundity and the idea was abandoned.

2.3.4. Mating Compatibility and Competitiveness of the BKF and the SEN Strains

In view of the marked differences between the ecosystems of Burkina Faso and Senegal, and the large genetic differences between the two populations (BKF and SEN) (Solano et al. 2010), it was important to assess under semi-natural conditions the presence or absence of any potential mating barriers between the BKF and SEN strains that could jeopardize the release component and hence the outcome of the eradication campaign.

The mating performance of the BKF strain was compared with that of the 'wildish' SEN strain (that was a few generations from the wild) in walk-in field cages. The laboratory-adapted BKF strain showed close to equal competitiveness and mating compatibility with the SEN strain, which indicated the potential of using BKF strain males for the SIT component against the *G. p. gambiensis* populations in the Niayes (Mutika et al. 2013). These data were later confirmed during pilot trials in the target area (Bouyer, unpublished data).

2.3.5. Development of Protocols to Irradiate and Transport Male Tsetse Pupae

After the decision to procure the sterile males from the CIRDES, the Government of Senegal requested the IPCL to develop irradiation and transport protocols that would allow the shipment of (only) male *G. p. gambiensis* pupae over long distances, whilst retaining the female flies in the colony at the CIRDES. As female tsetse flies emerge two days before male flies, a scheme was proposed that would expose the male pupae to low temperatures after most of the female flies had emerged. The low temperatures would arrest male emergence from the pupae, making transport of irradiated male pupae to Senegal possible, whilst maintaining the required low temperature.

In the first series of experiments, exposing male pupae of *G. p. gambiensis* to low temperatures (10 and 12.5°C) for 3, 5, or 7 days immediately prior to emergence had no effect on emergence of male flies, whereas emergence of flies held at 15°C started before the simulated transport period was over. Survival of the experimental males and fecundity of females inseminated by males that emerged from pupae held at low temperature for different periods varied within the experimental groups, but mating performance of the experimental males was not impaired (Mutika et al. 2014).

A second series of experiments assessed the combined effect of irradiation and low-temperature period. Emergence and survival of adult male flies which were irradiated as pupae with 70, 90, 110 and 130 Gy on days 25, 27, and 29 post-larviposition was similar to that of un-irradiated pupae. Males that were irradiated with 110 Gy 24 h after initial exposure to the low temperatures and chilled for 5 days at 10°C were as competitive as un-irradiated males of the same age when competing with them in walk-in field cages for virgin untreated females (Mutika et al., unpublished data).

In addition to pupal irradiation and low temperature during their transport, the release protocol required a chilling period for adult males to allow immobilization and collection immediately prior to the aerial release (Mubarqui et al. 2014). A significantly lower proportion of males that had been irradiated (110 Gy) and held at low temperature as pupae (10°C for 5 days) and adults (5.1 ± 0.02°C for 6 or 30 hours six days after emergence) succeeded in mating compared to untreated colony males. Female insemination levels were slightly lower for males held at low temperature for 30 h compared to 6 h or not exposed to low temperature (standard colony conditions). The data confirmed the feasibility of transporting irradiated pupae at low temperatures for long distances followed by releases of chilled males using an adult release system, but it was found necessary to minimize the time that the adults remain chilled (Mutika et al., unpublished data).

2.3.6. Validation of Protocol for Long-distance Shipment of Irradiated Male Pupae

The use of isothermal boxes that contained phase change material (Phase Change Material Products Limited, Cambridgeshire, UK) packs to transport the male pupae was validated during weekly shipments from 2011 to 2013. More than 900 000 *G. p. gambiensis* pupae were transported in 132 shipments from the CIRDES in Burkina Faso, the SAS in Slovakia, and the IPCL in Austria to the ISRA in Dakar, Senegal, using a commercial courier service. The average temperature and humidity inside the insulated transport boxes were 10.1 ± 2.3°C and 81.4 ± 14.3% relative humidity, respectively. Pupae were collected on different days at the source insectary and depending on the date of collection, they were kept for different periods at low temperatures (4°C).

At the emergence and dispersal centre in Senegal (ISRA), the emergence rate from pupae that had been chilled at 4°C for one day in the source insectary before transport (batch 2) was significantly higher than that of pupae that had been chilled at 4°C for two days in the source insectary before transport (batch 1), i.e. an average emergence rate (± SD) of 76.1 ± 13.2% and 72.2 ± 14.3% respectively, with a small proportion emerging during transport (0.7 ± 1.7% and 0.9 ± 2.9% respectively). Among the emerged flies at the dispersal centre, the percentage with deformed (not fully expanded) wings was significantly higher for flies from batch 1 (12.0 ± 6.3%) than from batch 2 (10.7 ± 7.5%). The quantity of sterile males available for release as a percentage of the total pupae shipped was 65.8 ± 13.3% and 61.7 ± 14.7% for batch 1 and 2 pupae, respectively. The results showed that the temperature inside the boxes, during shipment, must be controlled around 10°C with a maximal deviation of 3°C to maximize the male yield (Pagabeleguem et al. 2015).

2.3.7. Quality Control Procedures to Assess Sterile Male Quality after Long-distance Shipment

Routine quality control procedures were required to regularly monitor the biological quality of the shipped and received biological material. This was important to ensure that the flies that were released, especially those released by air, were adequately competitive. A quality control test derived from the one used in fruit flies in Central America (Enkerlin et al. 2015) was developed to monitor the quality of *G. p. gambiensis* males that emerged from pupae produced and irradiated in Burkina Faso (irradiation done at CIRDES) and Slovakia (irradiation done at the IPCL) and transported weekly under low temperature conditions to Dakar.

For each consignment, a subsample of 50 pupae was taken before shipment and at destination to assess emergence, flight ability of the adult flies from a cylinder and survival of the flyers without access to blood meals. The quality protocol proved a good proxy of fly quality, explaining a large part of the variances of emergence rates, percentage of flies with deformed wings and flight ability in the field. Initially only 35.8 ± 18.4% of the transported pupae produced sterile males that showed a propensity to fly, thereafter named "operational flies" (Seck et al. 2015). However, these operational males were very competitive after release, which has already resulted in eradication of some of the target populations (Bouyer et al. 2012). Over time, the handling procedures and transport protocols were fine-tuned, resulting in a significant improvement in the percentage of operational flies from an initial 36% (SD 18%) in 2012 to 59% (SD 15%) in 2016 (Fig. 4). Unfortunately, this percentage dropped again in 2017 and 2018, mainly due to problems with environmental control and blood-feeding in the mass-rearing facilities producing the flies. Improving the quality of the flies will be crucial to ensure the success of the operational phase, as a significant positive correlation was observed between the recapture rate of sterile males in the field and this quality indicator (Bouyer and Seck, unpublished data).

2.3.8. Environmental Suitability of Available Strains for Release in the Niayes

At the CIRAD in Montpellier, a study was carried out to determine the critical environmental thresholds for survival of *G. p. gambiensis* flies from the three strains (BKF, SEN and the introgressed SEN-BKF strain). The study provided information on which strain would be best adapted to a particular environment or ecosystem. The optimal temperatures for maintaining flies of the BKF, SEN-BKF and SEN strains were 25 ± 1, 24.6 ± 1 and 23.9 ± 1°C, respectively. The survival of this tsetse species was governed by temperature alone and unaffected by changing humidity within the tested range. The BKF strain better survived temperatures above these optima than the SEN and SEN-BKF strains, but a temperature of about 32°C was the limit for survival for all strains. The relative humidity ranging from 40 to 75% had no effect on productivity at 25-26°C (Pagabeleguem et al. 2016b).

2.3.9. Field Competitiveness of the BKF Strain after Release in the Niayes

The competitiveness, mortality and dispersal of BKF flies was measured in the field in 2010-2011 (Bouyer et al. 2012) using mark-release-recapture studies in four different ecosystems (Hann, Diaksao Peuhl, Pout and Kayar). Data were collected on recapture rate, trap efficiency, daily mortality of the sterile males, dispersal capacity and mating competitiveness in both space and time. Female abortion rates (i.e. rate of induced sterility) were assessed through dissection of all captured wild females (Van der Vloedt and Barnor 1984; Vreysen et al. 1996) and corrected for natural abortion rates using developed models.

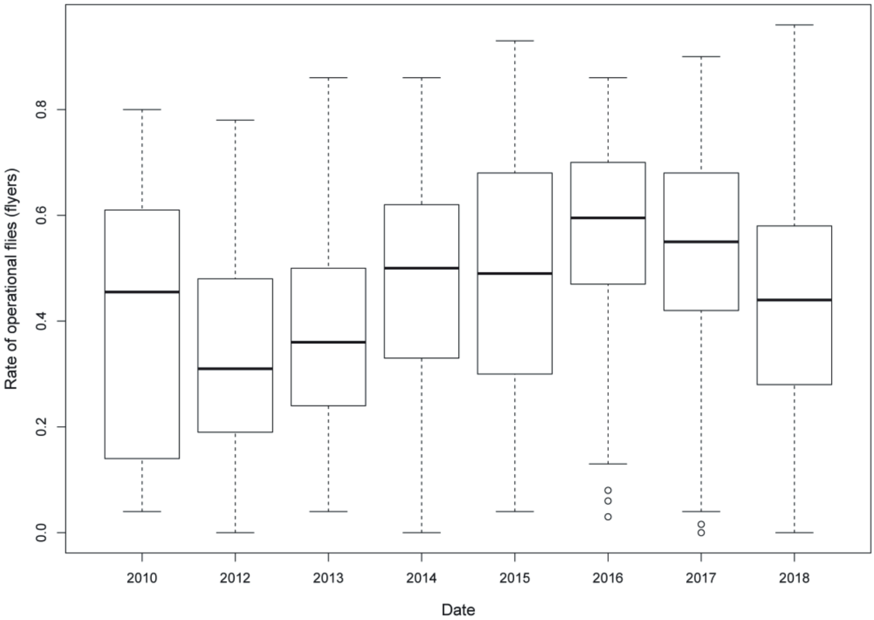

Figure 4. Rate of operational flies measured as the proportion of flies capable of flying out of a flight cylinder as part of routine quality tests at the ISRA (Institut Sénégalais de Recherches Agricoles) dispersal center (2012-2018). Boxplots present the median and quartiles and bars the 95% confidence intervals (updated from data published in Seck et al. 2015).

Trap efficiency (measured as the probability that a trap catches a fly present within 1 km^2 within 1 day (Barclay and Hargrove 2005)) was estimated at 0.03 (SD 0.04) and its variability in space and time was low. The daily mortality rate was quite homogeneous, but higher in the urban ecosystem (Parc de Hann) as compared with the more natural habitats. Although the dispersal rates were lower as compared with values obtained in riparian forests in Burkina Faso (Cuisance et al. 1984; Bouyer et al. 2007) they were, nonetheless, considered sufficient to obtain a homogeneous dispersal of sterile males using swaths of 500 m between aerial release lines. Finally, Fried indices obtained (Fried 1971) were high (> 0.35) but varied with the ecosystem. These data were instrumental in the development of an efficient release strategy for the sterile males.

2.3.10. Molecular Tools to Discriminate Sterile and Wild G. p. gambiensis *Flies*

In any AW-IPM project with a SIT component, the impact of the released sterile males needs to be assessed at regular intervals to monitor project progress and allow quick mitigation of emerging problems. Monitoring usually relies on an adult trapping system that captures both sterile and wild insects in a similar way (Vreysen 2021). This requires procedures that allow discriminating between the trapped wild and sterile male insects.

In the tsetse eradication project in Senegal, sterile adult male *G. p. gambiensis* were marked with a fluorescent dye powder (DayGlo®, 1% dye by weight mixed with sand) during emergence from the pupae (Parker 2005). A similar procedure was used in the *Glossina austeni* Newstead eradication project on Unguja Island of Zanzibar (Vreysen 1995). This type of marking is effective, although not infallible and in some cases, sterile male flies were only slightly marked; conversely, some wild flies could become contaminated with a few dye particles in the cages of the monitoring traps (which leads to incorrect interpretation of the trapping results).

In some cases, predatory ants also damaged the trapped flies, making discrimination between wild and sterile males using a fluorescence camera and / or a fluorescence microscope difficult.

A molecular technique, based on the determination of cytochrome oxidase haplotypes of *G. p. gambiensis*, was therefore developed to discriminate wild from sterile males with a high level of accuracy. DNA was isolated from the fly heads and a portion of the 5’ end of the mitochondrial gene cytochrome oxidase I was amplified for sequencing. All sterile males from the BKF strain displayed the same haplotype and differed from that of wild male flies trapped in Senegal (and in Burkina Faso). The method allowed complete and fail-proof discrimination between sterile and wild male *G. p. gambiensis* and might be used in other tsetse control campaigns with a SIT component (Pagabeleguem et al. 2016a).

2.3.11. Aerial Release Trials

Sterile male tsetse flies were released by air for the first time in the *G. austeni* eradication campaign on the Island of Unguja, Zanzibar (Vreysen et al. 2000), using biodegradable carton boxes that contained un-chilled sterile adult insects. The fixed-wing aircraft were equipped with an appropriate chute that allowed the cartons to be released through the fuselage of the aircraft (Vreysen et al. 2000).

In the Niayes project, the area that needed to be covered with sterile males was large enough to opt for aerial releases to efficiently disperse the sterile insects, rather than ground releases which were considered too costly, inefficient and not conducive to an area-wide coverage. The release vehicle of choice was the gyrocopter (Fig. 5), which was initially adapted to release sterile males in carton release containers. A gyrocopter is an autogyro that is characterized by a free-spinning rotor that turns because of the passage of air through the rotor from below which sustains the autogyro in the air, and a separate engine driven propeller that provides forward thrust (Wikipedia 2019).

Figure 5. Loading of the chilled adult aerial release device with immobilized adult tsetse males (top) and the gyrocopter ready for take-off to release the sterile insects (bottom).

Gyrocopters have been used for the SIT component in other AW-IPM projects, such as the release of sterile false codling moth, *Thaumatotibia leucotreta* Meyrick, in South Africa (Boersma, this volume).

The aerial release of sterile males using carton boxes was tested in a sub-unit of the first block (Kayar) along 4 release lines that were separated with a swath of 500 m. For 11 weeks (from March 2013 to June 2013), 32 boxes were released each week separated by a distance of 500 m over each release line. A total of 65 000 sterile males were released of which 316 flies (0.5%) were recaptured, giving an estimated daily mortality rate of 28% (SD 12%) and a mean daily displacement of 917 m (SD 477 m).

Although the release with carton boxes was very successful, a new approach for the aerial release of sterile tsetse flies was developed in collaboration with the Mubarqui group of Mexico (Mubarqui et al. 2014). This innovative system (Fig. 5) allowed the release of small numbers of tsetse flies per surface area (between 10–100 per km^2) and was based on the use of a vibrating mechanism. The device is guided by a GIS that can adjust flexibly the density of sterile males to be released depending on the requirements of the different target areas being treated. The GIS is installed on an android tablet which enables the pilot to concentrate on navigating the predefined release lines; the machine will automatically start releasing the required number of sterile insects for each target zone. However, the calibration of the release rate using the release machine proved challenging because of significant (unwanted) secondary vibrations of the gyrocopter. As a consequence, a new release device was designed, based on a rotating cylinder, which provided improved results (patent deposition number 1653994 by CIRAD and ISRA).

2.3.12. Use of a Maxent Distribution Model

All suppression and release activities were optimized using a Maxent distribution model that mixed high spatial resolution data (four supervised classifications of the vegetation Landsat 7ETM+ images from four seasons) with high temporal resolution data (MODIS images) that allowed a very good identification of suitable habitats (Dicko et al. 2014). The model was used to select and deploy insecticide-impregnated traps in suitable vegetation (see above), but also to adjust the release density of the sterile males in relation to the availability of suitable habitat (the reference was 10 and 100 sterile males per km^2 in unsuitable and suitable habitat, respectively).

2.4. Phase 4: Operational Phase (since 2011)

2.4.1. External Review of the Project

An external team of experts visited the project in May 2012 and reviewed all past activities since the initiation of the project. The evaluation team highlighted the thoroughness of the baseline data collection effort that enabled the project area to be defined. The reviewers likewise emphasized the good collaboration, complementarities and interaction between the persons involved in the project as a key factor for the project's success. The team concluded that the project was ready to enter the full operational eradication phase (unpublished report to the IAEA of an external review team–May 2012).

2.4.2. The "Rolling Carpet" Strategy

Although the *G. p. gambiensis* populations in the target area were genetically isolated from the remainder of the tsetse belt in the south-eastern part of Senegal, the lack of sufficient manpower in the field and insufficient numbers of sterile males available on a weekly basis made it impossible to tackle the entire project area at once.

During the baseline data collection, it became apparent that the project area contained three distinct tsetse populations in areas of suitable habitat that were separated from each other by zones of unsuitable habitat (or very fragmented suitable habitat), limiting the potential for tsetse dispersal. The project area was therefore divided into three main operational blocks, i.e. Kayar in the north (Block 1), Pout/Sebikotane/Diacksao Peulh in the middle (Block 2) and Dakar (Block 3B) and Thiès (Block 3B), west and east of Block 2, respectively (Fig. 6).

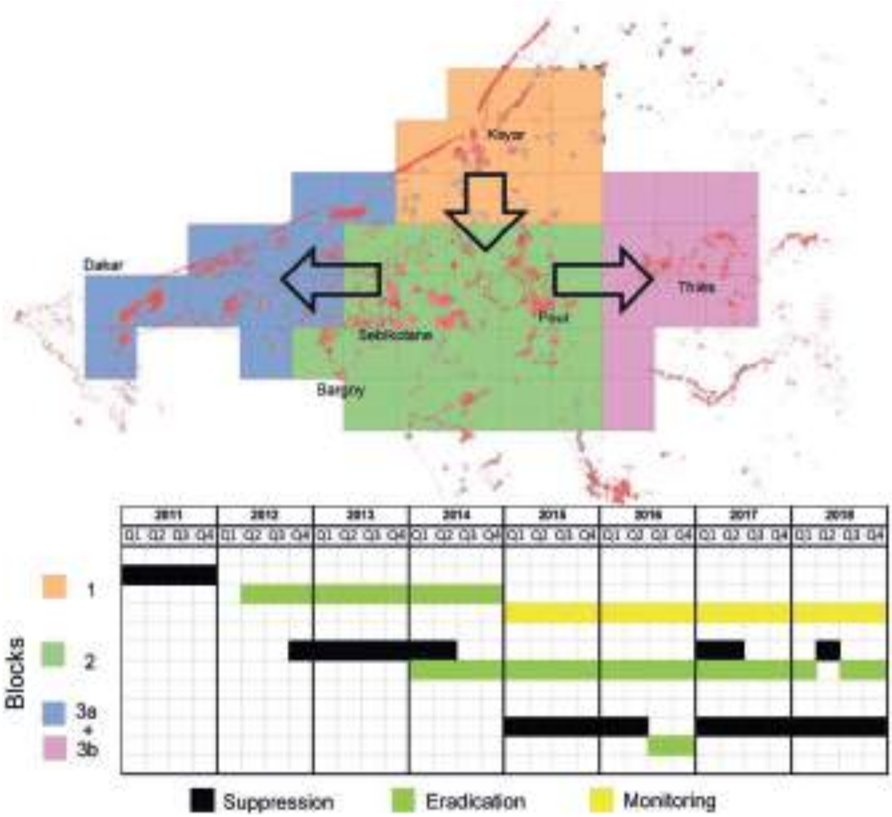

Figure 6. Map of project area with each grid cell corresponding to 5 x 5 km. Diagram of the three main activities of the operational phase in the different blocks of the project area.

An operational "rolling carpet" strategy (Hendrichs et al. 2021) was adopted and implemented whereby the different blocks were treated in sequence (suppression, followed by sterile male releases, and then monitoring of the status of eradication) (Fig. 6). In each block, insecticide-impregnated targets/traps were removed before the start of sterile male releases.

In Block 1, January 2011 marked the start of the operational phase of the project with the deployment of 269 insecticide-impregnated Vavoua traps (Laveissière and Grébaut 1990) in the favourable habitat areas, a density which corresponded to 19.4 traps per km^2 of suitable habitat. The apparent density of the *G. p. gambiensis* population dropped from an average of 0.42 (SD 0.39) flies per trap per day before the start of the suppression to an average of 0.04 (SD 0.11) flies per trap per day at the end of the trap deployment. This was followed by the aerial release of sterile males in March 2012 using biodegradable cardboard boxes over 185 release points, following 23 release lines over a total surface area of 72 km^2. In February 2014, the "boxed release" system was abandoned, and a "chilled adult" release system became operational (Mubarqui et al. 2014).

The apparent density of the *G. p. gambiensis* fly population was reduced to zero catches after six months of sterile male releases. In Block 1, the last wild fly was trapped on August 9, 2012, i.e. an old female (> 40 days), which was in her fourth oviposition cycle and which had an empty uterus. The next follicle in ovulation sequence was still immature and small, indicating an abortion of the larvae or an egg in embryonic arrest. This female showed a copulation scar and a spermathecal fill of 85%, indicating that its sterility was probably induced through a mating with a sterile male (Van der Vloedt and Barnor 1984; Vreysen et al. 1996).

From the beginning of the releases in Block 1 (March 16, 2012) to the date corresponding to the last capture, only three other wild females could be dissected, and all had indications of having mated with a sterile male. The average percentage of sterile males as a proportion of the total catch was then 99.2% (SD 1.6%), corresponding to a sterile-to-wild male ratio of 130:1. The percentage of sterile males remained 100% thereafter (no wild fly has been captured for the subsequent 78 weekly collections with 25 monitoring traps). Sterile male releases were suspended in late 2014 and as of January 2015, all sterile flies were released in Block 2.

The monitoring in Block 1 was continued on a monthly basis and is still ongoing at the time of writing. Since 2012, no wild flies have been trapped in Block 1, corresponding to a very high likelihood of eradication (probability of not detecting potential remaining flies $< 10^{-6}$ at the time of writing, considering that the population would have recovered to at least 10 flies during almost 2 years of monitoring without control) (Fig. 7, upper graph).

In Block 2, remote sensing and land cover maps were used to select 1205 suitable habitat sites for the deployment of insecticide-impregnated traps (corresponding to 16.7 traps per km^2 of suitable habitat and 2.7 traps per km^2 of the total targeted area). Deployment of the suppression traps in Block 2 was initiated in December 2012 and was supplemented with an additional 300 insecticide-impregnated traps in early 2013. In addition, at 6 monthly intervals, 2970 cattle were treated three times with a "pour-on" insecticide as a complementary method to suppress the *G. p. gambiensis* fly population.

In Block 2, the apparent fly density dropped from an average of 1.24 (SD 1.23) flies per trap per day before the suppression to an average of 0.005 (SD 0.017) flies per trap per day at the end of the suppression phase. Sterile male releases were started in Block 2 in February 2014, initially covering a quarter of the block, which was expanded based on sterile male availability to half of the block in April 2014. In January 2015, releases were expanded to cover the entire Block 2.

The apparent fly density was reduced to < 0.001 fly per trap per day by the end of 2018. The releases are scheduled to continue for another 10–12 months after the last wild fly has been trapped (Fig. 7, middle graph). In the beginning of 2017 and 2018 unexpected upsurges in the density of the wild fly population were observed in Block 2 in 3-5 areas. The reasons for these upsurges are not clear, but mitigating action was taken immediately, and suppression traps were deployed in the affected areas (Fig. 6). In addition, emergency insecticide spraying of *Euphorbia* hedges was carried out in selected areas, that brought the fly situation rapidly again under control. Depending on availability of sterile male flies, these areas received higher concentrations of sterile flies as compared to the rest of the area.

Ground releases were carried out in an area of 114 km^2 in Block 2, where recapture rates of sterile males released by air were consistently zero. This was later assumed to be correlated with the opening of a cement factory that apparently had a negative impact of fly survival. As a result, the aerial releases in that area were abandoned and replaced with releases from the ground. Additional ground releases were used in the hot spot areas of Block 2 (Pout and Diacksao Peulh) to supplement the aerial releases.

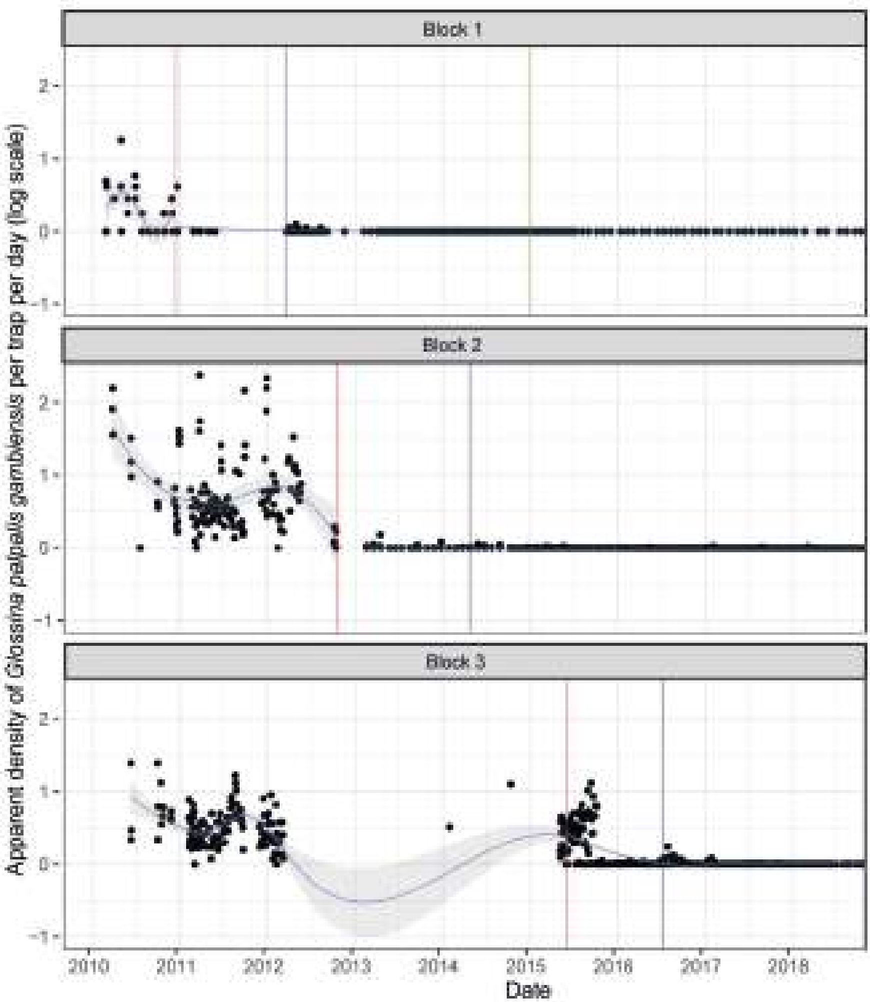

Figure 7. Apparent density (number of flies/trap/day) of the Glossina palpalis gambiensis *populations in Block 1, 2 and 3 of the Niayes during the pre-suppression, suppression and eradication activities. Pre-suppression activities are shown before the blue line, suppression between the blue and red lines, eradication activities with sterile male releases between the red and green lines, and post-SIT monitoring only after the green line.*

In Block 3, the suppression activities started in May 2015 with the deployment of 191 insecticide-impregnated traps in suitable habitat (12.6 traps per km^2 of suitable habitat), thereafter reinforced with an additional 43 traps. Before suppression, the initial apparent density of the fly population was 1.50 (SD 2.12) flies per trap per day; this dropped to 0.008 (SD 0.039) flies per trap per day in June 2016, i.e. a reduction of 99.4%. Sterile male releases were started in July 2016 in 100 km^2 of Block 3, but were suspended in early 2017, to accommodate the releases in the problem areas in Block 2 (Fig. 7, lower graph).

2.4.3. Monitoring the Progress of the Campaign

The Maxent distribution model was also used to guide the monitoring of the eradication campaign by deploying monitoring traps in suitable habitats (Dicko et al. 2014). As eradication was the selected strategy, the suitability threshold was set to provide a high sensitivity (0.96). The model was continuously improved during the project to increase its specificity from an initial 0.43 using the supervised classifications of the vegetation to 0.57 using the Maxent.

The areas around the monitoring traps were regularly cleared of vegetation and the monitoring traps were changed every 3 months. Moreover, monitoring traps in sites with no capture for one year were moved to other sites, but still within the predicted suitable habitats, and were labelled as temporary monitoring sites.

Regular parasitological monitoring of sentinel herds, each composed of ~100 tagged cattle, was carried out every year in three sites, of which one site was in a non-infested area and two were in the target area (in Blocks 1 and 2). In the non-infested area, the overall AAT seroprevalence remained below 5% between 2009 and 2017. In the target area, the AAT prevalence reduced quickly as control operations advanced ($p < 0.001$), i.e. from an initial value of >20% in 2009 to below 1% in 2014 in Block 1, and from 60-85% in 2009–2010 to below 5% in 2016 and 2017 in Block 2 (Bouyer and Seck, unpublished data).

In 2015, irregular sero-prevalence peaks of *T. vivax* were observed in both blocks, i.e. 12% in Block 1 and 16% in Block 2, which might be attributed to mechanical transmission (Desquesnes and Dia 2003; Desquesnes et al. 2009) facilitated by the presence of trypanosomes in tsetse in Block 3 and a small persistence in Block 2.

A blanket treatment of all cattle using trypanocidal drugs will be carried out in the Niayes area after tsetse eradication, to also ensure the eradication of trypanosomes.

3. DISCUSSION AND PERSPECTIVES

Many successful AW-IPM projects with a SIT component were or are implemented by management structures that were/are flexible and independent, with a high degree of financial and political freedom and not affected by strangling government bureaucracies and regulations (Vreysen et al. 2007). The New World Screwworm Commission, initially established between Mexico and the USA and later other countries in Central America, is a good example in this respect (Wyss 2006). The commission had to account for all financial, physical and human resources, could hire and fire staff based on merit and performance, and all staff were employed full-time without any other responsibilities (Vreysen et al. 2007).

Another good example is the Programa Moscamed, a cooperative agreement between Mexico, Guatemala and the USA, that has contained the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) for the last 30 years in Guatemala and has prevented its spread into Mexico and the USA, which are free from this pest (Enkerlin et al. 2015).

There are however examples of AW-IPM projects, albeit smaller than the examples given above, that operated successfully outside such an organization, e.g. the tsetse eradication project on Unguja Island of Zanzibar that was implemented within the Ministry of Agriculture, Livestock and Environment of Zanzibar (Vreysen et al. 2000). The success of the project, however, was made possible by the full autonomy and independence that was given to the senior project managers by the Government of Zanzibar to implement the project as required.

The project in the Niayes is likewise operated within the Ministries of Livestock and Agriculture (EXPO Milano 2015) and not implemented by an independent organization. The project adopted an "adaptive management" approach which included monthly project coordination meetings with the different stakeholders (Fig. 8). It is believed that this approach was critical to the project's success. This management approach involved all the stakeholders, including researchers, ensuring transparency and decision-making by consensus. The important decisions in the project were based on scientific principles (never political, personal, or emotional) and were guided by analysed field or other data. Day-to-day operational and financial problems were openly discussed, leading to consolidated solutions being found. Any decision that required follow-up actions was immediately acted upon and was always implemented according to plan, as the DSV and the ISRA had full authority over regional veterinary staff and technicians employed for the SIT component of the project, respectively. It is believed that the collaboration between the internal stakeholders, international partners and the policy of "non-interference" of the respective Ministries have been instrumental for the smooth implementation of the project.

The stability in project staffing, with basically no turn-over experienced in 12 years, both at the management and at the technical (insectary/field staff) level is considered another important factor for the project's success. This created a personnel culture of reliability, transparency and trust, and ensured the necessary institutional memory. The main outputs of the research component of this innovative project were the development of methods that allowed an optimization of the implementation of the SIT to eradicate the tsetse fly using an adaptive management scheme. The involvement of the public sector in the innovation processes guaranteed top-down control of the use of the technology from the central veterinary services to regional veterinary services or dedicated personnel (Devaux-Spatarakis et al. 2016).

All data generated within the project were transferred to and managed within a relational database that was accessible on the web with information displayed in graphs, featuring specific queries that allowed all stakeholders and the general public to make assessments of the progress of the project at any time and at a glance (Projet de Lutte contre la Mouche Tsé-tsé dans le Niayes 2019). This provided transparency on project progress for all stakeholders in the project and also facilitated statistical analyses of the field data to better inform the decision-making process.

Before the start of the operational phase of the project and after a critical review of its components, the Senegalese Ministry of the Environment issued a permit to implement the planned project, provided that it was accompanied by an environmental monitoring scheme for the entire life span of the project. This monitoring revealed a slight and transitory impact of the suppression activities on non-target fauna (Ciss et al. 2019). The removal of the tsetse fly and AAT from the Niayes is expected to result in an improvement of farming systems (i.e. a replacement of traditional, low-productive cattle with more productive cross- and/or exotic breeds–this replacement is already apparent in Block 1 and certain areas of Block 2), but at the same time in an anticipated reduction (up to 45%) of the average size of cattle herds (Bouyer et al. 2014, 2015a). This will actually significantly reduce overgrazing which is a major cause of land degradation in Senegal, and as such, the removal of the tsetse fly will have a positive impact on this already fragile ecosystem and environment (Budde et al. 2004). Despite the experienced upsurges of the wild fly population in the beginning of 2017-2018 period, the apparent density of the wild fly population has been significantly reduced in the entire project area and transmission of AAT has basically stopped in the Niayes at the time of writing. Consequently, milk production, resulting from an increased rate of replacement of local with exotic cattle, has significantly increased and milk import has significantly been reduced. In 2016-2017, Senegal imported more than 1000 exotic cattle into the Niayes area as compared to 100-200 in earlier years.

An important part of the operational funding was provided by international partners, such as the IAEA's Department of Technical Cooperation and the US Department of State's PUI. The socio-economic studies which were carried out documented the processes of innovation that increased the impact of the eradication project (Bouyer et al. 2014, 2015a), and the outcome of these studies were important to convince external partners to continue financing the project, even though it could take some time for the economic impact of the project to become visible.

Like many other AW-IPM projects with a SIT component, the AW-IPM campaign in the Niayes was accompanied by an extensive public relations campaign. The inhabitants of the Niayes were informed from the beginning and during the different phases of the project about the justification, activities, future advantages of the project through meetings organized by the Chiefs of the local veterinary centres in collaboration with administrative (sub-prefects) and local (village chiefs and locally elected politicians) authorities. It is believed that these meetings were instrumental in informing the general public about the project and soliciting their support. Even in the beginning of the project, the period of baseline data collection was taken as an opportunity to inform and intensify contacts with the local farming community regarding the project. In addition, T-shirts and hats were distributed that carried the logo of the project to increase the visibility of the project. Finally, two video films produced in 2012 and 2013 were aired on the national TV.

As was done for Block 1, probability models will also be used to verify eradication over the entire project area (Barclay and Hargrove 2005). These calculations might be complemented by a new innovative diagnostic technique that is based on the prevalence of specific antibodies against tsetse saliva in the host that can persist for 4-6 weeks, which is being developed as an indirect - but very sensitive - measure of tsetse presence (Somda et al. 2013, 2016).

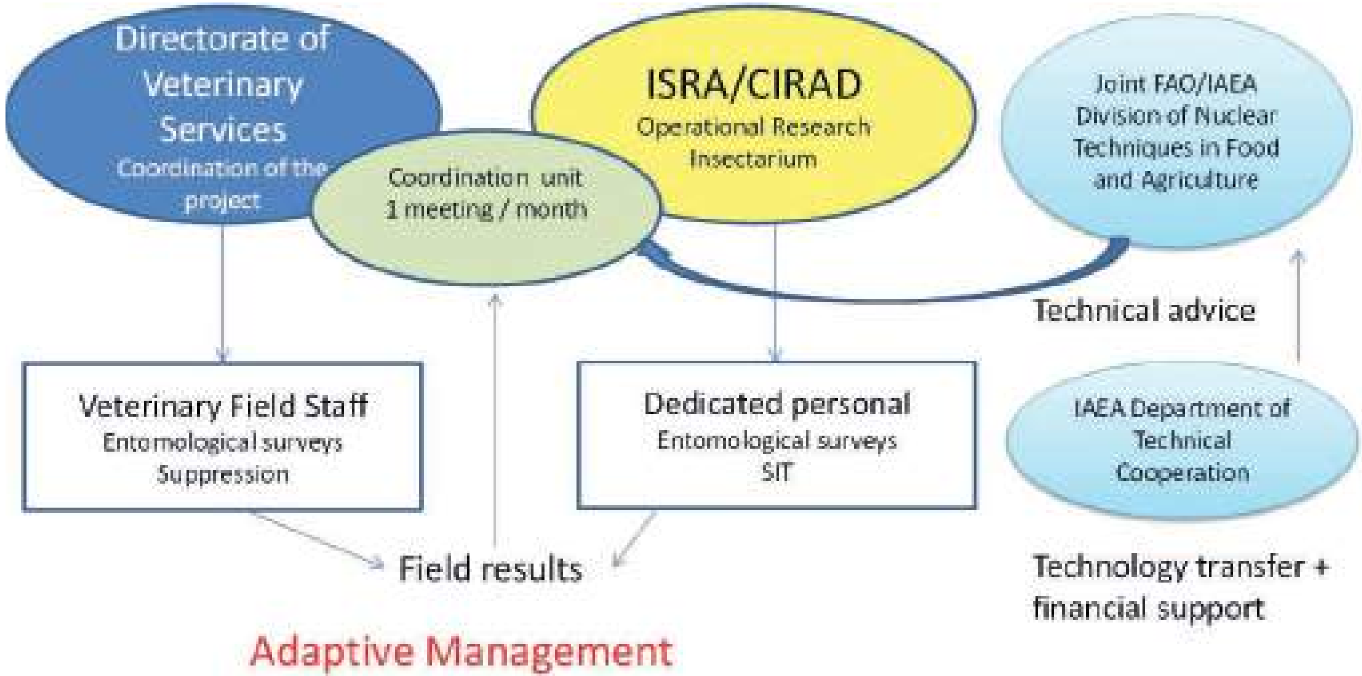

Figure 8. Organigramme of the adaptive management philosophy adopted by the project. (CIRAD = Centre de Coopération Internationale en Recherche Agronomique pour le Développement; ISRA = Institut Sénégalais de Recherches Agricoles; IAEA: International Atomic Energy Agency; FAO: Food and Agriculture Organization of the United Nations

The absence of circulation of the AAT parasites will also be used as an indirect measure of the absence of cyclical transmission and hence, the absence of tsetse flies. All these data will permit the provisional declaration of tsetse eradication after there have been zero fly catches for a period of six months and confirmed tsetse eradication if no wild tsetse are captured during for least one year after the end of control operations (Barclay et al. 2021).

Finally, friction models have been developed and used to identify other potential *G. p. gambiensis* populations that could be potential targets for eradication (Bouyer et al. 2015b). These models allow the resistance of the environment to tsetse dispersal to be mapped, i.e. natural barriers isolating sub-populations from the main tsetse belt. These "ecological islands" of suitable habitats might be good candidates for tsetse eradication projects, but feasibility studies similar to those described in the present paper will be necessary to accurately assess their boundaries and confirm their isolated status with respect to neighbouring populations. The method could be used to prioritize intervention areas elsewhere in Africa within the PATTEC initiative and is applicable to the strategic management of other vector and pest species.

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PHYLOGEOGRAPHY AND INSECTICIDE RESISTANCE OF THE NEW WORLD SCREWWORM FLY IN SOUTH AMERICA AND THE CARIBBEAN

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SUMMARY

Insect pests have a widespread negative impact on livestock production, resulting in large economic losses. Monitoring and surveillance of pest species are fundamental to manage their populations and reduce the damage they inflict on livestock. In addition, resistance to pest control methods, such as the use of insecticides, is becoming an increasingly important issue. Inferring population structure, the phylogeographic pattern of pest species, and the connectivity among populations is key to understanding migration patterns, which can be used to delineate area-wide pest surveillance and management schemes such as the Sterile Insect Technique (SIT). This review provides a summary of phylogeographic patterns of the New World screwworm (NWS) fly, *Cochliomyia hominivorax* Coquerel, a myiasis-causing fly that leads to significant losses in livestock production, based on molecular markers and the monitoring of insecticide resistance to improve its management. The species' current geographic distribution comprises most of the Neotropical region, having been eradicated in North and Central America after area-wide integration of the SIT with other methods. Introducing similar management programmes in South America and the Caribbean could be a strategic alternative to the permanent and exclusive use of insecticides, which has a negative environmental impact and is a growing challenge because of increasing resistance development in NWS. Such an area-wide approach requires NWS population delineation at regional and geographic scales, and the monitoring of mutations that are involved in insecticide resistance in natural populations.

Key Words: Cochliomyia hominivorax, myiasis, livestock, phylogeographic patterns, carboxylesterase, management unit, microsatellites, mitochondrial DNA, molecular markers, population structure, Neotropical region, Caribbean, South America

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1. INTRODUCTION

Successful eradication of the New World screwworm (NWS) fly *Cochliomyia hominivorax* Coquerel from North and Central America, using an area-wide integrated pest management (AW-IPM) (Klassen and Vreysen 2021) approach that included a Sterile Insect Technique (SIT) component, has triggered discussions about its potential eradication in the Caribbean and South America (Vargas-Terán et al. 2021). However, the high livestock density and wildlife distribution in the NWS fly's current habitat area, with the geographical and environmental settings including large rainforests, wetlands, and huge grasslands, make area-wide management and eventual eradication a great challenge.

The efficient area-wide management of a pest requires the control of all its target populations in a delimited geographic region, requiring a minimum area sufficiently large to guarantee that natural dispersion only occurs inside it (Klassen and Vreysen 2021). E. F. Knipling (1972) showed that the survival of a small remnant fraction of the population (i.e. 1% of the original population) is enough for it to recover to a density capable of causing economic damages in a few generations.

In this sense, the delimitation of adequate target regions and geographic scales is extremely important as well the understanding of gene flow pattern among populations (Tabachnick and Black 1995). Several studies, reviewed below, have aimed to characterize the structure of NWS fly populations and infer gene flow patterns at different geographic scales, from local to continental, providing a basis for distinct hypotheses about the distribution of genetic variability and its possible effects on control strategies.

Another important requisite for the effective application of the SIT is a low density of the target field populations (Knipling 1979). Due to the relatively high density of NWS populations in some local situations (Krafsur et al. 1979), complementary actions need to be taken to ensure their reduction prior to the release of sterile insects. Wound and myiasis treatment, which relies on the application of insecticides (e.g. organophosphates and pyrethroids), is the standard method to reduce NWS fly populations in the first step of a management programme (reviewed in Mangan and Bouyer 2021; Vargas-Terán et al. 2021). However, chemical treatment will not succeed if populations are resistant to the used compounds.

Thus, studies that aimed to discover the main genes involved in NWS fly insecticide resistance and to monitor the frequencies of mutations in the genes associated with this resistance in natural populations are also reviewed here.

2. POPULATION GENETICS AND PHYLOGEOGRAPHY

Over the last three decades, technological advances in molecular biology have led to the introduction of many types of molecular markers to assay genetic variation. Accompanying these advances, the genetic variability and structure of NWS fly natural populations in South America and the Caribbean region have been extensively studied and characterized (see Table 1 for a summary).

2.1. NWS Population Genetic Studies from South America and Caribbean Region

Restriction fragment length polymorphism of mitochondrial DNA (mtDNA RFLP) was the first method used and a seasonal analysis of a single population from Brazil (Caraguatatuba, São Paulo) indicated a high genetic heterogeneity for some restriction sites over time, with seven haplotypes exclusively found during summer and fall (Azeredo-Espin 1993). A study of four other populations from the same state, São Paulo, showed 15 haplotypes, with a small number of haplotypes widely distributed and a large number that appeared to be local (Infante-Vargas and Azeredo-Espin 1995). Similarly, Infante-Malachias et al. (1999) explored the nuclear genome with Random Amplified Polymorphic DNA (RAPD) markers, and detected moderate genetic differentiation among 6 populations from south-eastern Brazil and one from northern Argentina.

Table 1. NWS population genetic studies from South America and Caribbean region[*]

Reference	Region	Marker	Var.	F_{ST}
Azeredo-Espin (1993)	South-eastern BR	RFLP	-	-
Infante-Vargas and Azeredo-Espin (1995)	South-eastern BR	RFLP	H	-
Infante-Malachias et al. (1999)	Northern AR, south-eastern BR	RAPD	-	0.122
Taylor et al. (1996)	CB, CR, DR, JM, TT, southern BR	PCR-RFLP	H	-
Lyra et al. (2005)	UY	PCR-RFLP	H	0.145[**]
Torres et al. (2007)	UY	SSR	M	0.031
Torres and Azeredo-Espin (2009)	CB, DR, JM, TT	SSR	M/H	0.157
Griffiths et al. (2009)	BR, JM, TT, UY	SSR	M/H	-
McDonagh et al. (2009)	BR, CB, CO, DR, EC, JM, PE, TT, USA, UY, VE	mtDNA, Nuc	-	-
Lyra et al. (2009)	BR, CB, CO, DR, EC, JM, PY, TT, UY, VE	PCR-RFLP	L/H	0.130
Fresia et al. (2011)	AR and Lyra et al. (2009)	mtDNA	H	0.496
Fresia et al. (2013)	BL, CR, MX, US and Fresia et al. (2011)	mtDNA	-	0.155-0.718
Mastrangelo et al. (2014)	Amazon Basin BR	mtDNA, SSR	H	0.24(mtDNA) 0.099(SSR)
Fresia et al. (2014)	Fresia et al. (2011) and Mastrangelo et al. (2014)	mtDNA	-	-

[*] *Var., variability; H, high; M, moderate; L, low; F_{ST}, fixation index; SSR, microsatellites; mtDNA, mitochondrial DNA; Nuc, nuclear marker; AR, Argentina; BL, Belize; BR, Brazil; CO, Colombia; CB, Cuba; CR, Costa Rica; DR, Dominican Republic; EC, Ecuador; JM, Jamaica; MX, Mexico; PY, Paraguay; PE, Peru; TT, Trinidad and Tobago; UY, Uruguay; USA, United States of America; VE, Venezuela.*
[**] *Value not statistically significant*

Subsequently, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was used to characterize mtDNA variation in Caribbean, Central, and South American NWS fly populations (Taylor et al. 1996). Fourteen mtDNA haplotypes were observed among 18 flies, indicating high variability. These haplotypes, based on phenetic analysis, were divided into three discontinuous assemblages: "North and Central America", "South America", and "Jamaica". Notably, the Cuban sample seemed to be more closely related to Central American populations, while Dominican Republic samples were grouped with those from South America, suggesting a scenario of multiple origins of the NWS fly throughout the Caribbean.

The mtDNA variation was also investigated by PCR-RFLP in seven populations from Uruguay (Lyra et al. 2005). High genetic variability and no evidence of subpopulation differentiation were observed, indicating the existence of a single panmictic population. This lack of differentiation was attributed to the absence of geographic and/or climatic barriers and to the fact that Uruguay is almost at the southern extreme of the species' distribution. These same populations from Uruguay were also investigated by Torres et al. (2007) using nuclear microsatellites. A moderate degree of polymorphism and an excess of observed homozygosity were found, which could have been caused by demographic changes in response to the decrease in temperature and humidity in the Uruguayan winter and/or persistent insecticide treatment. It is likely that the low population differentiation was caused by passive migration of larvae through the movement of infested animals, as well as by recent recolonization events.

Microsatellite markers were also used to investigate ten populations from four Caribbean islands (Torres and Azeredo-Espin 2009) and, contrary to expectations, the level of genetic variability of some Caribbean populations was not lower than that of continental samples. In fact, moderate to high levels of genetic variability and a high level of population differentiation were found, even among populations within the same island.

Despite small sample sizes, an analysis of nine populations from South America and the Caribbean islands found microsatellite differences between Jamaica and Trinidad and Tobago, and in relation to the mainland (Griffiths et al. 2009). Population structure in mainland South America was more difficult to describe, but some weak signals of structure were detected, suggesting that population differentiation may exist between NWS flies from at least some areas.

McDonagh et al. (2009), utilizing the sequences of two mitochondrial (COI and 12S) and one nuclear (EF1α) gene investigated the phylogenetic relationship of NWS fly populations from the Caribbean, South America, and Texas ("historical" North American samples). This study found that NWS fly populations of the Caribbean islands were structured and suggested a period of isolation and/or founder effects following colonization from South America. The data did not support a North American origin of the Cuban NWS population, as previously hypothesized by Taylor et al. (1996). The NWS samples from Texas were in a different lineage as compared with South American and Caribbean samples, indicating a possible north-south division.

Lyra et al. (2009) conducted the first study on a continental-scale that encompassed NWS fly populations covering its entire distribution area. Thirty-four populations from 10 South American and Caribbean countries were analysed using mitochondrial PCR-RFLP. Population structure with significant fixation indices and low variability were found in the Caribbean, indicating that island populations have been evolving independently due to geographic isolation, but are connected by restricted gene flow. In contrast, mainland populations presented high genetic variability and low differentiation, with no correlation of genetic and geographic distances. The moderate and non-homogeneous level of genetic differentiation of the NWS fly in its current distribution area, as well as its high genetic variability, was described as being the product of several historical demographic processes.

In order to highlight and test the results obtained by Lyra et al. (2009), the same NWS fly samples and samples from four other populations were investigated using mtDNA sequences (Fresia et al. 2011). This study found that genetic diversity is distributed in four main groups of populations, corresponding to Cuba (CG), the Dominican Republic (DRG), and North and South Amazon regions (NAG and SAG, respectively). This phylogeographic structure of the NWS populations over its entire range was characterized by distinct historical events:

1. Island colonization from the mainland (a North American and/or Central American colonization was suggested for Cuba, whereas the other Caribbean islands were colonized from South America).

2. Recent separation of NAG and SAG probably associated to a barrier in the Amazon region resulting in separate populations in NAG and SAG.

3. Population expansion that started ca. 20–25 000 years ago and that increased exponentially up to date; it was probably linked with climatic oscillations in the late Pleistocene and resource availability. The population expansion probably caused the low divergence detected within SAG, erasing genetic and geographic correlations even among distant populations (maximum distance of 10 000 km).

In analysing mtDNA sequences from 60 populations (see Fig. 1), a north to south colonization was proposed for the continental Americas (Fresia et al. 2013). According to the best population divergence model chosen by Approximate Bayesian Computation (ABC), a first split occurred between North/Central American and South American populations at the end of the Last Glacial Maximum. A second split occurred between the North and South Amazonian populations in the transition between the Pleistocene and Holocene eras. The NWS fly went through a population expansion during its dispersal toward its current geographic range, with the strongest signals in SAG. This work concluded that climatic oscillations only were not sufficient to explain the phylogeographic patterns observed, and human activity might have played a crucial role in shaping the current distribution of the NWS fly.

The most recent survey of genetic variability was conducted on under-explored NWS populations of the highly important region in Amazonia, in an attempt to better understand the NAG-SAG evolutionary relationships (Mastrangelo et al. 2014). Based on 3 mtDNA genes and 8 microsatellite loci, a high genetic diversity and differentiation was revealed among 9 populations. These Amazonian populations only share mtDNA haplotypes with SAG, suggesting that the NAG-SAG split is the result of a barrier north of the Amazon Basin rather than of the basin environment itself.

Finally, pairwise F_{ST} among South American NWS fly populations were mapped with a geographic information system (GIS) on a friction layer derived from the Maxent niche modelling in order to identify connection corridors between NAG and SAG (Fresia et al. 2014). Despite methodological limitations, it was possible to identify two strong connections between the populations of the NAG and SAG: one along the Atlantic Ocean passing through the northwest of Brazil and the other passing through Peru. The main limitations for this approach are the sampling strategy based mainly on larvae, because it does not capture with precision the adults´ habitat, and the genetic distances estimation based only on mitochondrial DNA sequences.

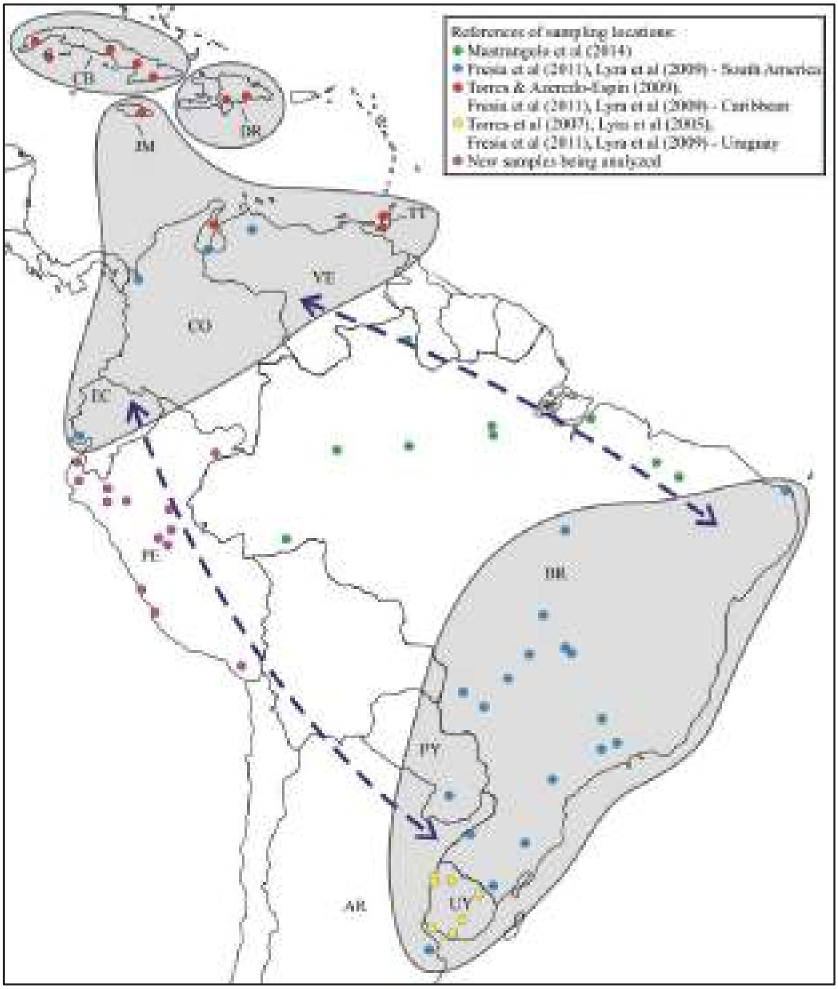

Figure 1. Consensus map showing sampled populations (coloured points), and current population structure scenario (the four main groups are highlighted in grey: Cuba, Dominican Republic, and North and South Amazon), and predicted connection corridors (dashed arrows) for NWS fly populations from the Caribbean and South America.

2.2. Consensus Scenario and Main Conclusions

Synthesizing the results from the previous studies presented above, we established the distribution of genetic diversity and population structure of the NWS populations in the Caribbean and South America (Fig. 1).

Caribbean populations are structured (Taylor et al. 1996; Griffiths et al. 2009; Lyra et al. 2009; McDonagh et al. 2009; Torres and Azeredo-Espin 2009; Fresia et al. 2011) and several events hypothetically resulted in their current distribution, such as Cuba having been originally colonized by North and/or Central American populations and the other Caribbean islands colonized by South American populations (Torres and Azeredo-Espin 2009; Fresia et al. 2011). However, the lack of congruence between nuclear (Torres and Azeredo-Espin 2009) and mtDNA (Lyra et al. 2009) genetic diversity in the Caribbean suggests a complex scenario of population structure.

Unlike the Caribbean populations, South American patterns of genetic variability and structure are not completely clear, but, in general, populations present a high genetic variability and low differentiation with no correlation to geographic distance. There are two distinct genetic groups, NAG and SAG (Fresia et al. 2011), probably separated by a barrier in the north of the Amazon Basin (Mastrangelo et al. 2014) during the transition between the Pleistocene and Holocene eras (Fresia et al. 2013). Populations experienced an expansion during the north-south colonization, mainly SAG, which is probably the cause of its low genetic divergence. All these historical factors and climatic oscillations are important to explain the pattern observed in South America, but current factors may also be influencing it, such as livestock movement and human activity.

Results of NWS phylogeography and population genetics studies can be of relevance to the operation of SIT programmes (Krafsur 1985). However, the significant genetic differences found in these studies do not result in mating incompatibility. Strains from three different locations in Brazil (i.e. Pará state, in the Amazon Basin; Piauí state, in the northeast; and São Paulo state, in the southeast) were crossed and showed no significant differences in all biological parameters assessed and no evidence of hybrid dysgenesis (Mastrangelo et al. 2014). Similarly, the crossing between a Brazilian strain from Goiás state in central Brazil, and the Jamaica-06 wt-strain, which is currently being mass-reared in Panama, did not show any evidence of genetic incompatibility or hybrid dysgenesis (Mastrangelo et al. 2012). The absence of mating incompatibility indicates that sterile males from the Jamaica strain reared in Panama could be used in future SIT-based control programmes throughout Brazil and, possibly, South America. Target management units still need to be determined within the NAG and SAG large geographic distribution area and a better understanding of the distribution of genetic variability in Amazonia is required before considering starting an AW-IPM programme against NWS in these regions. Regional-scale studies in South America were conducted only in Uruguay, a region that coincides with the southern-most distribution of the species, and different degrees of population polymorphism and structure were reported (Lyra et al. 2005; Torres et al. 2007). These differences can be associated with the distinct molecular markers used (Table 1), as they present different modes of inheritance (the effective size of mtDNA populations is one-quarter the size of nuclear DNA ones) and/or mtDNA can present a sex-biased gene flow among the populations.

2.3. Perspectives

In 2009, a study was carried out in a 100 x 60 km area situated at the Brazil-Uruguay border with samples collected during a pilot SIT project against the NWS (Pontes et al. 2009). The high genetic diversity and absence of population structure indicate that the target population limits are certainly larger than the pilot area, and consequently, the management unit should be larger than this pilot project.

Population analyses can be further refined through the use of new and more genetic markers. To reach this goal, we standardized a Genotyping-By-Sequencing (GBS) protocol for the species and the sequencing of the first library, which contains samples from one Uruguayan population, resulting in approximately 1000 filtered single-nucleotide polymorphisms (SNPs).

Another library is being constructed with individuals from the same population that were sampled one year later. After generating these data, we aim to evaluate if the obtained SNPs will give an increased resolution for temporal population genetic analyses in comparison to other molecular markers (mtDNA and microsatellites).

Recently the evolutionary relationships and the phylogeographic structure of populations from the northwest of Brazil and Peru (i.e. the predicted corridor connecting NAG and SAG (Fresia et al. 2014)) were investigated using samples of 13 NWS populations from Peru that were obtained with the assistance of the Servicio Nacional de Sanidad Agraria (SENASA) of Peru (Fig. 1), and three mitochondrial regions (COI, COII, and CR) are being sequenced. Preliminary results suggest the presence of genetically distinct groups with some geographic isolation, high haplotype diversity, low nucleotide diversity, and significant negative values of Tajima's D and Fu's Fs, indicating population expansion.

3. INSECTICIDE RESISTANCE

3.1. Investigation of the Molecular Basis of Resistance Mechanisms

NWS fly management throughout South America is mostly carried out independently on each farm and the farmer decides on the used control strategy. Topical insecticide application on livestock is the most popular and effective suppression method, and two main classes of compounds are used, i.e. organophosphates (OPs) and pyrethroids, which can be applied separately or in combination (Coronado and Kowalski 2009; SINDAN 2010).

A decrease in carboxylesterase activity has been observed in OP resistant strains of some arthropod species (Van Asperen and Oppenoorth 1959; Towsend and Busvine 1969; Hughes and Raftos 1985), that has resulted in the formulation of a mutant ali-esterase hypothesis. This suggests that a structural mutation in a carboxylesterase results in a reduced ability to hydrolyse aliphatic ester substrates, but also in an acquired ability to hydrolyse OP substrates (Claudianos et al. 1999).

In the Australian sheep blowfly, *Lucilia cuprina* (Wiedemann), which belongs to the same family Calliphoridae as the NWS fly, the *LcαE7* gene encodes the ali-esterase E3 isozyme. Biochemical assays with proteins produced by different *LcαE7* alleles showed that an amino acid substitution at position 137 (Gly137Asp) abolished the ali-esterase activity and increased diethyl-OP hydrolase activity, while a second amino acid substitution (Trp251Leu) increased dimethyl-OP hydrolase activity (Campbell et al. 1998). These two amino acid substitutions confer insecticide resistance because they are part of the active site of the enzyme (Newcomb et al. 1997, Campbell et al. 1998).

Based on these previous studies and in view that OPs are commonly used to suppress the NWS fly, the E3 gene in this species (*ChαE7*) was partially characterized (Carvalho et al. 2006, Carvalho et al. 2009). Mutations at the positions responsible for conferring OP resistance in *L. cuprina* (Gly137 and Trp251) were identified, but unlike with *L. cuprina*, NWS fly samples with a mutation in the Trp251 residue showed the substitution of a tryptophan for a serine. It is suggested that this new substitution has the same effect of reducing esterase activity (Taşkin et al. 2004) and may also be involved in pyrethroid resistance and be the molecular basis of cross-resistance between OPs and pyrethroids (Heidari et al. 2005). The strong association between this mutation (Trp251Ser) and dimethyl-OP resistance was later confirmed (Carvalho et al. 2010a).

Population genetic analyses assessed the selective pressures that have shaped carboxylesterase E3 evolution in NWS (Bergamo et al. 2015) and found a negative association between the Gly137Asp and Trp251Ser mutations. Fay & Wu's H value was significantly negative for the exons in which these mutations occur, which suggests that the E3 gene has evolved under positive selection, which is indirect evidence of its role in insecticide resistance.

This association between carboxylesterase E3 mutations and insecticide resistance were not directly proven by bioassays. Only the study involving bioassays by Silva and Azeredo-Espin (2009) indicated a correlation between the Trp251Ser mutation and moderate resistance to the pyrethroid cypermethrin. However, the high conservation of mutations in this gene among dipteran species suggests that the same resistance mechanism could have evolved in the NWS fly. Moreover, mutation-mediated resistance conferred by the E3 gene appears to be the main resistance mechanism selected in this species.

Other mechanisms of insecticide resistance were also investigated for the NWS fly: point mutations in the sodium channel, known as "knockdown resistance" (kdr) (Silva and Azeredo-Espin 2009); point mutations in acetylcholinesterase (AChE) (Carvalho et al. 2010a, Silva et al. 2011); changes in the expression levels of glutathione S-transferases and cytochrome P450 monooxygenases (Carvalho et al. 2010a); and glutamate-gated chloride channels (Lopes et al. 2014). However, no evidence of their association to insecticide resistance was detected.

3.2. *Field Monitoring of Mutations in the Carboxylesterase E3 Gene Associated with Organophosphate Insecticide Resistance in South America*

In view of the mutations of the carboxylesterase E3 gene that were identified as an important insecticide resistance mechanism in the NWS fly, the characterization of this gene in natural populations of the species throughout its current geographic distribution area can be an important tool for area-wide monitoring of resistance to insecticides. This information can then be used to select and implement more effective pest management programmes.

The Trp251Ser and Gly137Asp mutations were screened in ten NWS fly populations from Brazil, Colombia, Cuba, Paraguay, Uruguay and Venezuela (Silva and Azeredo-Espin 2009; Silva et al. 2011, respectively). Although sample size was small, with only one population from each country (except for Brazil), the Trp251Ser mutation was detected in all populations. In Brazil, allelic frequencies varied from 15.6% to 46.7%. In Cuba, the frequency was 16.7%. In Uruguay, where the use of pyrethroids seems to be common, the frequency was 28.1%, while the highest frequencies were found in Colombia and Venezuela (93.7% and 100%, respectively). The Gly137Asp mutation, however, was not detected in Colombia, Cuba, and Venezuela, although it was present in high frequencies in Brazilian and Uruguayan NWS populations.

The changes in the frequency of both mutations in three different regions of Uruguay in two years (2003 and 2009) were investigated by Carvalho et al. (2010b). The NWS populations of the three regions showed high frequencies of mutated alleles, but whereas the frequency of the Gly137Asp mutation was reduced in 2009 as compared with 2003, the frequency of the Trp251Ser mutation was significantly higher in 2009. This change is probably associated with the current intense use of pyrethroids and dimethyl-OP compounds for NWS fly control in Uruguay.

Analysis of the structure of 21 NWS populations in the SAG area showed three distinct population groups when considering the carboxylesterase E3 gene, with some differences related to both mutation frequencies (Bergamo et al. 2015). Resistant genotypes were observed in high frequencies in all sampled areas, but the frequency of the Trp251Ser and Gly137Asp mutations was higher at lower and higher latitudes.

There is a need for further resistance monitoring studies that would cover the largest possible area of the current distribution of the NWS fly, in addition to studies that would measure changes in temporal frequencies of mutations associated with insecticide resistance. However, the studies presented above clearly indicate that insecticide resistance is widespread throughout studied South American NWS populations.

3.3. *Perspectives*

Frequencies of both mutations of the E3 gene associated with OP resistance are being monitored in strategic regions of South America that have not been analysed before. The first region of interest is Amazonia, whose NWS populations showed, based on our preliminary results, a considerable frequency of mutant individuals (24% and 16% of the Gly137Asp and Trp251Ser mutations, respectively).

The other important region that is currently being analysed for both E3 mutations is Peru, which is located along a putative connection corridor for the species (Fresia et al. 2014) and consequently can be a key region for the spread of resistance mutations among populations.

4. CONCLUSIONS

Identification of isolated populations or groups of populations is very important to determine target management units for effective AW-IPM programmes of the NWS fly in its current geographic distribution area. Many insights on genetic variability, population structure, and even migration patterns have been obtained, but, except for the Caribbean islands, the identified mainland areas (NAG and SAG regions) are very large and have no identifiable barriers that limit NWS dispersion. The identification of restricted areas and populations within NAG and SAG will be essential for the success of NWS area-wide programmes, both for managing the logistics of implementing the SIT and other suppression methods, and also for the economic implications.

Furthermore, monitoring the spread of insecticide resistance among NWS fly natural populations is equally important, as the effective use of insecticides will be necessary for population suppression activities as part of future area-wide management programmes that integrate the SIT. However, already the current resistance scenario represents a significant challenge.

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AREA-WIDE MOSQUITO MANAGEMENT IN LEE COUNTY, FLORIDA, USA

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SUMMARY

Located in South Florida, the Lee County Mosquito Control District (LCMCD) is the largest single county mosquito abatement programme in the USA based on sheer necessity to combat the extremely high populations of mosquitoes found naturally in the area. South Florida is one of the largest, flattest, wettest, subtropical areas on the planet, making it prime habitat to produce enormous numbers of mosquitoes. LCMCD operates independently an integrated mosquito management (IMM) programme, funded by local taxation, which effectively and responsibly controls mosquitoes minimizing risk to human health, while reducing the environmental footprint. LCMCD incorporates a broad-based approach of control measures ranging from physical or mechanical control, to biological control, larviciding, and adulticiding, as well as mosquito and arbovirus surveillance, public education, and comprehensive evaluation of products and techniques. LCMCD also strives to be at the forefront of advancing technologies, such as the Sterile Insect Technique (SIT) and unmanned aerial systems to assist with the implementation of ongoing suppression efforts. LCMCD continues to be a leader state- and nation-wide with a focus on sound and effective mosquito control for the citizens of Lee County, Florida since 1958.

Key Words: Mosquito abatement programme, control district, integrated mosquito management (IMM), arbovirus surveillance, *Aedes aegypti*, *Aedes albopictus*, *Aedes taeniorhynchus*, *Culex nigripalpus*, *Culex quinquefasciatus*, *Psorophora columbiae*, *Toxorhynchites rutilus rutilus*, *Gambusia holbrooki*

1. LEE COUNTY MOSQUITO CONTROL DISTRICT

Mosquitoes have played a prominent role in Florida's history (Patterson 2004). The discovery that yellow fever, malaria, and dengue fever were mosquito-borne diseases prompted the formation of the Florida State Board of Health in 1889 and the establishment of the Florida Anti-Mosquito Association in 1922, followed shortly by legislation allowing the creation of mosquito control Special Taxing Districts (Connelly and Carlson 2009).

J. Hendrichs, R. Pereira and M. J. B. Vreysen (eds.), Area-Wide Integrated Pest Management: Development and Field Application, pp. 319–338. CRC Press, Boca Raton, Florida, USA.

Lee County Mosquito Control District (LCMCD) was established as an independent taxing district in 1958 by an act of the Florida Legislature, and has been providing mosquito control services to the citizens of Lee County for over sixty years. Additionally, the Lee County Hyacinth Control District was formed by the Florida Legislature in 1961 to serve Lee County in controlling water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), both mosquito-breeding plants, and other noxious aquatic weeds impeding navigation in the Caloosahatchee River and within other water bodies located in Lee County.

Both the mosquito and hyacinth control districts are situated at the same physical location and governed by the same seven-member board of commissioners; commissioners are elected to serve a four-year term. Both independent districts collect *ad valorem* taxes needed to perform their respective control activities. LCMCD is governed according to the laws of Florida, Statue Chapter 388 and the rules of the Florida Department of Agricultural and Consumer Service Administration Code 5E/13. Act 98-462, Laws of Florida, is the enabling legislation creating Lee County Hyacinth Control District. The districts are led by a single executive director.

Lee County Mosquito Control District is the largest single county mosquito-abatement district of more than 700 districts and programmes in the USA, of which 66 are in Florida (Challet 1994; McKenna 2016; Kerzee 2019). With an annual budget of ca. USD 24 million, LCMCD has remained at the forefront of mosquito control by helping to develop control technologies that are effective and sensitive to Florida's unique natural habitats and wildlife. Over 97 per cent of Lee County's mosquitoes are controlled by LCMCD, the rest are controlled by the Ft. Myers Beach Mosquito Control District, formed in 1949 by referendum election for the purpose of providing mosquito control for the town of Ft. Myers Beach. The creation of Ft. Myers Beach Mosquito Control District precedes the formation of LCMCD by nine years.

Lee County, Florida is located in the south-eastern USA on the south-western coast of Florida (Fig. 1). Bordered by the Gulf of Mexico on the west, Charlotte County to the north and Collier County to the south. Lee County is known for its popular white sandy beaches and its large estuary habitat at the base of the Caloosahatchee River. With over 56 000 acres (22 662 ha) of salt marsh mangrove habitat and several large, populated barrier islands, Lee County is unique in the scale of mosquito breeding habitats that are in close proximity to urbanized environments.

1.1. Conservation and Land Management Agencies

As concern for conservation increases, a large portion of land in Lee County is protected by various land management agencies, such as the Florida Department of Environmental Protection (FDEP), the Florida Fish and Wildlife Conservation Commission (FFWCC), the U.S. Fish and Wildlife Service (USFWS), and the Environmental Protection Agency (US-EPA) (Connelly and Carlson 2009). LCMCD collaborates with several local, state, and federal land managers to conduct mosquito abatement activities on these lands (Fig. 2). Due to the biodiversity and individual geographic challenges, many of these lands have their own individual management requirements and restrictions pertaining to mosquito abatement (Batzer and Resh 1992).

To better work together towards a common goal, LCMCD holds annual meetings each spring with all land managing agencies to discuss any issues brought forth from the previous year. During this time, future projects and operations are discussed as a way to develop future operations and build working relationships between agencies and LCMCD.

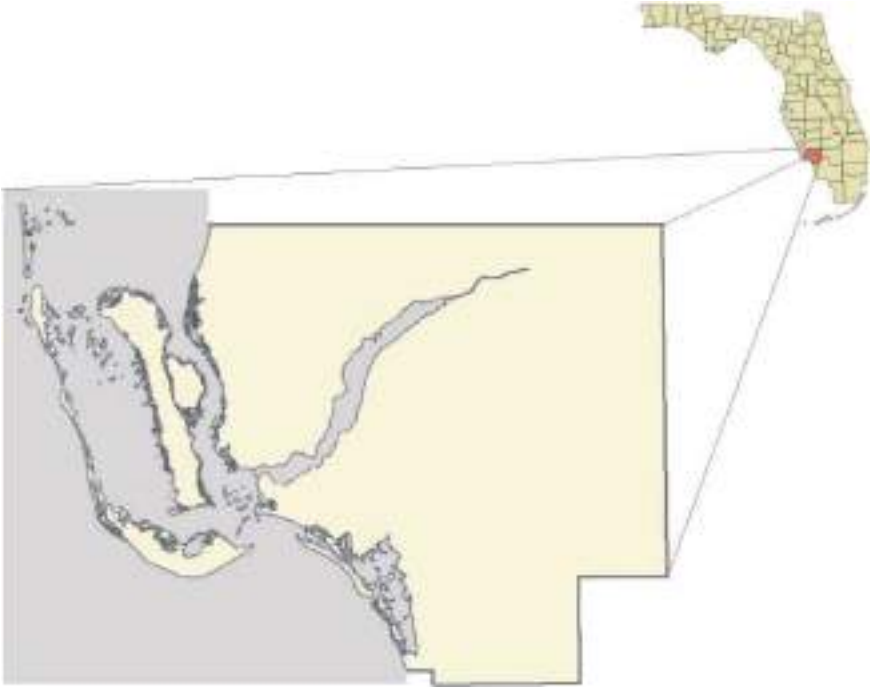

Figure 1. Map showing the location of Lee County in south-western Florida, USA.

1.2. Public Education

As Lee County is one of the counties with the fastest growing human population in the USA, LCMCD dedicates significant resources to educate the public concerning the importance of a strong mosquito abatement programme and why it is needed to live comfortably in south-western Florida. LCMCD believes in strong community engagement and participates in public outreach events throughout the year.

LCMCD also aims to produce a more informed community through a hands-on approach by collaborating with the local Lee County School District and employing a team of licensed educators. LCMCD has developed a unique working relationship with the local school district to fund licensed teachers that offer courses in the school district classrooms across the county teaching mosquito biology and mosquito control essentials to students from kindergarten through high school. All curricula follow the most current standards put forward by the state of Florida and engages students in real world science focused on mosquito control. This instrumental programme gets students excited about science at an early age. LCMCD teachers developed coursework that incorporates biology, ecology, and chemistry with mosquito control, helping the students recognize the science behind what it takes to control mosquitoes. The result is an educated Lee County population, knowledgeable of mosquito control, understanding of why operations occur, the environmental protections in place and judicious use of insecticides.

Figure 2. Map of land managing partners in Lee County, Florida. Red represents FDEP lands, blue represents USFWS lands, and yellow represents FFWCC lands.

1.3. Primary Mosquito Species

The black salt marsh mosquito, *Aedes taeniorhynchus* (Wiedemann) (Agramonte and Connelly 2014), reproduces in extremely high numbers in the 56 000 acres of protected salt marsh within LCMCD (Fig. 3). The aquatic habitat for this species is vast and covers a significant portion of LCMCD's 450 000 acres (1821 km^2). This species is known to oviposit up to 45 000 eggs per square foot (0.1 m^2) (Provost 1969) and is capable of autogeny, the ability to lay an initial batch of eggs without the benefit of a blood meal, believed to be a survival mechanism when hosts are scarce. After developing into adults, *Ae. taeniorhynchus* fly from 20 to 30 miles (32.2 to 48.3 km) in search of a blood meal. It is an aggressive biter and is a major pest along the coastal areas of LCMCD, primary being a nuisance biter and a vector of dog heartworm (*Dirofilaria immitis* Leidy) (Nayar and Connelly 2017), as well as a potential vector for eastern equine encephalitis (EEE) (Agramonte and Connelly 2014).

High tides that flood coastal marsh areas and summer rains cause explosive production of these *Ae. taeniorhynchus* mosquitoes. With a potential of 2 billion eggs per acre (0.4 ha), managing this mosquito on an area-wide basis over the large salt marsh surfaces is of great importance.

Figure 3. Inspector, Sean Christman, searches for Ae. taeniorhynchus *larvae in salt marsh habitat.*

Culex nigripalpus Theobald is a freshwater species with larvae found in roadside ditches, retention/detention ponds, agricultural fields and flooded areas (Day 2017). These sites develop more decomposing organic material later in the rainy season that become increasingly attractive to these mosquitoes. This species is responsible for the transmission of Saint Louis encephalitis and West Nile virus (Day and Curtis 1999). *Cx. nigripalpus* is a major health threat to residents of LCMCD and is a priority for control.

Culex quinquefasciatus Say, the southern house mosquito, is a freshwater species most notable for their association with residential habitats and can be readily found in Lee County (CABI 2019). Similar to *Cx. nigripalpus*, this species can be found in roadside ditches as well as storm drains, containers, and other sites with high organic matter. In contrast to *Cx. nigripalpus*, the larvae of this species are able to survive in waters with higher levels of pollution. *Cx. quinquefasciatus* is the primary vector for St. Louis encephalitis virus throughout the southern USA as well as a potential vector for West Nile virus (Hill and Connelly 2009).

Aedes aegypti (L.), the yellow fever mosquito, is a dusk and dawn biting species, which can also be found biting during the daytime in the shade. This species is closely associated with natural and artificial containers (Zettel and Kaufman 2019). Females have a relatively short flight range (100-500 m) (McDonald 1977; Muir and Kay 1998) and are typically found close to a nearby water source. This species is responsible for the transmission of several disease agents such as yellow fever, dengue, chikungunya and Zika viruses. *Ae. aegypti* is a health threat to residents of LCMCD and is a priority for control.

Psorophora columbiae (Dyar & Knab) is a pestiferous freshwater species (Bibbs et al. 2019). It is found extensively throughout the county in roadside ditches, retention/detention ponds, irrigated agricultural fields, pastures, and low-lying areas that regularly flood, both within and surrounding LCMCD. A floodwater mosquito, it is produced in large numbers as water levels rise and low areas flood during the rainy season and is a major nuisance in the inland areas of LCMCD.

Other nuisance species that sometimes occur in high numbers include *Psorophora ferox* Humboldt, *Ps. ciliata* Fabricius, *Anopheles quadrimaculatus* Say, *An. atropos* Dyar & Knab, *An. cruicians*, *Mansonia titillans* (Walker), *Mn. dyari* Belkin, Heinemann & Page, *Aedes albopictus* (Skuse), and others.

1.4. Weather

Lee County has a subtropical climate, distinguished by warm humid weather year-round, with minimal temperature differences between seasons. Mosquito production in southern Florida is dependent on the presence of standing water throughout the year. Even during the dry winter season, temperatures are rarely sustained low enough to prevent larval development or cause mortality in adults. Seasonal summer rains begin in May or June in south-western Florida and continue through September or October. While the average annual rainfall per year is 53 inches (1.35 m), this amount can be exceptionally variable, especially after hurricanes or tropical storms, and contributes to mosquito production year-round. Rainfall totals and tidal activity are monitored all year with increased monitoring throughout the summer months.

2. SURVEILLANCE

2.1. Population Surveillance

Due to south-western Florida's subtropical climate and mild winters, mosquito surveillance is conducted year-round with increased mosquito collections in the summer months. To address the over 45 mosquito species present in Lee county, an illustration of the nuisance problem being faced, LCMCD deploys multiple trap systems that include eighteen Centers for Disease Control and Prevention (CDC) light traps (Kline 2006; AMCA 2017), seven Biogents BG-Sentinel traps (Regensburg, Germany) (Rose et al. 2006; AMCA 2017), and 6 trap trucks (Fig. 4) to survey 54 pre-determined routes for collecting mosquitoes in flight. CDC and BGS traps are set on a weekly basis, while the trap trucks operate across the county every night from early May through 30 October.

CDC light traps are baited with carbon dioxide in the form of dry ice blocks and set for two trap nights/week. Trap collections are identified the following morning. BGS traps are utilized in urban/suburban areas once per week for *Ae. aegypti* and *Ae. albopictus* surveillance. Along with carbon dioxide, these traps are baited with octenol lures, as well as proprietary BG-lures. BGS traps are very effective in collecting these day-biting mosquitoes.

LCMCD conducts a one-step, Triplex Real-Time PCR (polymerase chain reaction) assay on *Ae. aegypti* and *Ae. albopictus* mosquitoes collected in the weekly BGS trappings. Collected mosquitoes are tested biweekly for the presence of dengue virus, chikungunya virus, and Zika virus.

Figure 4. Trap truck for collecting mosquitoes in flight along pre-determined routes.

Between the months of May and October, LCMCD operates an extensive trap truck programme. An LCMCD trap truck consists of a large conical shaped collection screen (7 feet (2.13 m) wide by 2.5 feet (0.76 m) tall) affixed atop a vehicle (Fig. 4). The trap body measures 11 feet (3.35 m) long and tapers rearward to a 6 by 6-inch (15 by 15 cm) outlet at the rear of the vehicle. At the start of each predetermined route, a collection bag is secured over the collection screen outlets. Routes are driven at a speed of 20 miles (32.2 km) per hour for a three-mile (4.8 km) run and bags collected immediately after finishing. Rainfall gauges are stationed at the beginning and ending locations of each route, providing additional precipitation data important to mosquito production.

2.2. *Arbovirus Surveillance*

LCMCD maintains 16 sentinel chicken coops stationed around the county for the purpose of monitoring arbovirus transmission (Fig. 5). Six birds are kept at each location with blood samples taken once every two weeks and sent to the Florida Department of Health Laboratory in Tampa for analysis.

Figure 5. Sentinel chicken coop on location.

Blood is collected over the course of two days and processed in the LCMCD laboratory prior to shipping to the state laboratory. Half of each processed blood sample is reserved for in-house ELISA testing separate from the state laboratory. Testing samples independently allows for a quicker turnaround time for operational response if a location indicates the presence of an arbovirus. However, the samples sent to the state laboratory are considered the official record of arbovirus detection for the state.

2.3. Landing Rates

Landing rates are an effective and quick tool to determine the scale of a mosquito problem in a specific area (Connelly and Carlson 2009; AMCA 2017). Measuring landing rates involves an inspector visiting a citizen complaint location and counting the number of host-seeking adult mosquitoes within a sixty-second period. This relativity simple technique allows for a good understanding of mosquito bite pressure in an area. The landing rate surveillance method allows a single inspector to cover a larger geographic area more efficiently than setting collection traps overnight.

2.4. Service Request Calls

Concerned citizens are encouraged to call our office and enter a request for service if they are experiencing a mosquito problem at their residence. LCMCD logs all of the calls into our database and uses them as another form of surveillance. Citizens are also able to enter a request through our website (LCMCD 2019) rather than calling directly if they prefer. By mapping the callers address into our geographic information system-based data management programme, LCMCD is able to use these requests as a way to view problematic areas and dispatch inspectors accordingly.

Inspectors responding to individual calls make every effort to meet with the callers directly and search for mosquito problems on their property. If mosquito breeding is found, the inspectors take the time to educate the homeowner on proactive steps they can take to limit future problems. Inspectors log their findings on laptop computers before moving onto the next site. Service request calls sometimes identify areas needing treatment.

2.5. Field Validation

Field validation at LCMCD is a comprehensive programme designed to evaluate new products and technologies, monitor for the development of insecticide resistance and conduct droplet size characterization on all adulticiding equipment used. The field validation programme also maintains laboratory colonies of four different mosquitoes to include the locally pestiferous or disease vectoring species of *Ae. taeniorhynchus, Cx. quinquefasciatus, Ae. albopictus*, and *Ae. aegypti.*

In addition, the field validation programme maintains colonies of the predatory mosquito *Toxorhynchites rutilus rutilus* (Coquillett) and the predatory mosquito fish *Gambusia holbrooki* Girard that are used for biological control of larval mosquitoes; it also oversees the releases of these predators.

LCMCD conducts bioassays on both larval and adult mosquitoes to evaluate product efficacy in controlling local mosquito populations. Products used to control adult mosquitoes are evaluated using the CDC bottle bioassay protocol (Connelly and Carlson 2009). Products used to control larval mosquitoes are evaluated using a serial dilution larval assay (WHO 2016). Laboratory colony susceptibilities are compared against results of wild mosquitoes to establish a control baseline.

The field validation programme is responsible for conducting droplet characterization on all adulticiding spray systems annually to ensure equipment is in proper working order prior to use. Droplet characterization is conducted using one inch (2.54 cm) and three-millimetre Teflon-coated slides to capture droplets for analysis. Using automated computer software, the slides are analysed under a compound microscope to determine droplet Volume Median Diameter (VMD) (Connelly and Carlson 2009). Droplet sizes must fall within an acceptable range as determined by product label and approved by EPA. Droplet characterization is conducted any time an adulticiding spray system is altered with a minimum of once per year to ensure the equipment is working properly before use.

Additionally, the field validation programme evaluates new products prior to their incorporation into field operations. New product formulations are first evaluated under laboratory conditions to establish the appropriate application rate under ideal conditions. Products with favourable laboratory results are then applied in small-scale field sites for possible operational selection. During these trials, products are monitored for efficacy, duration, and any potential adverse effects to local non-target species. These trials are crucial to determine how a product is going to work under local conditions prior to their implementation as part of the LCMCD treatment programme.

3. BIOLOGICAL CONTROL

Biological control is a vital component of an integrated mosquito management (IMM) programme of any size. Therefore, LCMCD also incorporates biological methods for mosquito control to minimize the use of insecticides. LCMCD accomplishes this by introducing the predatory native mosquito fish (*G. holbrooki*) into mosquito breeding areas (Cassiano et al. 2018) and releasing the predatory mosquito *Tx. rutilus rutilus* (Focks et al. 1980).

In addition, mosquito ditches were installed throughout the 1960s as a form of water management that provides access of natural predators into mosquito breeding habit during times of high tides (Fig. 6). When water levels rise during a high tide, these ditches can introduce juvenile fish species into areas that otherwise would have been inaccessible. As water levels recede these species make their way back to the safety of the ditches.

LCMCD has a mosquito fish programme designed to raise native *G. holbrooki* for release into problematic areas (Fig. 7). In 2019, LCMCD released around one thousand fish into various sites with the goal of natural larval suppression. *Gambusia* is a native freshwater genus of fish that are ferocious predators of mosquito larvae. Often, once this species establishes breeding populations in a body of water, they will suppress mosquito larvae to levels where insecticides are no longer needed.

Figure 6. Network of mosquito ditches on Pine Island, Florida. Darker green foliage shows area where ditches are present.

LCMCD also maintains a colonized population of *Tx. rutilus rutilus* for the purpose of biological control. Native to south-western Florida, these beneficial mosquitoes are predatory on other mosquito larvae and adults do not require a blood meal for reproduction (Focks et al. 1980). By introducing these beneficial mosquitoes into isolated habitats, such as abandoned properties and cemeteries, the goal is to promote the natural suppression of sanguivorous mosquito species.

Figure 7. Biologist, Kara Tyler-Julian, tends to Gambusia *fish rearing tanks.*

4. MOSQUITO BREEDING SOURCE REDUCTION

Source reduction is an important component of an IMM programme. LCMCD inspectors work in the field everyday surveying for larvae. When appropriate, all known larval sources are inspected to determine if the breeding site can be reduced or eliminated before considering chemical and biological treatment methods. This can include, but is not limited to, filling-in tree holes, dumping buckets/containers, removing waste that holds water, drilling holes to drain containers, placing screen covers over rain barrels, etc.

Shortly after LCMCD was established in 1958, crews began work installing mosquito drainage ditches across much of its salt marsh habitat. Digging was accomplished via dragline machinery to depths of five by six feet (1.52 by 1.83 m) wide. By the early 1970s, LCMCD had installed a complex network of canals through much of its problematic coastal areas with the purpose of removing water from the marsh during periods of low tide. By allowing water a place to recede naturally, it limits mosquito breeding habitat and greatly reduces the amount of pesticide needed for control. Although these ditches were installed up to sixty years ago, they continue to function as designed and remain a valuable mosquito control tool in Lee County (Fig. 8).

Figure 8. Mosquito ditch on Pine Island, Florida.

LCMCD continues to implement manual control methods in areas where applicable. Recently, LCMCD collaborated with the National Wildlife Refuge (NWR) of the USFWS to control mosquitoes on a remote island used as an active rookery for several species of shore birds. A depression in the interior of the island would fill with water in the summer months and breed *Ae. taeniorhynchus* mosquitoes. Due to the sensitive nature of nesting birds, getting access to inspect and treat the island was virtually impossible. In 2017, crews from the NWR and LCMCD met at the remote island and hand dug a ditch from the exterior of the island towards the problematic interior. In a couple of hours, a mosquito ditch was installed that drained the stagnant water from the interior of the island. During a high tide, the ditch can introduce natural predators such as fish and other macroinvertebrates into the ecosystem. As the tide retreats, the natural predators leave the island along with the water and mosquito larvae, virtually eliminating mosquitoes naturally.

5. LARVAL MOSQUITO CONTROL

5.1. Ground Larviciding

The ground larviciding programme at LCMCD focuses mainly on inland roadside ditches and residential neighbourhoods. Although some areas are affected by tidal fluctuations, most of ground larviciding is conducted in response to rainfall events for freshwater mosquito species. Ground larviciding crews survey areas of recent rainfall and treat with a variety of methods including a truck-mounted spray system, handheld equipment (backpack unit or squirt bottle) or single use treatment items (water-soluble pouches or briquette-formulated larvicides).

Vehicle-mounted spray systems (Fig. 9) primarily utilize products with the active ingredient temephos for larval control.

Figure 9. Vehicles used for ground larviciding.

Products dispensed with handheld equipment ranges widely depending on the situation, but mostly consists of monomolecular films, larviciding oils, and *Lysinibacillus (Bacillus) sphaericus*. Various formulations of *L. sphaericus*, spinosad, and methoprene products are available for single use treatment items ranging from 30 to 150 days of residual control and are used to treat more permanent bodies of water that will be problematic throughout the season. *Bacillus thuringiensis* is seldom used in ground larviciding operations, but it is commonly used in aerial larviciding operations at LCMCD.

All ground larviciding vehicles are equipped with a Global Positioning System (GPS) monitoring device to record vehicle location and speed. This system also records the activity of the vehicle-mounted spray system. All inspection and treatment information is recorded by the technician onsite with a laptop computer.

In the dry season, ground larviciding crews continue to survey for mosquitoes often found in breeding sites such as containers, tyres, and neighbourhood drainage basins. Without the consistent summer afternoon rains to flush these habitats, *Culex* species become established and cause problems for nearby residences. Consistent surveillance and treatment are critical to control mosquitoes in urbanized ecosystems.

To address more cryptic mosquito habitats with limited inspector access, LCMCD uses a truck-mounted A1 mist sprayer (A1 Mist Sprayers Resources, Inc., Ponca, Nebraska, USA). By driving residential roadways in the evening hours, this unit treats for mosquito larvae by blowing small droplets of liquid larvicide upwards of 50 feet (15.24 m) into the air enabling it to drift into residential areas that would otherwise be difficult to access. LCMCD has integrated this technique as a way to efficiently treat for mosquito larvae that otherwise would require a team of individuals going door-to-door to inspect and treat cryptic breeding sites in areas that may have limited access.

5.2. Aerial Larviciding

Impoundments are areas of salt marsh surrounded by a dike to allow control of water levels for mosquito control, thereby negatively affecting wetland function and vegetation (Rey and Rutledge 2006). Even though Lee County is unique in having over 56 000 acres of mangrove salt marsh habitat, little of it is managed through the use of impoundments, as is more common in other parts of the state. This habitat is home to several species of mosquitoes, most notably *Ae. taeniorhynchus*. This species is a ferocious biter and a prolific breeder with an extensive flight range that extends across the county (Provost 1952; Elmore Jr. and Schoof 1963). To best target these mosquitoes, LCMCD operates a robust aerial larviciding programme aimed at controlling these mosquitoes at their source while in their juvenile life stage.

LCMCD owns and operates a fleet of six Airbus (Herndon, Virginia, USA) H125 helicopters for the purpose of accessing and treating remote breeding sites (Fig. 10). LCMCD biologists constantly monitor salt marsh habitat for rainfall and tidal fluctuations throughout the year. Following a high tide or rain event, biologists fly to remote landing sites to inspect the new water for the presence of newly hatched juvenile mosquitoes. They will take various water samples around a geographic area and check for the presence of mosquito larvae. If larval densities exceed individual site thresholds, biologists record inspection data and schedule the area for treatment.

Figure 10. LCMCD Inspector searching for mosquito larvae in salt marsh habitat.

Treatments are conducted once the biologists are able to develop a site-specific treatment plan, often as soon as that same day. LCMCD also owns eight remote heliport locations along the western edge of the county bordering salt marsh habitat. These locations serve as secure outpost facilities to refuel and reload products onto helicopters in areas closest to treatment sites. Computers equipped at these locations give biologists the ability to develop treatment plans on site without the need to return to LCMCD. Depending on site-specific needs, a variety of products are available for use, including temephos, *B. thuringiensis israelensis, L. sphaericus*, spinosad, methoprene, and larviciding oil. Product formulations also range from liquid to granular formulations as well as single use products providing 30-day residual control.

All helicopters are equipped with an on-board computer to control the helicopters' spray system. This system works harmoniously to upload the individualized treatment polygons with spraying turning on when the helicopter flies into the targeted polygon. Once the pilot exits the pre-programmed treatment zone, the spray system turns off. This GIS-based system operates with pinpoint accuracy that increases pilot safety by simplifying inflight procedures and prevents spraying of off-target sites, saving insecticide and money.

Following a treatment, biologists will return to their inspection site to complete a post-treatment inspection. All inspection data are recorded onsite at time of collection with a custom iPhone application. Once synchronised, all data are available for viewing at the office and are recorded in an organized format. All treatment data are captured by the system's on-board computer and are available for viewing post-treatment in a similar fashion.

6. ADULT MOSQUITO CONTROL

6.1. Ground Adulticiding

LCMCD operates 13 vehicles equipped with ultra-low volume (ULV) spray systems used to target adult mosquitoes in and around neighbourhoods (Fig. 11). Ground adulticiding missions are conducted between sunset and 02:00 to target flying mosquitoes when they are most active. Formulated products are applied without dilution or mixing, and equipment is calibrated to treat a 300-feet (91.4 m) swath at a speed of 10 miles (16.1 km) per hour. A variable flow spray system is equipped to keep the targeted application rate even when the vehicle speed increases or decreases from the 10 miles per hour targeted spray rate. As the vehicle changes speed, within a range of 2-20 miles (3.2-32.2 km) per hour, the appropriate amount of product is dispensed according to label directions. When the vehicle speed surpasses 20 miles per hour, the spray system shuts off preventing spray.

Figure 11. Ground adulticiding vehicles with rear-mounted ULV machine.

The vehicle's spray system is operated remotely from inside the cab of the vehicle with a handheld controller. This design prevents the driver from coming into contact with chemicals during spray operations and limits exposure. The spray system also records various parameters throughout the evening such as vehicle speed, vehicle location, spray activity, miles sprayed, acres treated, and total chemical dispensed. Chemical usage information is compared each morning to the amount recorded by the driver at the start and end of their shift to ensure proper calibration.

Small isolated locations that are not large enough to warrant a ULV truck application are easily treated with small handheld ULV sprayers. These units are typically reserved for areas with easy access that can be walked by a technician. Common treatment sites for such handheld applications include used tyre shops or dumps targeting *Ae. aegypti* and small natural areas targeting freshly emerged *Ae. taeniorhynchus* to prevent dispersal.

6.2. Aerial Adulticiding

The aerial adulticiding operations in Lee County is an important programme designed to efficiently control biting mosquitoes across large geographic areas. LCMCD owns and operates a fleet of eight fixed-wing airplanes outfitted with spray equipment designed to target flying adult mosquitoes (Fig. 12). Adulticiding missions are conducted at night between the hours of 21:00 and 02:00 when night-active mosquitoes are typically most active. Applications are made at an altitude of 350 feet (107 m) above ground level and pilots are equipped with night vision goggles for maximum visibility.

Figure 12. Douglas DC3-TP with four 50-gallon chemical tanks used for aerial adulticiding.

Similar to the LCMCD aerial larviciding system, the adulticiding spray system is controlled via an on-board computer with a pre-programmed mission. Once pilots arrive on-site the spray system is automatically turned on and remains spraying until the pilot exits the treatment area. This automation increases precision of the application and enhances pilot safety when flying in such an unconventional manner. Depending on how large the problematic area is, treatments sites can be as large as 23 000 acres per mission per aircraft. Flights typically occur at 130 or 150 knots, depending on aircraft type and chemical flow rate.

LCMCD primarily utilizes naled and malathion for aerial adulticiding. Products are dispensed with a high-pressure nozzle system or with a rotary atomizer at a rate of 0.5 oz/acre, 0.66 oz/acre, or 1.5 oz/acre depending on the pesticide used and the targeted mosquito species. LCMCD does not utilize set treatment frequencies for scheduling treatments of any kind, but rather relies entirely on surveillance data to determine if treatments are warranted. Each surveillance method has an associated treatment threshold that must be met based on inspection type and location baselines.

For an aerial adulticiding treatment to be conducted, surveillance data are evaluated first and considered prior to scheduling. Surveillance methods include landing rate counts, truck trap collections, spray zone thresholds (that were obtained over many years of trap data), arbovirus detection in sentinel chicken flocks, and mosquito trapping results. If criteria mandated by the state of Florida are met and a wide-scale problem is determined, an aerial adulticiding application is scheduled as early as that same night.

7. NOVEL TECHNIQUES

It is essential to keep up with evolving mosquito populations, increasingly sophisticated control technologies, climate change, a constantly increasing human population density, and increases in exotic disease agents and vector invasions. As such, a programme can fall behind and become less efficient than it once was if these changes are not taken into account. To best combat these dynamic circumstances, LCMCD is committed to staying abreast of new technology and the advancement of various control measures.

Applying sterilisation techniques for the control of insect populations is not a new concept, however the application of it on mosquitoes is an emerging field (Lees et al. 2021; Baton et al., this volume). The Sterile Insect Technique (SIT) was first utilized in the late 1950s to successfully control the screwworm fly (*Cochliomyia hominivorax* Coquerel) on the isolated habitat of Sanibel Island in Lee County, Florida (Bushland and Hopkins 1953; Bushland 1960). Since the first trial on Sanibel Island, the SIT has been employed to effectively suppress, contain and eradicate a variety of medically- and agriculturally-important insects (Dyck et al. 2021).

LCMCD is currently in the process of establishing the first SIT programme for *Ae. aegypti* solely operated by a mosquito control district in the state of Florida. This novel programme aims to reduce *Ae. aegypti* in Lee County using X-ray irradiation for sterilisation. To accomplish this, LCMCD will be mass-rearing locally collected populations of *Ae. aegypti*, irradiating the mosquitoes using X-rays, and releasing the sterilized male adults on an area-wide basis into the field to breed with wild female populations. The goal of this programme is to become a valuable complement to traditional mosquito control techniques in the fight to prevent the spread of diseases such as Zika, dengue, yellow fever, and chikungunya, which are transmitted via the bite of the *Ae. aegypti* mosquito.

LCMCD is also interested in using more conventional technology in innovative ways to improve operations. Unmanned aerial systems (UAS) technology has been available to the commercial market for several years now and is utilized primarily for their photographic abilities. LCMCD owns two UAS for the purpose of aiding in inspections of mosquito breeding habitat and have recently purchased one UAS capable of carrying and spraying a payload of insecticide. As the rules and regulations surrounding UAS continue to evolve (Benavente et al., this volume), LCMCD plans on being there along the way to incorporate these new technologies into the mosquito control industry as a part of its commitment to protect the health of the citizens of Lee County.

8. CONCLUSIONS

LCMCD operates a comprehensive IMM programme in an effort to provide the most effective mosquito abatement possible for the citizens of Lee County. As with any IMM programme, the efficient integration of all methods together achieves the most advantageous results.

The larviciding programme, aimed at suppressing mosquitoes in their juvenile life stage, offers the most efficient means of control by targeting mosquitoes when they are at their most concentrated state of development and unable to bite. Source reduction and biological control measures, although varying differently in application, offer a natural and potentially longer duration of control than insecticides.

A strong adulticiding programme plays a vital role in suppressing the biting pressure on the local population and interrupts the disease transmission in the event of an arbovirus outbreak.

The implementation of novel control measures, such as the SIT, complement conventional control methods to aid in the control of disease vectoring agents. All of these methods offer specific advantages, however, if utilized on their own they would prove wildly inadequate. The harmonious integration of all control measures is best supported with a backbone of strong surveillance and a well-educated staff to oversee its implementation.

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