BUFFALO FLIES (*Haematobia exigua*) EXPANDING THEIR RANGE IN AUSTRALIA FACILITATED BY CLIMATE CHANGE: THE OPPORTUNITY FOR AREA-WIDE CONTROLS

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SUMMARY

Buffalo flies *Haematobia exigua* de Meijere were introduced to Australia in 1838 and have become major cattle pests in Australia's northern cattle industries. They have been steadily expanding their range southward and their spread is likely to be further facilitated by climate change. Control programmes consisting of compulsory chemical treatments and regulated cattle movements have proven unsuccessful in preventing the spread of buffalo flies and, without area-wide intervention, they are likely to become major cattle pests in Australia's southern beef and dairy industries. Buffalo flies do not have a pupal overwintering strategy but survive winter in localised foci of slowly cycling low level populations of flies. Populations increase and spread to infest surrounding areas when weather becomes favourable in summer. This suggests the potential for an area-wide control approach, targeting overwintering foci of the flies. A project has been initiated to transinfect buffalo flies with *Wolbachia*, determine the effects of *Wolbachia* infection in the flies, and assess the feasibility of control by *Wolbachia*-based approaches directly targeting overwintering foci of the flies.

Key Words: *Bos indicus*, *Bos taurus*, *Bubalus bubalis*, *Chrysomya bezziana*, *Haematobia irritans*, *Lucilia cuprina*, *Stephanofilaria*, *Wolbachia*, Muscidae, biological control, cattle, horn flies, invasive species

1. BUFFALO FLIES AND HORN FLIES

Buffalo flies *Haematobia exigua* de Meijere and horn flies *Haematobia irritans* (L.) are obligate parasites, living most of their lives on cattle, and leaving only to oviposit when cattle defecate. Both the male and the female subsist completely on blood, using their sharp mouthparts to pierce the animal's skin. If uncontrolled, infestations may reach several thousand flies per animal, each feeding up to 40 times daily, irritating cattle and causing production loss and welfare impacts.

Horn flies have been estimated to cost the North American and Brazilian cattle industries close to USD 1000 million (Cupp et al. 1998) and USD 2540 million (Grisi et al. 2014) per annum respectively, whereas buffalo flies are estimated to cost the Australian beef cattle industry AUD 99 million annually with losses presently confined mainly to the northern part of the country (Lane et al. 2015).

Buffalo fly feeding can lead to the development of lesions that are of significant welfare concern (Jonsson and Matchoss 1998), reduce hide value and make cattle less acceptable for the market (Guglielmone et al. 1999; Lane et al. 2015). These lesions can range in nature from dry and alopecic or scab encrusted, to severe open areas of ulceration (Johnson 1989). They are found most commonly beneath the eyes of cattle, but can also be prevalent on the neck, dewlap, belly and flanks (Sutherst et al. 2006) and their development and persistence has been associated with a currently unnamed species of filarial nematode (*Stephanofilaria* sp.), transmitted by buffalo flies (Johnson 1989; Shaw and Sutherland 2006). Although similar lesions are found associated with horn fly feeding, they are mainly abdominal in distribution and generally not nearly as severe as those associated with buffalo flies (Hibler 1966; Silva et al. 2010).

Skin lesions are most widespread in northern areas of Australia, where buffalo flies are present throughout the year, with up to 95% of cattle affected (Johnson et al. 1986). It is expected that the prevalence of lesions will increase in more southern parts of the fly range as global warming extends the length of the buffalo fly season and the intensity of fly attack. In a survey of Queensland dairy producers, buffalo flies were considered to be a greater problem for production than cattle ticks, and when asked what aspect of infestation concerned them most, 42% of producers noted the welfare effects (Jonsson and Matchoss 1998). In addition, the lesions present a potential focus for strikes by Old World screwworm *Chrysomya bezziana* (Villeneuve) flies, which are endemic in a number of Australia's nearest northern neighbouring countries and which are considered a major biosecurity risk for northern Australia (AHA 2017).

2. TAXONOMIC STATUS

Buffalo flies and horn flies are very closely related and have variously been considered as sub-species (Zumpt 1973; Pont 1973) and separate species (Skidmore 1985). The larval stages of the two species are extremely similar and are probably morphologically indistinguishable (Pont 1973). Morphological differentiation of the adults is also difficult, and the main distinguishing feature in the flies is the presence of 4 to 6 long curled hairs on the hind tarsi of male *H. exigua*, which are not found in *H. irritans* (Mackerras 1933; Iwasa and Ishiguro 2010). Kano et al. (1972) suggested a number of other morphological distinguishing features, but Iwasa and Ishiguro (2010) indicated that these varied with latitudinal gradient within each species. Snyder (1965), who studied many specimens from Micronesia, stated that even the bristling on the hind tarsi of *H. exigua* is variable (Zumpt 1973).

Urech et al. (2005), who measured buffalo flies cultured on cattle in Australia and horn flies from a laboratory colony in Florida, indicated that there were distinct differences in the cuticular hydrocarbons of the two groups of flies and suggested that these differences may support their status as separate species.

Iwasa and Ishiguro (2010) examined the mtDNA in the COI to COII genes of horn flies collected from two sites in Japan and buffalo flies from sites in Taiwan and Viet Nam and found sequence divergence of 1.8% - 1.9% between the two species. They concluded that the relative genetic divergence observed between and within the two species may indicate an intermediate status in species development. From a more recent study of molecular differentiation of the two species, using the mtDNA COI, cytochrome B (Cytb), NADH dehydrogenase subunit 5 (ND5) genes, and the nuclear and 18S and 28S ribosomal RNA regions, Low et al. (2014, 2017) concluded that the two species are genetically distinct and that the COI and Cytb genes were the most informative for distinguishing the two species. Regardless of whether or not buffalo and horn flies can be considered separate species, all indications are that they are extremely closely related.

3. INVASION AND DISTRIBUTION

Both buffalo and horn flies have proven to be extremely invasive species. Either *H. irritans* or *H. exigua* is now present in most major cattle production areas of the world, with the exception of sub-Saharan Africa, where the species *Haematobia thirouxipotans* (Bezzi) and *Haematobia minuta* (Bezzi) occupy this niche (Zumpt 1973) (Fig. 1).

Figure 1. World distribution of horn fly *H. irritans* and buffalo fly *H. exigua*.

Horn flies were introduced to the east-coast of North America from Europe on imported cattle in 1885-86 (Butler and Okine 1999). They spread rapidly to reach California by 1893 and by 1900 had been reported from most of the USA, Canada and Puerto Rico. They were reported in South America from cattle in Colombia, Ecuador and Venezuela by 1937 (Mancebo et al. 2001), and were first reported in Brazil's northern-most State, Roraima in 1956.

By 1980, horn flies had spread south of the Amazon into Goiás state (Mancebo et al. 2001), by 1991 had reached the south of Brazil (Mancebo et al. 2001), and by 1993 had spread through Uruguay and all of the major cattle production areas in Argentina (22°S to 44°S) (Anziani et al. 1993; Guglielmone 1999).

On the western side of the continent horn flies were found in Bolivia before 1955 (Munro 1960), in Peru by at the latest 1973 (Zumpt 1973), and in Chile in 1967 (Gonzalez 1967), although it appears that they did not become a significant pest in Chile until 1993 (Campano and Avalos 1994). These records suggest that the southerly spread of horn flies in South America may have occurred independently on both the west and east sides of the continent.

3.1. *Spread of Buffalo Flies in Australia*

Buffalo flies have been similarly invasive in Australia, although their spread has occurred more slowly than for horn flies in the Americas, and has been limited at its southern extent by the inability of buffalo flies to undergo a winter pupal dormant phase, as occurs in horn flies (Ferrar 1969; Cook and Spain 1982). Buffalo flies entered mainland Australia near Darwin (12.5° S, 130.8° E) in 1838, probably on water buffalos (*Bubalus bubalis* L. 1758) introduced from Timor in 1825 (Tillyard 1931). Early spread occurred very slowly and coincided closely with the spread of buffalos (Hill 1917), which appear to be the preferred native host of the flies in Asia (Iwasa and Ishigura 2010) (Fig. 2).

It wasn't until 1928 that buffalo flies reached the Queensland border, approximately 1300 km southeast of their original point of introduction (Seddon 1967), subsequently spreading across the dry stretch of land south of the Gulf of Carpentaria to eastern Queensland during a series of wet years in 1939-41. From there, they spread rapidly to the east coast of Cape York in northern Australia and southwards along the eastern coast until they appeared to reach a southerly limit just north of Bundaberg (24.8°S latitude) by 1946. Here the spread paused, and no further southerly spread was observed for the next 30 years (Fig. 2).

Following a series of mild winters and wet years from 1973 onwards, changes to buffalo flies and tick regulatory programmes, possibly aided by changes in the chemicals used for cattle tick *Rhipicephalus australis* Fuller treatment, southerly range expansion recommenced and buffalo flies reached the Brisbane Valley and Nambour in 1977, the Tweed Valley in New South Wales in April 1978, and Bonville, south of Coffs harbour (30.4°S) in 1982 (Williams et al. 1985). Since then, the flies have continued their southerly spread with infestations seen as far south as Dubbo, Narromine and Maitland (32.7°S) in 2011 (Fig. 2). This represents an increase in their southerly range of approximately 1000 km in the last 40 years.

Following their first detection in New South Wales in 1978, the flies now survive the winter in many eastern parts of the state and have become a significant endemic cattle pest in these areas.

The impact of buffalo flies and the area affected in Australia varies significantly with season and weather conditions. During warm wet summers the distribution of the flies increases significantly in northern and north-eastern areas, and they may spread to affect cattle in an area potentially more than two times larger than the permanently infested range (Fig. 2).

Figure 2: Spread over time of the buffalo fly *H. exigua* in Australia.

3.2. Effects of Climate Change

All indications to date suggest that climate change effects in Australia will facilitate the continued spread of buffalo flies into new areas and will increase the economic and welfare impacts in the southern parts of their current range (CSIRO/BOM 2016). Rising temperatures will enable more rapid *H. exigua* population growth, an increased number of generations each year, greater fly activity in many areas and longer seasons of cattle challenge. In addition, predicted rises in minimum temperatures and a reduction in the frequency of frosts will favour survival in marginal areas and further southerly extension of the flies' range. A possible increase in the summer incidence of rainfall in some areas of Australia may also favour the flies' breeding.

The results of CLIMEX modelling (R. Dobson personal communication 2015) suggest greater impacts from buffalo flies in the southern parts of their current range, including the potential for persisting fly populations to establish through most of the moist coastal belt of New South Wales and in foci as far south as South Australia and southern Western Australia (Fig. 3). In addition, increased weather variability and extreme rainfall events predicted under climate change may assist the spread of flies across inhospitable areas to new foci suitable for winter fly survival. Once established in these areas, new overwintering foci would provide a source for more extensive incursions during warm wet periods, similar to that seen in northern Australia.

The CLIMEX modelling does not account for factors such as a changing resource base, microclimate effects or changes in pest biology. In southern areas, the cattle industry is based largely on *Bos taurus* L. breeds that are more susceptible to buffalo flies than the *Bos indicus* L. cattle that predominate in northern areas (Frisch et al. 2000). In addition, northern cattle are normally treated to control cattle ticks, which can also impact on buffalo fly numbers, whereas few parasite treatments are applied to southern cattle. Thus, the southern beef and dairy industries provide a susceptible and largely untreated host resource extremely favourable for invasion by buffalo flies. Furthermore, adaption of insects at the edge of their range can be an important contributing factor in new pest invasions (Hill et al. 2011).

Figure 3. CLIMEX predictions of areas suitable for the establishment of persisting buffalo fly *H. exigua* populations under predicted climate change. Size of solid circles indicates degree of favourability of areas for buffalo fly persistence; crosses indicate weather station sites not suitable for buffalo flies' persistence; the large open ellipses indicate areas of most significant range expansion (credit Rob Dobson).

The degree to which genetic adaptation of buffalo flies to cooler temperatures has contributed to their southerly spread is uncertain. However, Iwasa and Ishigura (2010) note that the flies in their native range appear to prefer buffalo to cattle as hosts, and a period of adaptation to cattle and cooler Australian conditions may have contributed to their spread. Development of pupal overwintering capacity as they move south, a possibility given their close genetic relatedness with horn flies, is a concerning prospect and could see the species develop a temperate distribution in Australia, similar to the distribution seen for horn flies in the northern hemisphere and South America.

4. POTENTIAL FOR THE USE OF AREA-WIDE APPROACHES AGAINST BUFFALO FLIES IN AUSTRALIA

Currently, control of buffalo flies in Australia depends largely on chemical treatments, although techniques such as buffalo fly traps (Sutherst and Tozer 1995) and selection of more tolerant *Bos indicus* breeds (Frisch et al. 2000) are also used. In addition, dung beetles may assist the regulation of buffalo fly populations under some circumstances (Doubé 1986). Treatments are applied almost exclusively on a herd-by-herd basis. However, modelling studies indicate the likely inefficiency of herd-by-herd approaches showing that the effects of invasion of pests from untreated areas can be devastating in compromising the effectiveness of control measures (Knippling 1972a). Application of control techniques on an area-wide basis, targeting the entire population rather than just individual properties or herds, can be much more efficient than more intensive programmes applied on a herd-by-herd basis. Area-wide approaches are expected to be particularly advantageous when pests are mobile and can readily auto-disseminate and therefore may not be easily controlled by property-based or herd-based programmes (Hendrichs et al. 2007), such as is the case with buffalo flies.

4.1. Chemical-based Programmes

Area-wide control programmes have historically been based mainly on the application of chemical insecticides by methods such as aerial spraying or intensive ground spraying, or in the case of diseases of livestock, by individual animal or herd treatments with quarantine controls and movement restrictions (Graham and Hourigan 1977).

At various stages in the spread of buffalo flies in Australia, regulatory programmes, supported by legislation, and which included movement controls and compulsory spraying of relocated cattle with insecticides, were used in an attempt to prevent their southerly incursion (Parliament of Queensland 1965). However, these programmes were not effective in stemming the southward spread of buffalo flies (Anonymous 1934; Roberts 1946; Eastaway 1974) and all were eventually abandoned in 1978 (Williams et al. 1985). However, buffalo flies remain a legislatively specified notifiable disease in some southern states of Australia, where the flies are not currently present (DAWR 2017).

4.2. Autocidal and Biologically-based Approaches

Programmes which require widespread application of insecticides are increasingly unacceptable on a community basis and can be compromised by the development of resistance or resurgence of pests from cattle that are not treated or where treatments are poorly applied. More biologically-based, species-specific and environment-friendly techniques which operate by disrupting biological processes of pests, generally find wider community acceptance and are often more effective than insecticide applications (Bourtzis et al. 2016). In addition, because of the ability of released insects to disperse into all areas occupied by the target field population and to actively search out and mate with target insects, biologically-based methods are often more effective against pests that can disperse autonomously, or which survive in cryptic habitats that are hard to reach with chemical sprays.

The most well-known of these approaches is the Sterile Insect Technique (SIT) in which insects of the target species are mass-reared and sterilised using low level ionizing radiation, followed by inundative releases of the sterilised insects (usually males) on an area-wide basis over the entire area of the target population (Vreysen and Robinson 2011; Dyck et al. 2021). The sterile males mate with field females, which consequently produce infertile eggs and through sequential releases the target population is suppressed, or under certain favourable conditions, eradicated.

Some of the most significant successes with the SIT have involved insect pests of livestock, including the eradication of New World screwworm *Cochliomyia hominivorax* (Coquerel) from North and Central America (Wyss 2000), eradication of an incursion of this pest in Libya (Lindquist et al. 1992), and removal of the tsetse fly *Glossina austeni* (Newstead) from the Island of Unguja in the Zanzibar archipelago (Vreysen et al. 2000, 2014). Localised eradication or suppression using the SIT has also been achieved on a number of occasions with other tsetse species in Africa (Vreysen et al. 2013). Successful eradication using this approach can be extremely cost efficient. For example, in the New World screwworm programme in the Americas it has been calculated that the direct benefits achieved each year from the programme are equal to or greater than the total cost of the sterile male release programme over the fifty years of its operation (Vreysen and Robinson 2011).

Horn flies are one of the species suggested by Knipling (1972b) as likely candidates for control by the SIT. Knipling considered that the close association of the flies with cattle and its consequent accessibility to control meant that fly populations could be readily reduced by insecticide treatment of cattle, then the remaining population eliminated using the SIT. In early trials with horn flies, cattle were sprayed with topical insecticides to reduce fly numbers. However, the subsequent sterile insect releases were compromised because the released flies were more susceptible to the insecticides used than were the field flies (Eschle et al. 1973, 1977). This was overcome by using methoprene, an insect growth regulator administered in drinking water, which targeted the larval stages of horn flies, and had no effect on the released adult flies. Trials on the isolated Kalaupapa peninsula of Molokai in Hawaii subsequently confirmed that a semi-isolated population could be effectively eradicated using this method, even in the very horn fly-favourable environment of Hawaii (Eschle et al. 1977). Unfortunately, the area was later reinfested by the introduction of infested cattle into the area.

Although the SIT is by far the most widely known and successful genetic technique used against livestock pests to date, SIT application is not always feasible and a range of other genetically-based techniques have also been tested, or are under contemplation. For example, in continental Australia the extensive areas of livestock production and the wide distribution of associated pest species, together with few natural geographic boundaries, made the use of the SIT impractical or at least of dubious cost-benefit for use against many livestock pests, as in the case of sheep blowfly *Lucilia cuprina* (Wiedemann). As a consequence, a range of other genetically-based techniques such as the use of compound chromosome and sex-linked translocation strains (field female killing systems), which were predicted to be more effective at lower release ratios, were developed and tested (Foster et al. 1985, 1988, 1991). Field testing showed promise for these other approaches, but for a number of reasons discussed by Scott (2014), they were never implemented for widespread use.

More recently, transgenic sexing strains of *L. cuprina* have been developed that carry a tetracycline-repressible female lethal genetic system that could form the basis for mass-production of only males of *L. cuprina*, and potentially other fly species, for use in genetic control programmes (Scott 2014). A range of other techniques such as the Release of Insects carrying Dominant Lethal (RIDL) genes, RNAi and homing endonuclease genes (HEG) are now also being considered for use with mosquitoes, tsetse flies and other species (reviewed by McGraw and O'Neill 2013; Bourtzis et al. 2016) and have proceeded to field testing in some instances (Harris et al. 2011). With increasing access to sequenced insect pest genomes (International *Glossina* Genome Initiative 2014; Anstead et al. 2015) and rapid advances in molecular technology, most notably the availability of new gene editing techniques such as CRISPR-Cas9, many new, purpose-designed approaches for control will likely emerge.

5. *Wolbachia* AND AREA-WIDE CONTROL OF BUFFALO FLIES

Other technologies of much current interest for use in area-wide control programmes are symbiont-based approaches (Bourtzis 2008; Bourtzis et al. 2016; McGraw and O'Neill 2013; Wilke and Marelli 2015), in particular the use of *Wolbachia*. *Wolbachia* are maternally transmitted intracellular bacteria in the family Alphabacteria, estimated to infect 40% of terrestrial arthropod species (Zug and Hammerstein 2012). *Wolbachia* are capable of spreading through insect populations by manipulating host reproductive processes and have many and varied other effects that present potential for use in buffalo fly control programmes (Hoffmann et al. 2015). These can be considered in three main groups:

1. Cytoplasmic incompatibility, which can be harnessed for population suppression, population replacement or potentially population elimination
2. Fitness effects induced by *Wolbachia* infection, and
3. Transmission blocking of secondary pathogens.

These strategies are considered below for their potential to reduce the impacts of buffalo flies or interrupt their spread into uninfected areas.

5.1. Cytoplasmic Incompatibility and Incompatible Insect Technique (IIT)

Wolbachia infection can interfere with insect reproduction in several ways, including through the induction of cytoplasmic incompatibility whereby matings between infected males and non-infected females or between males and females infected with incompatible *Wolbachia* strains (bidirectional incompatibility), produce infertile eggs. This approach when used as an insect suppression or eradication strategy has been termed the Incompatible Insect Technique (IIT) (Zabalou et al. 2009). The IIT method is similar in approach to SIT, with *Wolbachia*-infected males used as *de facto* sterile males. Since *Wolbachia* is not paternally transmitted, as long as similarly infected females are not also released, the *Wolbachia* strain present in the released males does not establish in the target population in the field. Thus, serial release of only the infected males can lead to population suppression or eradication.

The effectiveness of using *Wolbachia*-induced cytoplasmic incompatibility in this way was demonstrated as early as the 1960s when release of *Wolbachia*-infected male *Culex quinquefasciatus* Say mosquitoes, vectors of human filariasis, led to local eradication of this species from areas in Myanmar (Laven 1967). Since then, studies towards the use of IIT have been conducted with a range of mosquito species, including *Aedes polynesiensis* Marks (Brelsfoard et al. 2009; O'Connor et al. 2012), *Aedes albopictus* (Skuse) (Calvitti et al. 2010), *Anopheles stephensi* Liston (Bian et al. 2013) and *Culex pipiens pallens* (Coquillett) (Chen et al. 2013), as well as the veterinary pests *Glossina morsitans* Westwood (Alam et al. 2011; Bourtzis et al. 2016) and *Stomoxys calcitrans* (L.) (Kusmintarsih 2009).

Use of an IIT approach could be applicable for eradication of confined foci of overwintering populations of buffalo flies to prevent or retard southerly spread or to slow rates of re-colonisation of favourable northern areas in summer. The IIT method could also be used to eradicate buffalo flies that become established in relatively isolated areas as a result of climate change, such as those predicted in South Australia and south-western Western Australia (Fig. 2).

Ideally only male *Wolbachia*-infected buffalo flies would be released, but to date no method for accurate mass-sexing of horn or buffalo flies has been reported. In the case of the Hawaii SIT trials with horn flies, irradiated flies of both sexes were released (Eschle et al. 1977). Although this is usually undesirable, because it increases competition with field females for mates and can temporarily increase fly pressure on cattle, it did not compromise success in the case of the Hawaiian trial and may not be a consideration if used against low-level populations present in overwintering foci of buffalo flies.

Reduction of male mating competitiveness from the effects of irradiation is one of the difficulties sometimes experienced in SIT programmes (Zhang et al. 2015). As female flies are often sterilised at levels of radiation below that which causes reduction of competitiveness in males, this has led to the suggestion of the complementary simultaneous use of the SIT and the IIT, with *Wolbachia* used to induce functional sterility in the males and low-level irradiation used to sterilise the females thereby also assuring that the *Wolbachia* strain present in the released males does not establish in the target pest population (Brelsfoard et al. 2009; Zhang et al. 2015; Bourtzis et al. 2016). In the absence of a practical sexing method, a similar approach could be considered for buffalo flies.

Alternatively, the development of a self-sexing strain in stable flies *S. calcitrans* (Seawright et al. 1986), which are in the same subfamily as buffalo flies, the determination of a near infrared (NIR)-based method for sexing tsetse fly pupae (Dowell et al. 2005; Moran and Parker 2016), and the rapid advances with molecular techniques currently being made in other species (Scott 2014), suggest significant potential for the future development of a sexing method for buffalo flies.

Notwithstanding the potential added difficulties for artificial rearing, the use of a strain of *Wolbachia* that also confers a fitness disadvantage or inability to overwinter in infected flies, such as wMelPop (see below), is a further possibility to guard against the effects of inadvertent female release in a *Wolbachia*-based IIT programme.

5.2. Using *Wolbachia*-Induced Fitness Effects to Collapse Overwintering Populations of Buffalo Flies

Different strains of *Wolbachia* can induce a range of different effects on the fitness of infected hosts (Hoffmann et al. 2015). Some of these effects include reduced life span (McMeniman et al. 2009), mortality of eggs (McMeniman and O'Neill 2010), slowed larval development (Ross et al. 2014), and reduced overall fitness (Yeap et al. 2011, 2014; Ross et al. 2015). Infection with *Wolbachia* has also been shown to interfere with blood-feeding efficiency in mosquitoes (Moreira et al. 2009; Turley et al. 2009), and to affect locomotor activity in parasitoid wasps, *Drosophila* species, and some mosquitoes (Fleury et al. 2000; Peng et al. 2008; Evans et al. 2009). Similar effects in buffalo flies could also have deleterious effects on survival and mating efficiency, as well as the persistence of their populations, particularly during winter.

The most profound deleterious effects described have been from the 'popcorn' (wMelPop) strain of *Wolbachia*, initially isolated from laboratory populations of *Drosophila melanogaster* Meigen (Min and Benzer 1997). The wMelPop strain replicates in host cells, causing cellular damage, characteristic morphological changes in infected tissues, and a range of physiological effects. These effects reduce life span by approximately one-half in *D. melanogaster* and transinfected mosquitoes (Min and Benzer 1997; McMeniman et al. 2009). Reductions of life span of this magnitude, and other fitness characters, can have profound effects on the population dynamics of a species, particularly during unfavourable times of the year (Rasic et al. 2014). However, the effects of *Wolbachia* are highly strain-, host- and environment-dependent, and less profound effects on fitness have also been observed in other *Wolbachia*-host associations (Hoffmann et al. 2015).

Modelling conducted by Rasic et al. (2014) demonstrated potential for using fitness reductions induced by *Wolbachia* to suppress or eliminate *Aedes aegypti* L. populations, particularly in locally or seasonally variable environments. Their results suggested that the effects of wMelPop were not sufficient to reduce persistence of mosquito populations in the very favourable climates of north Queensland, but they were likely to cause local extinctions in the more mosquito-marginal environments of central Queensland. These predictions were supported by semi-field cage studies, which showed that reductions in the survival of desiccation-resistant eggs resulting from wMelPop infection, eliminated populations of *Ae. aegypti* during extended dry periods (Ritchie et al. 2015).

Wolbachia could also be used to drive co-inherited deleterious ‘payload genes’ in the genome of infected insects into the target pest population (Curtis and Sinkins 1998; Hoffmann and Turelli 2013; Champer et al. 2016). These genes could confer reduced fitness or conditionally lethal effects such as cold temperature sensitivity or insecticide susceptibility. Conversely the use of linked traits that confer a fitness advantage in certain circumstances might be used to facilitate the spread of *Wolbachia* strains into a population. For example, insecticide resistance that confers a competitive advantage under a spraying regime could be used to assist the spread of a *wMelPop*-infected strain that confers a pathogen blocking capability or seasonal lethality (Hoffmann and Turelli 2013).

As more pest insect genomes are characterised, along with the rapid advancement in molecular transformation technologies, it is expected that possibilities for this approach will grow rapidly. Using *Wolbachia* as the driving mechanism is expected to have greater public acceptance and less potential for unanticipated effects than transgenic gene drives (Champer et al. 2016). An attractive alternative approach is the direct transformation of *Wolbachia* genomes with genes to be driven into a pest population. Until recently, successful genetic transformation of *Wolbachia* had proved elusive, but the recent reporting of a phage-mediated system for the genetic modification of *Wolbachia* (Bordenstein and Bordenstein 2017) offers exciting possibilities in this area.

5.3. *Stephanofilaria Blocking*

Buffalo fly-associated lesions are of significant welfare and economic concern, with estimates of over 95% of cattle affected in northern areas of Australia (Johnson 1989). Although the exact etiology of buffalo fly-associated lesions is unclear, an unnamed species of filarial nematode (*Stephanofilaria* sp.), transmitted by buffalo flies and found in the lesions, is thought to play a role (Johnson et al. 1986; Johnson 1989). Surveys of buffalo flies collected from near Townsville in the 1980s found a 2.9% (range 0% - 9.3%) prevalence of *Stephanofilaria* in female flies (Johnson 1989), whereas a more recent study in 2004 measured infection rates between 29% and 57% in flies collected from four sites near Rockhampton (Shaw and Sutherland 2006).

Wolbachia infection has been demonstrated to reduce vectorial capacity of various species of mosquitoes for a range of pathogens, including filarial nematodes. Inhibition of development of filarial nematodes was seen with both *wMelPop* in *Ae. aegypti* (Kambris et al. 2009) and *wAlbB* in *Ae. polynesiensis* (Andrews et al. 2012) and resulted in a reduction in the prevalence of infective third stage nematodes in the mosquitoes. The mechanism of pathogen blocking is not completely understood but may be due to competition for host resources or modulation of host immune response, in particular reduction in levels of reactive oxygen species (Andrews et al. 2012). The *wMelPop* strain of *Wolbachia* also reduces the efficiency of disease transmission by shortening the life span of vectors and reducing the likelihood that a pathogen will be able to complete its required extrinsic incubation period before host mortality.

The shortest incubation period seen for *Stephanofilaria* sp. in buffalo flies was 7 days (Johnson 1989), suggesting that the life-shortening effects of *wMelPop*

Wolbachia could also significantly affect the transmission dynamics of this filarial nematode species.

Lesions associated with horn fly-transmitted *Stephanofilaria stilesi* in North America appear to be less extensive and severe than buffalo fly-associated lesions in Australia (Hibler 1966). As horn flies are infected with *Wolbachia*, but buffalo flies are not, it is tempting to hypothesise that this difference may be associated with differences in the efficiency of *Stephanofilaria* transmission, although many other factors could also be involved. Disruption of the spread of *Stephanofilaria* or reduction in the severity of lesions by the introduction of a transmission-blocking *Wolbachia* strain into buffalo flies, would be a significant outcome for the Australian cattle industries from both economic and welfare perspectives.

6. BUFFALO FLY OVERWINTERING, A SUSCEPTIBLE STAGE FOR AREA-WIDE APPROACHES?

Horn flies have the ability to overwinter in the pupal phase, as pharate adults, whereas buffalo flies do not (Ferrar et al. 1969; Cook and Spain 1982), which is a major difference between the two species (Showler et al. 2014). In the northerly part of their range in North America, adult horn flies begin to disappear from cattle in autumn and do not reappear until the next spring. Overwintering dormancy allows horn flies to emerge and rapidly re-establish throughout the previous season's range when conditions become suitable in spring or summer. There is, however, significant plasticity in this response and at warmer latitudes horn fly populations continue cycling throughout the year (Showler et al. 2014).

In more marginal areas, horn flies may survive winter both as adults, with reduced activity, and in the pupal stage, with various levels of dormancy. Mendes and Linhares (1999) working in a warm winter climate in Brazil (21°30'S), verified diapause in 9.1% of winter pupae, even though horn flies were present on cattle year-round. These authors note that this dual overwintering mechanism could present difficulties for the design of cost-efficient eradication programmes for horn flies. The plasticity in overwintering response has most likely been a key factor allowing horn flies to disperse and become established in a wide range of environments.

In contrast to horn flies, buffalo flies die out through much of their summer range in winter (Fig. 2). Their range at the southern and continental edges in Australia is limited by cooler temperatures and low moisture levels in dung during winter (Cook and Spain 1982). Low temperatures either prevent development completely, or they slow the development of the larval stages to a degree that they can't be completed before moisture content in dung falls to lethal levels. The occurrence of frosts can also have a devastating effect on the survival of the soil stages, i.e. larvae and pupae (Cook and Spain 1982).

Williams et al. (1985) found that buffalo flies overwintered at the edge of their winter range as slowly cycling, low level fly populations in local areas of moderate microclimates. Most of these overwintering foci were in hilly, heavily timbered areas that were well-watered from either creeks, dams or swamps, and less exposed to low minimum temperatures or frosts than the low-lying surrounding areas. Nearly all of the overwintering sites identified were within 40 km of the coast, where temperatures

were likely moderated by coastal influences. Re-colonisation of summer-suitable areas and southern range extension relied on overwintering of buffalo flies in these foci. When conditions became favourable each year, the flies built-up in numbers and either dispersed from these areas autonomously or were transported by cattle movements to reinfest their summer range (Fig. 2).

These localised overwintering foci provide a potential target for the application of *Wolbachia*-based approaches. The use of *Wolbachia* in either an IIT approach, to compromise *Stephanofilaria* transmission or to introduce a deleterious fitness factor, is likely to be most efficiently achieved at times of low fly populations, such as during overwintering, when suitable release ratios will be most readily achieved. Indeed, SIT and IIT approaches are often initiated when target populations are low, or involve population reduction by insecticide treatments prior to the release of infected flies.

Persistence of buffalo fly populations in overwintering foci is precarious and it is the soil stages that are most subject to adverse effects from low temperatures and dryness. Adult flies living with the warmth and blood provided by their cattle hosts are less affected by adverse winter conditions. Therefore, it is likely that released adult flies will be less exposed to the effects of winter conditions than the soil stages and able to persist for sufficient time to mate with overwintering adult flies and either interrupt reproduction or spread *Wolbachia* infection.

7. TOWARDS A *Wolbachia*-BASED APPROACH TO CONTROLLING BUFFALO FLIES

The effects of *Wolbachia* are most profound in new host associations (McGraw et al. 2002) and *Wolbachia*-based approaches to control require either the transinfection of *Wolbachia* into uninfected host populations, or transinfection of already infected populations with different strains of *Wolbachia* (O'Connor et al. 2012). Transinfection has most often been achieved by embryonic microinjection, but adult microinjection has also been successful in some instances (Hughes and Rasgon 2014). The success rate of microinjection is generally low, with subsequent loss of infection in newly injected hosts common, particularly in more distantly related host species. This is thought to be due to inability of the injected *Wolbachia* to adapt quickly enough to the new host environment. However, the probability of success can be increased by prior adaptation of *Wolbachia* in target host cell lines (McMeniman et al. 2008, 2009).

Although *Wolbachia* has not been found in buffalo flies, it is found widely in horn flies (Jeyaprasak and Hoy 2000; Floate et al. 2006; Zhang et al. 2009). The very close relatedness of buffalo and horn flies suggests it likely that the former will be a competent host for *Wolbachia* and that the likelihood of successful transinfection with suitable strains of *Wolbachia* is high.

We have successfully established cell lines for both horn and buffalo flies and have achieved persisting infections of *wAlbB*, *wMel*, and *wMelPop* in cell lines for both species, also suggesting good potential for the successful transinfection of buffalo flies with *Wolbachia*.

We are undertaking a programme of microinjection towards the stable transinfection of buffalo flies with these three *Wolbachia* strains. We have also

recently developed laboratory rearing methods for buffalo flies and have established a stable persisting laboratory colony (James et al. 2013). These methods will facilitate maintenance of transinfected strains and studies to determine the effects induced in these flies by infection with *wAbB*, *wMel* and *wMelPop*, with a view to develop *Wolbachia*-based strategies for reducing buffalo fly spread and impacts.

8. CONCLUSION

Without intervention, buffalo flies are likely to become major cattle pests in Australia's southern beef and dairy industries, and also increase their impacts in northern herds. Their further southerly invasion is likely to be facilitated by the effects of climate change, together with the availability of a large, susceptible and mostly unprotected *Bos taurus* cattle population in the southern areas of Australia.

Previous regulatory procedures, based on spraying and cattle movement controls, have failed to prevent the southward spread of buffalo flies. Using an integrated area-wide approach incorporating use of a biological agent, such as *Wolbachia*, and focusing on the pest population rather than cattle, avoids potential disadvantages associated to widespread chemical use. In addition, *Wolbachia* are vertically transmitted from female flies to their eggs and restricted to living exclusively within host cells, thus minimising the potential for non-target effects. The use of *Wolbachia* has had good community acceptance in Australia to date (Kolopack et al. 2015) and importantly, a legislative framework for the release of *Wolbachia*-transinfected strains already exists in Australia (De Barro et al. 2011).

The design of optimal strategies will rely on a knowledge of the biological effects of candidate strains of *Wolbachia* in buffalo flies. A number of critical steps towards this end have been completed, including the establishment of an *in vitro* colony of buffalo flies as well as *Haematobia* cell lines transinfected with the *wAlb*, *wMel* and *wMelPop* strains of *Wolbachia*.

We are currently undertaking embryonic and adult microinjection with these strains towards the establishment of transinfected buffalo fly lines. Successful completion of this step will allow characterisation of the effects of *Wolbachia* in buffalo flies towards the design of potential *Wolbachia*-based control strategies and an initial assessment of the likely feasibility of using a *Wolbachia*-based area-wide approach to reduce buffalo fly impacts in endemic areas and interrupt the southerly encroachment of buffalo flies.

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