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A Review of Sheep Blowfly Pathogen Control

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1. Introduction

1.1 Sheep strike

Blowfly strike has been a significant animal health issue for the Australian sheep industry since the late 1800s (Sandeman *et al*., 2014). Despite decades of research and investment into an array of control options, which can often be used in combination, blowflies remain a serious problem and cost Australian wool producers close to A$300M annually (www.flyboss.com). Summaries of the available control options for flystrike can be found in Phillips (2009) and Sandeman *et al.* (2014), with the list including the use of insecticides, breeding sheep with reduced susceptibility, genetic manipulation of the flies, utilising the sheep's immune system through either genetic selection or vaccination, trapping flies and biological control.

Despite the previous investment in blowfly control research, the need for new and innovative control options is as important, today, as it has ever been. This current need is being driven by changes in the fly populations (i.e. the development of resistance to insecticides) and changes in the demands of the human (consumer) population in terms of both animal and environmental welfare.

Resistance by blowflies to the insecticides used against them has been known since the 1950s and has seen a steady progression of development following the introduction of each new class of compounds (Sandeman *et al.*, 2014; Heath and Levot, 2015). Although there are several compounds available today to which resistance has not yet developed, there is every likelihood that eventually it will. With chemicals being the mainstay of sheep protection in many countries, long term solutions will require either a continuous supply of new compounds with novel modes of action, or a reduction in the selective forces applied to the flies to adapt. The latter will probably require a reduced reliance on prophylactic chemical use in favour of alternative control options.

There is a growing global interest in animal welfare standards, with consumers increasingly showing interest in the welfare of animals which produce the products they buy. Of particular relevance here is the practice of mulesing, which involves cutting the loose skin near the breech of the sheep that subsequently heals to leave a bare, stretched area which remains free from contamination with urine and faeces (Philips, 2009; Sandeman *et al.*, 2014). Despite being effective at reducing the incidence of strike, the practice is being phased out in Australia in response to concerns regarding its' ethical acceptability (Sneddon and Rollin, 2010). A complete removal of the mulesing practice from Australian farms will necessitate its replacement with equally effective (and cost-effective) options for flystrike control if current production and welfare levels are to be maintained (Phillips, 2009).

Finally, there has for some time, been a growing interest amongst the human population in the non-target impacts of chemical inputs into farming systems (Bishop *et al.*, 1996; Zhang *et al.*, 2018), and this has included sheep ectoparasiticides (Savage, 1998). Over time, there has been a shift away from widespread use of the more toxic classes of compounds such as the organophosphates and pyrethroids to the much safer insect growth regulators such as diflubenzuron and dicyclanil, and this has reduced the risk to the farmer/operator. However, the organophosphate and carbamate compounds are still used to some extent (Wall, 2012) and the insect growth regulators do have considerable potential for non-target effects, especially in waterways following the scouring process (Robinson and Scott, 1995). There can be no doubt that there is increasing consumer interest in the influence of residues in products and the environment (Köhler and Triebskorn, 2013; Koch *et al.*, 2017) which are likely to pose future challenges for farmers at many levels.

With these challenges currently facing the sheep industry, it is clear the availability of a range of flystrike control strategies other than insecticides would be highly desirable in the future (Sandeman *et al*., 2014). Given that flystrike incidence is determined largely by two factors, the number of susceptible sheep and the number of flies available to oviposit, then control strategies fall logically into two approaches; reducing sheep susceptibility and reducing fly abundance to a level that reduces challenge (Wall, 2012). The focus of this review is the potential of biological control, in particular the use of pathogens, as future tools capable of reducing the abundance of flies, especially *Lucilia cuprina*.

1.2 Biopesticides

The term biopesticide has been used to cover a wide range of approaches and organisms, which may include the use of entomopathogenic viruses, bacteria, protozoa, fungi, nematodes and plant secondary metabolites (Copping and Menn, 2000; Senthil-Nathan, 2015). Some authors also included the compounds derived from these organisms, despite the final product being a synthetic derivative (Copping and Menn, 2000). For example, the commercial insecticide spinosad was developed from an active compound, spinosyn, originally isolated from the soil actinomycete *Saccharopolyspora spinosa*. Thus, the line between what constitutes a biopesticide and what is a synthetic pesticide is not always clear. Regardless of the definition, insect pathogens are potentially useful in their own right as biopesticides, and also as a source of new bioactive proteins or chemistry.

While biopesticides represent only a small proportion of the global pesticide market, perhaps no more than 4%, their market size is expected to grow at more than twice the rate of synthetic pesticides (Ruiu, 2015). The traditionally low market share for biopesticides probably reflects their disadvantages when compared with synthetic insecticides. For example, they are often more difficult to produce in volume, have shorter shelf lives being live organisms, and tend to control a narrower range of target species (Jaronski, 2010; Acharya *et al.*, 2015). However, modern technology and a greater recognition of their advantages is leading to an increase in their appeal. Biologicals are generally considered to have fewer toxicity issues to non-target organisms (Khachatourians, 1986), due to their often narrow host range, and are widely regarded as being more eco-friendly than many synthetic compounds. They also have few if any residue issues and are generally considered acceptable for 'Organic' production systems. Interest in biopesticides has increased in parallel with consumer interest in 'safer' foods and the increasing levels of resistance to conventional chemical pesticides in the target organism (Copping and Menn, 2000; Senthil-Nathan, 2015). Of course, advances in production and formulation technologies are overcoming some of the earlier disadvantages associated with biologicals. There is also a view that because biopesticides are based on biological organisms, and have a complex mode of action, that selection of resistance to them is less likely than with conventional synthetic chemistry (Ruiu, 2015).

For the purposes of this review, we will consider only pathogens, with a particular interest in those which have shown (or might reasonably be expected to show) efficacy against Diptera.

1.3 Initial investigations of biocontrol

Early attempts at biological control of blowflies included the introduction of pupal and larval parasitoids such as *Alysia manducator* and *Nasonia vitripennis*, but these were not successful (Sandeman *et al.*, 2014). In New Zealand, several species of parasitoid were introduced, and along with several endemic species were subsequently identified from blowfly larvae in the field. However, the prevalence at which these occurred is likely too low to be of any practical value without *in vitro* culturing and field release to augment natural populations (Bishop *et al*., 1996).

A microsporidian, *Octosporea muscaedomesticae*, was imported into Australia as a potential biocontrol agent against *L. cuprina* (Cooper and Pinnock, 1983) but this was never developed and commercialised. Similarly, early studies with *Bacillus thuringiensis* (Bt) found isolates that were toxic to blowflies, and these were shown to confer a level of protection against strike when applied to the fleece of sheep (Cooper *et al.*, 1985). However, no products based on this bacterium, or it's associated toxin, have been commercialised. Since that time, advances have been made in science and technology, potentially opening new avenues for the development of commercially viable biopesticides for blowfly control.

2. Pathogen of Flies

A range of pathogenic microorganisms have been described for blowflies and related species, however, there has been no commercial development of biopesticides for their control. A survey of 121 mycoinsecticide products commercially available between 1977 and 2007 reported only nine products registered for the control of all Diptera, including three for the control of Muscidae. There were no products registered for the control of the Calliphoridae (Faria *et al.*, 2007). Products based on twelve fungal species were described, but the majority were based on two species; *Beauveria bassiana* (34%) and *Metarhizium anisopliae* (34%). Several bacterial pathogens, most notably *Bacillus thuringiensis*, have also been described in blowfly and related species. While all of these pathogens cause mortality, the period from infection to death is generally considered to be too long for effective control of adult fly populations. However, recent studies have identified sublethal effects of infection including reduced egg laying capacity and a reduction in the development of larvae, and these should be considered in addition to direct mortality when assessing the overall efficacy of pathogens for the control of fly populations.

2.1 Fungi

2.1.1 Beauveria and Metarhizium

Studies with commercially available products containing *Beauveria bassiana* (BotaniGardtm ES, Mycotrol® O), *Metarhizium anisopliae* (baLence™) and *Metarhizium brunneum* (Met52®EC) showed statistically significant reductions in the half-life (LT_{50}) of adult house fly (*Musca domestica*) and stable fly (*Stomoxys calcitrans*) with a reduction of housefly LT_{50} from 8 days to 5 days and stable fly to 7 days (Weeks *et al.,* 2017). Alone, this reduction in LT_{50} would have little effect on the overall population due to the ability of infected females to continue egg laying post infection, however, some biopesticides appear to have further effects on behaviour and development. Houseflies laid significantly fewer eggs on Met52 treated surfaces than surfaces treated with other biopesticide products; Botanigard™, Mycotrol® O, and baLance™, which in turn had significantly fewer eggs deposited than non-treated surfaces. A reduction in the number of eggs laid on treated surfaces was also observed with stable flies. It is not clear what caused these reductions, but fungal pathogens themselves may cause aversion, or formulation excipients such as oils may have a deterrent effect. Further testing with non-formulated actives would be necessary to clarify the cause. There were also effects on larval development. When housefly eggs were placed on surfaces treated with fungal-based biopesticides there was a significant reduction in pupal emergence. Of the products tested Met52 caused the highest reduction in emergence (85% reduction at 109 conidia/ml) and was the most efficacious at lower doses (35% at 108 conidia/ml) (Machtinger *et al.,* 2016). Further, efficacy may be achieved by using combinations of chemical and biological actives. Mwamburi *et al.* (2009) found *B. bassiana* alone was ineffective against housefly but when applied along with Bt or cyromazine (Lavadex) an additive

effect on larval development and emergence was observed. Similarly, Farooq *et al.* (2018) found an additive effect of *B. bassiana* and imidacloprid combinations when applied to *M. domestica*.

Studies with *Lucilia* spp. have found *M. anisopliae* efficacious against adult stages of both *L. cuprina* (Leemon and Jonsson, 2012) and *Lucilia sericata* (Wright *et al.,* 2004). When applied topically to *L. cuprina* there was a distinct dose response with LT_{50} ranging from 4.8 days (at 109/spores/ml) to 12.1 days (107 spores/ml), but when conidia were ingested with food there was a much flatter response with LT_{50} of between 2.3 days (100 spores) and 3.9 days (1 spore) indicating different modes of action with different infection routes (Leemon and Jonsson, 2012). Similarly, different methods of inoculation affected the mortality rate of *L. sericata* with high levels of mortality (up to 100%) achieved by immersion in spore suspension or by direct application of spores to the insect body. Inoculation by tarsal contact with an infected surface, considered to be a more real-life scenario, caused mortality rates of between 30 and 70% (Wright *et al.,* 2004) which may be sufficient to meet the 20-30% daily mortality required to suppress populations of *L. sericata* in the field (Wall and Smith, 1997). These studies suggest that dosing spores through food or tarsal contact using lure and kill strategies targeting adult flies might be an effective control solution. The behavioural and developmental changes in egg laying and emergence observed with houseflies have not been demonstrated with *Lucilia* spp. to date.

2.1.2 Other fungi

Tolypocladium cylindrosporum was found to cause a 100% reduction in the emergence of adult *L. sericata*, *L. cuprina*, and *Ch. rufifacies* as well an 80% reduction in the emergence of *C. stygia* in laboratory assays with doses of $2x10^8$ spores per ml applied to soil (Wright *et al.,* 2009). No dose titration was conducted but may be worthwhile considering Barson *et al.* (1994) found *T. cylindrosporum* prevented emergence of housefly at spore concentrations above 10^4/ml.

The microsporidium *Octosporea muscaedomesticae* causes death of both larvae and adult *L. cuprina* (Cooper and Pinnock, 1983) and was considered for inoculative release against *L. cuprina*, however, the period for disease progression (several days) combined with difficulties maintaining sufficient transmission levels led to the abandonment of this approach (Anstead *et al.,* 2017). Because infected adult flies pass spores in faeces, it has been suggested that if adults could be infected by attracting them to an infected bait, they may spread the disease through the wider population via transfer at other feeding sites (Cooper and Pinnock, 1983). However, the feasibility of this approach has not been evaluated.

Adult house flies are susceptible to *Entomophthora muscae* and *E. schizophorae* (Geden, 2012) and *E. muscae* is reported to be a common pathogen of *L. cuprina* throughout southeast Australia (Glare and Milner, 1987). Mass rearing methods to produce large number of infected flies have been developed and field releases have resulted in increased disease prevalence, however, the potential of *E. muscae* for fly suppression is limited by short lived conidia, intolerance to high temperatures and by the requirement for large populations to sustain epizootics (Geden *et al.,* 1995).

2.2 Bacteria

2.2.1 Bacillus thuringiensis (Bt)

Although a wide range of Bt strains have been shown to have activity against *L. cuprina* larvae (Gough *et al.,* 2005; Kongsuwan *et al.,* 2005; Heath *et al.,* 2004) and other related species (Gough *et al.,* 2002; Hodgman *et al.,* 1993) no commercial products have become available for sheep ectoparasite control (Sandeman *et al.,* 2014).

A survey of sheep fleece microbiota from commercial farms in South and Western Australia found Bt present in 92% of samples. Of 120 isolations, 27 showed larvicidal activity to *L. cuprina* indicating that larvicidal strains of Bt appear to form part of the natural biota of sheep fleece in Australia. This provides a potential explanation for the observed natural resistance to flystrike in some sheep (Lyness *et al.,* 1994), however, further surveys to find a correlation and better understand possible impacts are necessary. Interestingly, endospore forming *Bacillus* spp. were uncommon in New Zealand sheep fleece (Jackson *et al.,* 2002).

While spore/crystal preparations have been demonstrated to protect against myiasis (see below), cells applied to the fleece in the vegetative state were unable to establish and develop to the spore/crystal stage and subsequently prevent myiasis (Heath *et al.,* 2004). Vegetative cells are also susceptible to the high temperatures that may occur in fleece (Lyness *et al.,* 1994). So, while precipitation may not affect the level of toxin in the fleece (Heath *et al.,* 2004) the induction of spore germination in a wet fleece may result in reduced efficacy. The use of formulation strategies such as microencapsulation or oil-based carriers may have potential to protect spores from moisture to prevent germination and extend efficacy.

2.2.2 Brevibacillus laterosporus

Br. laterosporus has been demonstrated to have larvicidal activity against *L. cuprina*, with twelve isolates causing mortality of between 29% and 55% in bioassays. While no sublethal effects were observed, an influence on sex ratio was reported. The efficacy against *L. cuprina* larvae did not appear related to spore density (Pessanha *et al.,* 2015) and while the mode of action was not determined, it is indicative of a secreted toxin. In contrast, mortality of *Chrysomya putoria* and *M. domestica* was proportional to spore concentration suggesting a different mode of action in these species. These studies also reported sublethal effects related to feeding and development, including reduction in larval weight gain probably by inhibition of feeding and variation in the duration of developmental stages (Pereira *et al.,* 2018; Ruiu *et al.,* 2006).

2.2.3 Serratia

Based on reports of toxicity to other dipteran species including tsetse and stable flies Garnham *et al.* (1991) demonstrated that several isolates of *Serratia marcescens* caused significant reductions in the LT_{50} of *L. sericata, Calliphora vicina*, and *C. stygia*. O'Callaghan *et al.* (1996) found eight *S. marcescens* and one *S. liquefaciens* isolate caused significant mortality compared to untreated controls when fed as a sugar solution to adult *L. sericata*. *Serratia* species do not form spores and the vegetative cells are extremely sensitive to environmental stresses presenting a number of difficult technical challenges for biopesticide development.

3. Modes of Action

3.1 Fungi

Unlike entomopathogenic bacteria or viruses which need to be ingested to cause effect most fungal pathogens infect the host through direct penetration of the insect cuticle. Fungal pathogenesis typically follows a stepwise path from spore adhesion and host recognition. Stages such as conidial/spore adhesion, cuticle degradation and nutrient assimilation have been reported during fungal pathogenesis (Butt *et al.,* 2016). In *B. bassiana* and *M. anisopliae*, adhesion-related genes (Wang and St Leger, 2007; Cho *et al.,* 2007; Li *et al.,* 2010) and cuticle-degradation genes (Fan *et al.,* 2007) have been identified. In *Metarhizium* adhesion is mediated by hydrophobins / adhesions MAD1 and MAD2. The MAD1 adhesion mediates insect cuticular adhesion while MAD2 is associated with adhesion to the plant as a precursor to endophytic association (Wang and Wang, 2017). Post adhesion the formation of a specialised hyphal structure, the appressoria, primes the fungus to gain entry through the insect cuticle. A build-up of turgor pressure within the appressoria combined with the apical production of enzymes (extracellular lipases, peptidases, proteases and chitinases) enable the localised dissolution via the cleaving and degradation of the insect cuticular proteins, chitin and lipids (Gillespie *et al.,* 2000; St. Leger, 1995; Ortiz-Urquiza and Keyhani, 2013). From here the appressoria can penetrate allowing hyphal ingress. Laboratory based studies suggest that *M. anisopliae* and *B. bassiana* derived proteases are first produced, followed by chitinases and lipases (St Leger *et al.,* 1986).

Validation of the role of lipases in infection is provided by the lipase inhibitor Ebelactone B, which can prevent *M. anisopliae* infection of the tick host (da Silva *et al.,* 2009). There is also a positive correlation between pathogenicity and increased enzyme production (Kaur and Padmaja, 2009; Castellanos-Moguel *et al.,* 2007). Post entry of the invasive hyphae to the cuticle the fungus combats the immune system and grows to cause mycosis and host death (Pedrini *et al.,* 2007).

It is thought that the complexity and differences in the insect cuticle composition and immune system impart host specificity of fungal species (Boguś *et al.,* 2007). In addition, the presence of cuticular fatty acids, methyl esters and alcohols with antimicrobial properties, and some others exert inhibitory or stimulatory effects on fungal germination, growth and virulence (Kerwin, 1984; Boguś *et al.,* 2010; Gołębiowski *et al.,* 2011). Some insects, such as stink bug and red flour beetle, secrete a wide range of antifungal compounds such as benzoquinone. In some instances, these can be degraded by enzymes such as 1,4-benzoquinone oxidoreductase produced by *B. bassiana* allowing its ingress. Like pathogenic bacteria, fungi may have to overcome host derived reactive oxygen species (Wang and Wang, 2017).

In some circumstances, only a specific life stage of the host insect might be susceptible. The soil fungus *Conidiobolus coronatus* is highly virulent against adults, but not larvae or pupae of *Calliphora vicina*, *Calliphora vomitoria, L. sericata* and *M. domestica*. Cell free supernatants of this fungus have been found to contain elastase, N-acetylglucosaminidase, chitobiosidase and lipase (Boguś *et al.,* 2017). The authors found that the *C. coronatus* derived proteases and chitinases hydrolysed all adult and larval cuticles tested in vitro and observed differences in degradation rates between different species. The isolated lipases were able to hydrolyse various life stages of *C. vomitoria* and *C. vicina* but unable to hydrolyse cuticle from the wings of *L. sericata* or larvae of *M. domestica*.

Though infrequent, fungal infection by the oral route has been documented. The proboscis has been defined as an infection route of the *Anopheles* mosquito for *B. bassiana* (Ishii *et al.,* 2017) and for *B.*

bassiana and *M. anisopliae* against the stored grain pest wheat weevil *Sitophilus granarius* (Batta, 2018). Infection by ingestion is thought to be rare, wherein digestive proteolytic enzymes specifically in the mesenteron are likely to degrade the fungal spores (Ravallec *et al.,* 1989).

Mode of action studies on the fungus *T. cylindrosporum* against pest fly species such as *L. cuprina* have yet to be undertaken. Studies of *T. cylindrosporum* against the mosquito *Aedes aegypti* larvae, found that the fungus typically invaded the larval head at the base of the mandibles or maxillae. Secondary infection by *T. cylindrosporum* was through penetration of the pharynx or cuticle at moulting (Goettel, 1988) which was found to be the most susceptible phase. Soares (1982) identified that *T. cylindrosporum* can penetrate both the cuticle and the larval intestinal tract. Under laboratory conditions at 15^{o}C *T. cylindrosporum* was able to infect mosquito eggs affecting egg hatch (Flor-Weiler *et al.,* 2017). Initial infection of mosquito larvae by *T. cylindrosporum* is unusual in that no appressoria like structure is observed. It is theorised that the hyphae of this species generate strong adhesive forces enabling site specific penetration (Ravallec *et al.,* 1989; Soares, 1982). In relation to insect active metabolites, aged cultures of *T. cylindrosporum* and *Tolypocladium infatum* were found to produce an entomotoxic metabolite termed Tolypin with activity towards the fruit fly *Drosphila melanogaster* and mosquito larvae *Culex pipiens* (Weiser and Maťha, 1998). *Tolypocladium* sp. has also been found to produce 15 amino acid residue linear peptide molecules termed efrapeptin (Krasnoff and Gupta, 1991), with insect and miticidal activities and antimicrobial properties. Efrapeptins are thought to be inhibitors of intracellular protein transport system and mitochondrial ATPase (Charnley, 2003). Similar to destruxins, efrapeptin causes antifeedant, growth and development effects.

Other dipteran active fungal metabolites include destruxins a family (A-E) comprising variants of a structural backbone of five amino acids and an α-hydroxyl acid. Destruxins were first identified in *M. anisopliae* and later reported in a range of entomopathogenic fungi, and some plant pathogenic fungi (Pedras *et al.,* 2002). Members of the destruxins have insecticidal, antiviral, and phytotoxic properties. Their mode of action has yet to be fully elucidated, but they have been implicated in weakening the host immune defence, muscular and digestive systems (Schrank and Vainstein, 2010; Wang *et al.,* 2012). They are also known to inhibit nucleic acid and protein synthesis (Krasnoff and Gupta, 1991). Post ingestion they reduce feeding and growth development. The entomopathogenic fungus *Hirsutella thompsonii* produces hirsutellin, a cytolytic thermo stable non-glycosylated protein ribotoxin, which has oral and injected activity against fruit flies, aphids and mites. Hirsutellin cleaves rRNA preventing protein synthesis. In some instances, contact toxicity is also observed (Omoto and McCoy, 1998; Mazet and Vey, 1995).

The induction of fungal metabolites in the laboratory has been demonstrated via several routes, including the alteration of growth conditions, with *Tolypocladium geodes* (Kebede *et al.*, 2017). In other systems, the *M. anisopliae* chymoelastase (Pr1), and a trypsin like protease (Pr2), were induced using cuticle extracts of three mosquito species. Pr1 was proposed to be the main factor implicated in mosquitocidal activity (Mohanty *et al.,* 2008). These methods, combined with fungal stress screening assays (Liu *et al.,* 2017; Zhang *et al.,* 2009) and the *L. cuprina* feeding assays developed by Gough *et al.* (2005) and Guo *et al.* (2018) would allow the rapid identification of new putative *L. cuprina* bioactives.

3.2 Bacteria

Lipopolysaccharide from *Serratia marcescens* and *Pseudomonasa aeruginosa* was found to induce dose dependent changes on the heart rate of the blowfly (*Phaenicia sericata*, synonym of *L. sericata*) and fruit fly (*D. melanogaster*) (Anyagaligbo *et al.,* 2017). Also, the bacterium *Pseudomonas entomophila*

produces a gut acting monolysin pore-forming toxin with activity against *D. melanogaster* (Opota *et al.,* 2011).

Bacillus thuringiensis subsp. *israelensis* produces a range of toxins with activity against mosquito, black fly and fruit fly, with activity attributed to a range of Cry toxins (Ben-Dov *et al.,* 2014). Though the mode of action has yet to be elucidated, the *B. thuringiensis* CryB1 endotoxin protein has been found to be present in all *B. thuringiensis* isolates with activity towards *M. domestica* (Lysyk *et al.,* 2010). Crude extracts of *B. thuringiensis* derived CryB1a and *Escherichia coli* expressed CryB1a endotoxin were active against *L. cuprina* larvae (Heath *et al.,* 2004). The *B. thuringiensis* CAA890 δ-endotoxin Cry47Aa is also toxic to *L. cuprina* larvae (Kongsuwan *et al.,* 2005).

Additional options for the control of *L. cuprina* may be through the manipulation of the fly microbiome. Recent studies of microbiomes from the blowflies *Chrysomya megacephala* and housefly *M. domestica* revealed a high prevalence of the endosymbiont *Wolbachia* in the fly population (Junqueira *et al.,* 2017). Recently, *Wolbachia* has been identified as being common in the Australian blowfly population (Perry *et al.,* 2018. Presentation to the AWI Breech Flystrike Review Workshop, Sydney, December 2018). The bacterium *Wolbachia* has been implicated in the manipulation or alteration of invertebrate reproductive biology, altering the gender, cytoplasmic incompatibility (Werren *et al.,* 2008) eluding to a further avenue for control. Although not a pathogen of flies, the potential importance of this bacterium, and other microbes, in the biology of blowflies warrants further investigation.

3.3 Genomics

The recent sequencing of the *L. cuprina* genome (Anstead *et al.,* 2016) will enable more detailed mode of action/resistance studies, specifically transcriptome assessments of pathogen host interaction and provide the foundation for genetic alterations deploying technologies such as RNAi-based, CRISPR/cas9. The comparative assessment of invertebrate pest genomes from *L. cuprina* and tsetse fly *Glossina morsitans*, along with other fly species, may elude to shared mechanisms of animal host attack providing a target for future manipulation (Anstead *et al.,* 2016). In addition to this, genome assessments of some other invertebrate pest species have facilitated vaccine development for the pest, or its vectored disease. The targeted silencing of the Southern cattle fever tick *Rhipiaephalus microplus* Aquaporin 2 (AQP2) gene by RNA interference reduced fitness and offers an opportunity for the development of a reverse vaccinology strategy (Guerrero *et al.,* 2014; Hussein *et al.,* 2015).
The recent genome sequencing of several entomopathogenic fungi (Lee *et al.,* 2018; Sbaraini *et al.,* 2016; Wang and Wang, 2017) has provided further insight into their virulence and metabolic potential. The increasing power of bioinformatics has enabled the rapid detection of putative virulence genes with chitinases and trypsinases being conserved within *B. bassiana* (Lee *et al.,* 2018). Though the fly active *T. cylindrosporum* (Wright *et al.,* 2009) genome has yet to be determined, other members of this genus have recently been sequenced e.g., *Tolypocladium inflatum* (Bushley *et al.,* 2013) and the truffle-parasite *Tolypocladium ophioglossoides* (Quandt *et al.,* 2015). These genomes combined transcriptomic studies and established bioassays (Gough *et al.,* 2005; Guo *et al.,* 2018) which will expediate the discovery of new fungal actives and allow the detection and temporal expression of virulence, metabolic and sporulation genes (Wang *et al.,* 2017; Sbaraini *et al.,* 2016).

4. Applications

4.1 Method of delivery

4.1.1 Application to pasture

The most common method of application for biopesticides is undoubtedly the broad-acre application to pastures and crops where the target organisms live. More targeted environmental applications have been described including the application of a *B. bassiana*-based biopesticide to manure piles containing breeding flies to control the house fly *M. domestica* in poultry houses (Kaufman *et al.,* 2005). A variation on this approach was to feed a diet containing Bt to chickens so that their faeces contained the Bt-toxin for control of *M. domestica* (Merdan, 2012).

For blowfly control, the only stages present on pasture (i.e. in soil) are the pre-pupal and pupal stages. The fungus *T. cylindrosporum* was isolated from *Lucilia* spp. prepupae cadavers collected from soil at the Flock House Research Farm in New Zealand. Subsequent *in vitro* studies showed that application of the fungus to containers of soil prior to the addition of wandering-stage fly larvae resulted in 100% kill of *L. cuprina* and *L. sericata* (Wright *et al.,* 2009). Several other potential blowfly pathogens are also common soil dwelling fungi (e.g. *Metarhizium* spp.) which raises the possibility of applying one or more pathogens to soil, where the environment may allow for them to persist naturally for some length of time. As far as we can ascertain no published studies have investigated or contemplated the application of pathogens to soil for blowfly control. The potential of applying *T. cylindrosporum* and other likely pathogens to soil in late autumn, when the entire fly population will be overwintering as prepupae, is worthy of consideration.

4.1.2 Application to sheep

Inundative application of biopesticides to sheep (equivalent to modern insecticides) has been trialled using *B. thuringiensis* toxins on several occasions (Cooper and Pinnock, 1983; Heath *et al.,* 2004). Trials with Bt variants in larval implant studies demonstrated potential of this approach to kill larvae on the sheep's back (Cooper and Pinnock, 1983). When applied as crude spore/crystal preparations to sheep fleece, Bt isolates containing the Cry1B endotoxin provided 3-6 weeks protection against experimentally induced fly strike on most test animals, a level of protection comparable to diazinon (Heath *et al.,* 2004). The longevity of protection may be related to the strength of the solution applied, with reports of protection for up to 11 weeks when high concentration bentonite: spore preparations were jetted onto fleece (Lyness *et al.,* 1994). However, for toxicity to occur, larvae must ingest the toxin which must be near the skin where larvae are feeding. The most important factor affecting residual efficacy appears to be the movement of Bt away from the skin as wool grows. Although the distal wool portions remain potent, the interception of toxin by larvae is reduced with distance from the skin (Heath *et al.,* 2004). The use of stickers and spreaders to enhance retention in fleece and skin lipids may extend the persistence of Bt preparations and extend flystrike protection (Heath *et al.,* 2004) but as yet these possibilities remain untested.

Another variable which may limit the utility of biopesticides applied to sheep fleece is temperature. Sheep body temperature is maintained at about 39^{o}C which may be excessive for some pathogen species (Lyness *et al.,* 1994; Geden *et al.,* 1995). For example, Herrero *et al.* (2011) demonstrated inhibition of growth of *T. cylindrosporum* at 30^{o}C, suggesting that this fungus would not function

optimally if applied to sheep. It seems likely that optimal use of at least some pathogen species would be in targeting flies off the host, as they are probably not suitable as prophylactic treatments applied to sheep fleece.

4.1.3 Application to treated surfaces

The application of infective agents applied to treated surfaces has been utilized for flies, especially in pig and poultry houses, clearly demonstrating the potential of infection through tarsal contact alone (Acharya *et al.,* 2015). Similarly, Wright *et al.* (2004) demonstrated that *L. sericata* adults could become infected via tarsal contact after only 20 seconds of exposure to surfaces treated with conidia of *M. anisopliae*. Adults of *L. cuprina* were infected and killed by incorporating *M. anisopliae* conidia into sugar on which the flies were fed (Leemon and Jonsson, 2012), however, it was not established whether the conidia were ingested or invaded the host through the cuticle.

4.1.4 Lure and kill

The high mobility and low population densities of fly populations has been recognized as a limitation of pathogen spread within a population, however, the use of lures to focus flies into small areas to enable/enhance pathogen transmission has been proposed as a possible solution (Cooper and Pinnock, 1983; Wright *et al.,* 2004; Leemon and Jonsson, 2012). Baits have been used successfully to deliver fungal agents to the caterpillar of porina moth in New Zealand (Brownbridge *et al.,* 2008). A range of visual and semiochemical cues have been identified which might prove useful for attracting *Lucilia* spp. (Wall *et al.,* 1992; Ashworth and Wall, 1994; Morris *et al.,* 1998). The volatiles of various bacteria species, specifically *Providencia rettgeri*, have been found to be attractive to oviposition of gravid females of the blowfly *Cochliomyia hominivorax* (DeVaney *et al.,* 1973; Eddy *et al.,* 1975). Strains of *Proteus mirabilus* have been used to inoculate baits as attractants for blowflies in sheep pasture (Morris *et al.,* 1998). What is more, recent advances in metabolomics technology raises the feasibility of finding a range of new potent attractants for blowflies, potentially targeting the gravid females searching for a host.

An additional interest, here, is the potential to include biopesticides into baits which have previously been excluded on the basis that they are also attractive to beneficial insects (e.g. honey bees). Whereas sugar baits treated with synthetic insecticides would largely be unacceptable because of the risk to bees, the same constraints may not apply to pathogens delivered in sugar. For example, strips coated with *M. anisopliae* conidia, which have demonstrated efficacy against *L. cuprina* (Leemon and Jonsson, 2012), have been used successfully to control varroa mites in honey bee colonies in the U.S. (Kanga *et al.,* 2006), implying that the bees are not affected by the fungus. Therefore, the narrow host range associated with many pathogens may open the door to use of a range of lures and baits which have not previously been considered for use against blowflies.

4.2 Use strategies

4.2.1 Combining pathogens

Combining two or more pathogens into a single product can extend its host range, thereby overcoming one of the disadvantages associated with some single-pathogen pesticides (Sayed and Behle, 2017). Alternatively, multiple pathogens may be formulated into a single product to increase efficacy against a single target species. For example, Sayed and Behle (2017) combined *B. thuringiensis* and *B. bassiana* and found that a 50:50 mix of the two species was significantly more efficacious than either used alone, even though the components in the mixture were applied at half the dose rate of the single actives. This was interpreted as indicating a synergist interaction between the two pathogens (i.e. the combination was more effective than the sum of its parts). The limited number of studies of this type suggest that synergy between pathogens may be common against a range of insect pests (Wraight and Ramos, 2005). This approach may also have the advantage of decreasing the limitations imposed by environmental factors. For example, temperature and moisture optima may differ between agents, resulting in a combination having a broader environmental range of activity. With respect to *Lucilia* spp. both *M. anisopliae* and *T. cylindrosporum* are naturally occurring soil fungi, with efficacy against *L. sericata* and *L. cuprina* (Wright *et al.,* 2009). Combining these together may be an attractive option.

4.2.2 Combining with synthetic pesticides

Concurrent application with synthetic neurotoxic insecticides may have advantages, because the microbial agents are unlikely to be affected by the insecticide (Wright *et al.,* 2004). This approach is also growing in popularity as some modern chemicals are not as toxic to natural enemies as older compounds. The concurrent or alternate use of synthetic chemicals with biological agents, can therefore, enable a level of biological control to be achieved either between or concurrently with treatments with synthetic pesticides. For example, some insecticides commonly used for mosquito control are primarily active against adults, whereas the biopesticide containing *B. thuringiensis israeliensis* has persistent activity against larval stages. Combined application of both the synthetic and biopesticide was able to achieve a better and more sustained control of mosquito than either used alone (Seleena *et al.,* 1999).

4.2.3 Combining with other biocontrol agents

Co-administration of pathogens with other biocontrol methods are likely to enhance control, assuming the activity of the agents is independent. As an example, Alma *et al.* (2007) investigated the use of a biopesticide based on the fungus *Paecilomyces fumosoroseus* and a generalist insect predator (which was not affected by the fungus) on whitefly populations. They found the activity of the two agents was independent of the other and that the level of control achieved was effectively the sum of the two agents working independently.

5. Summary

The search for novel control tools for use against sheep blowflies is being driven by the steady progression of insecticide resistance in the fly populations and a growing consumer interest in food and environmental safety, and animal welfare issues. Pathogen based biopesticides offer numerous advantages in response to these issues. Clearly, there are pathogens available which can cause significant mortality of fly populations, with the likelihood that resistance to them will be slower to develop. The narrow host range of many pathogens results in low toxicity to the user and fewer issues of non-target effects. Biopesticides appear to have ample potential for useful integration into an integrated pest management (IPM) package targeting blowflies.

A range of pathogens have been shown to cause significant mortality of *Lucilia* species, predominantly *L. cuprina*. These include the fungi *M. anisoplae, O. muscaedomesticae, T. cylindrosprum, E. muscae* and *C. coronatus* along with the bacteria *B. thuringiensis, Br. laterosporus, Serratia marcescens* and *S. liquefaciens*. Each of these, used alone or in combination, has the potential to be a useful adjunct to blowfly control if they can be delivered in an appropriate format. The importance of combining biopesticide use with other control options in some form of IPM package is self-evident. The targeting of specific fly life stages through lure and kill strategies or inoculation of pathogens into soil seems achievable given recent advances in analytical and formulation technologies. Further, the rapidly growing stock-pile of bioactive compounds discovered through investigations of the pathogen-host interaction shows considerable promise. Technological advances, especially in high through put sequencing and metabolomics combined with improved bioassays increases the likelihood of discovering more effective lures and kairomones as attractants for blowflies. In addition, advances in genomics and transcriptomics has the potential to uncover a variety of sensitive targets within the flies themselves, or indeed within their microbial communities allowing their targeted manipulation. Given this array of possibilities, there can be little doubt that the study of blowfly pathogens, their application and mechanisms of action, along with the genetics, physiology and behaviour of the flies themselves offers fertile ground for the discovery of new and powerful tools for the future control of these important parasites.

6. References

Acharya, N., Seliga, R.A., Rajotte, E.G., Jenkins, N.E., Thomas, M.B., 2015. Persistence and efficacy of a *Beauveria bassiana* biopesticide against the house fly, *Musca domestica*, on typical structural substrates of poultry houses. Biocontrol Science and Technology 25, 697-715.

Alma, C.R., Goettel, M.S., Roitberg, B.D., Gillespie, D.R., 2007. Combined effects of the entomopathogenic fungus, *Paecilomyces fumosoroseus* Apopka-97, and the generalist predator, *Dicyphus hesperus*, on whitefly populations. BioControl 52, 669-681.

Anstead, C.A., Batterham, P., Korhonen, P.K., Young, N.D., Hall, R.S., Bowles, V.M., Richards, S., Scott, M.J., Gasser, R.B., 2016. A blow to the fly - *Lucilia cuprina* draft genome and transcriptome to support advances in biology and biotechnology. Biotechnology Advances 34, 605-620.

Anstead, C.A., Perry, T., Richards, S., Korhonen, P.K., Young, N.D., Bowles, V.M., Batterham, P., Gasser, R.B., 2017. The battle against flystrike – past research and new prospects through genomics. Advances in Parasitology 98:227-281.

Anyagaligbo, O., Bernard, J., Greenhalgh, A., Cooper, R.L., 2017. The effects of bacterial endotoxin (LPS) on cardiac function in a medicinal blow fly (*Phaenicia sericata*) and a fruit fly (*Drosophila melanogaster*). Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology 217, 15-24.

Ashworth, J.R., Wall, R., 1994. Responses of the sheep blowflies *L. sericata* and *L. cuprina* to odour and the development of semiochemical baits. Medical and Veterinary Entomology 8, 303-309.

Barson, G., Renn, N., Bywater, A.F., 1994. Laboratory evaluation of six species of entomopathogenic fungi for the control of the house fly (*Musca domestica* L.), a pest of intensive animal units. Journal of Invertebrate Pathology 64: 107-113.

Batta, Y.A., 2018. Efficacy of two species of entomopathogenic fungi against the stored-grain pest, *Sitophilus granarius* L. (Curculionidae: Coleoptera), via oral ingestion. Egyptian Journal of Biological Pest Control 28, 44.

Ben-Dov., E., 2014. *Bacillus thuringiensis* subsp. *israelensis* and its Dipteran-specific toxins. Toxins (Basel) 6, 1222-1243.

Bishop, D.M., Heath, A.C.G., Haack, N.A., 1996. Distribution, prevalence and host associations of Hymenoptera parasitic on Calliphoridae occurring in flystrike in New Zealand. Medical and Veterinary Entomology 10, 365-370.

Brownbridge, M., Ferguson, C., Saville, D.J., Swaminathan, J., Hurst, M.R.H., Jackson, T.A., 2008. Potential for biological control of porina (*Wiseana* spp.) with a novel insecticidal bacterium, Yersinia sp. (MH96) EN65 strain. New Zealand Plant Protection 61, 229-235.

Boguś, M.I., Czygier, M., Gołębiowski, M., Kedra, E., Kucińska, J., Mazgajska, J., Samborski, J., Wieloch, W., Włóka, E., 2010. Effects of insect cuticular fatty acids on in vitro growth and pathogenicity of the entomopathogenic fungus *Conidiobolus coronatus*. Experimental Parasitology 125, 400-408.

Boguś, M.I., Kędra, E., Bania, J., Szczepanik, M., Czygier, M., Jabłoński, P., Pasztaleniec, A., Samborski, J., Mazgajska, J., Polanowski, A., 2007. Different defense strategies of *Dendrolimus pini, Galleria mellonella*, and *Calliphora vicina* against fungal infection. Journal of Insect Physiology 53, 909-922.

Boguś, M.I., Włóka, E., Wrońska, A., Kaczmarek, A., Kazek, M., Zalewska, K., Ligęza-Żuber, M., Gołębiowski, M., 2017. Cuticle hydrolysis in four medically important fly species by enzymes of the entomopathogenic fungus *Conidiobolus coronatus*. Medical and Veterinary Entomology 31:23-35.

Bushley, K.E., Raja, R., Jaiswal, P., Cumbie, J.S., Nonogaki, M., Boyd, A.E., Owensby, C.A., Knaus, B.J., Elser, J., Miller, D., Di, Y., McPhail, K.L., Spatafora, J.W., 2013. The Genome of *Tolypocladium inflatum*: Evolution, organization, and expression of the Cyclosporin biosynthetic gene cluster. PLoS Genet 9, e1003496. https://doi.org/10.1371/journal.pgen.1003496

Butt, T., Coates, C., Dubovskiy, I., Ratcliffe, N., 2016. Entomopathogenic fungi: new insights into host–pathogen interactions. Advances in Genetics 94, 307-364.

Castellanos-Moguel, J., González-Barajas, M., Mier, T., Reyes-Montes Mdel, R., Aranda, E., Toriello, C., 2007. Virulence testing and extracellular subtilisin-like (Pr1) and trypsin-like (Pr2) activity during propagule production of *Paecilomyces fumosoroseus* isolates from whiteflies (Homoptera: Aleyrodidae). Revista Iberoamericana de Micología 24, 62-68.

Charnley, A.K., 2003. Fungal pathogens of insects: Cuticle degrading enzymes and toxins. Advances in Botanical Research 40, 241-321.

Cho, E.M., Kirkland, B.H., Holder, D.J., Keyhani, N.O., 2007. Phage display cDNA cloning and expression analysis of hydrophobins from the entomopathogenic fungus *Beauveria* (*Cordyceps*) *bassiana*. Microbiology 153, 3438-3447.

Cooper, D.J., Pinnock, D.E., 1983. The role of pathogens in the suppression of *Lucilia cuprina*. In: Raadsma HW (Ed.) Second National conference on sheep blowfly and flystrike in sheep. Department of Agriculture, NSW, Sydney pp 237-241.

Cooper, D.J., Pinnock, D.E., Were, S.T., 1985. Bacterial pathogens of the Australian sheep blowfly *Lucilia cuprina*. In: Proceedings of the 4th Australasian conference on Grassland Invertebrate Ecology.

Copping, L.G., Menn, J.J., 2000. Biopesticides: a review of their action, applications and efficacy. Pest Management Science 56, 651-676.

Da Silva, W.O.B., Santi, L., Schrank, A., Vainstein, M.H., 2009. *Metarhizium anisopliae* lipolytic activity plays a pivotal role in Rhipicephalus (Boophilus) microplus infection. Fungal Biology 114, 10-15.

DeVaney, J.A., Eddy, G.W., Ellis, E.M., Harrington, R. Jr., 1973. Attractancy of inoculated and incubated bovine blood fractions to screwworm flies (Diptera: Calliphoridae): role of bacteria. Journal of Medical Entomology 30, 591-595.

Eddy, G.W., DeVaney, J.A., Handke, B.D., 1975. Response of the adult screwworm (Diptera: Calliphoridae) to bacteria-inoculated and incubated bovine blood in olfactometer and oviposition tests. Journal of Medical Entomology 25,379-381.

Fan, Y., Fang, W., Guo, S., Pei, X., Zhang, Y., Xiao, Y., Li, D., Jin, K., Bidochka, M. J., Pei, Y., 2007. Increased insect virulence in *Beauveria bassiana*strains overexpressing an engineered chitinase. Applied and Environmental Microbiology 73, 295-302.

Faria, M.R., Wraight, S.P., 2007. Mycoinsecticides and Mycoaracaricides: A comprehensive list with worldwide coverage and international classification of formulation types. Biological Control 43, 237-256.

Farooq, M., Steenberg, T., Hojiland, D.H., Freed, S., 2018. Impact of sequential exposure to *Beauveria bassiana* and imididacloprid against susceptible and resistant strains of *Musca domestica*. BioControl 63, 707-718.

Flor-Weiler, L.B., Rooney, A.P., Behle, R.W., Muturi, E.J., 2017. Characterization of *Tolypocladium cylindrosporum* (Hypocreales: Ophiocordycipitaceae) and its impact against *Aedes aegypti* and *Aedes albopictus* eggs at low temperature. Journal of the American Mosquito Control Association 33, 184-192.

Garnham, M.L., Pearson, J.F., Jackson, T.A., 1991. Bacterial control of the sheep blowfly. Proceedings of the 44th New Zealand Weed and Pest Control Conference 201-204.

Geden, C.J., 2012. Status of biopesticides for control of house flies. Journal of Biopesticides 5 (Supplementary) 1-11.

Geden, C.J., Rutz, D.A., Steinkraus, D.C., 1995. Virulence of different isolates and formulations of *Beauveria bassiana* for house flies and the parasitoid *Muscidifurax raptor*. Biological Control 5, 615-621.

Gillespie, J.P., Bailey, A.M., Cobb, B., Vilcinskas, A., 2000. Fungi as elicitors of insect immune responses. Archives of Insect Biochemistry and Physiology 44, 49-68.

Glare, T.R., Milner, R.J., 1987. New records of Entomophthoran fungi in from insects in Australia. Australian Journal of Botany 35, 69-77.

Goettel, M.S., 1988. Pathogenesis of the Hyphomycete *Tolypocladium cylindrosporum* in the mosquito *Aedes aegypti*. Journal of Invertebrate Pathology 51, 259-274.

Gołębiowski, M., Boguś, M.I., Paszkiewicz, M., Stepnowski, P., 2011. Cuticular lipids of insects as a potential biofungicides: methods of lipids composition analysis. Analytical and Bioanalytical Chemistry 399, 3177-3191.

Gough, J.M., Akhurst, R.J., Eller, D.J., Kemp, D.H., Wijffels, G.L., 2002. New isolates of *Bacillus thuringiensis* for control of livestock ectoparasites. Biological Control 23, 179-189.

Gough, J.M., Kemp, D.H., Akhurst, R.J., Pearson, R.D., Konsuwan, K., 2005. Identification and characterization of proteins with high toxic activity against the sheep blowfly, *Lucilia cuprina.* Journal of Invertebrate Pathology 90, 39-46.

Guerrero, F.D., Andreotti, R., Bendele, K.G., Cunha, R.C., Miller, R.J., Yeater, K., de León, A.A. P., 2014. *Rhipicephalus* (Boophilus) *microplus* aquaporin as an effective vaccine antigen to protect against cattle tick infestations. Parasites & Vectors 7, 475.

Guo, S., Herzig, V., King, G.F., 2018. Dipteran toxicity assays for determining the oral insecticidal activity of venoms and toxins. Toxicon 150, 297-303.

Heath, A.C.G., Levot, G.W., 2015. Parasiticide resistance in flies, lice and ticks in New Zealand and Australia: mechanisms, prevalence and prevention. New Zealand Veterinary Journal 63, 199-210.

Heath, A.C.G., Broadwell, A.H., Chillcott, C.N., Wigley, P.J., Shoemaker, C.B., 2004. Efficacy of native and recombinant Cry1B protein against experimentally induced and naturally acquired ovine myiasis (flystrike) in sheep. Journal of Economic Entomology 97, 1797-1804.

Herrero, N., Perez-Sanchez, R., Oleaga, A., Zabalgogeazcoa, I., 2011. Tick pathogenicity, thermal tolerance and virus infection in *Tolypocladium cylindrosporum*. Annals of Applied Biology 159, 192-201.

Hodgman, T.C., Ziniu, Y., Ming, S., Sawyer, T., Nicholls, C.M., Ellar, D.J., 1993. Characterization of a *Bacillus thuringiensis* strain which is toxic to the housefly *Musca domestica*. FEMS Microbiology Letters 114, 17-22.

Hussein, H.E., Scoles, G.A., Ueti, M.W., Suarez, C.E., Adham, F.K., Guerrero, F.D., Bastos, R.G., 2015. Targeted silencing of the Aquaporin 2 gene of *Rhipicephalus* (Boophilus) *microplus* reduces tick fitness. Parasites & Vectors 8, 618.

Ishii, M., Kanuka, H., Badolo, A., Sagnon, N., Guelbeogo, W. M., Koike, M., Aiuchi, D., 2017. Proboscis infection route of *Beauveria bassiana* triggers early death of Anopheles mosquito. Scientific reports *7* 3476. doi:10.1038/s41598-017-03720-x.

Jackson, T.A., Pearson, J.F., Young, S.D., Armstrong, J., O'Callaghan, M., 2002. Abundance and distribution of microbial populations in sheep fleece. New Zealand Journal of Agricultural Research 45, 49-55.

Jaronski, S.T., 2010. Ecological factors in the inundative use of fungal entomopathogens. BioControl 55, 159-185.

Junqueira, A.C.M., Ratan, A., Acerbi, E., Drautz-Moses, D.I., Premkrishnan, B.N.V., Costea, P.I., Linz, B., Purbojati, R.W., Paulo, D.F., Gaultier, N.E., Subramanian, P., Hasan, N.A., Colwell, R.R., Bork, P., Azeredo-Espin, A.M.L., Bryant, D.A., Schuster, S.C., 2017. The microbiomes of blowflies and houseflies as bacterial transmission reservoirs. Scientific Reports 7, 16324.

Kanga, L.H., Jones, W.A., Gracia, C., 2006. Efficacy of strips coated with *Metarhizium anisopliae* for control of Varroa destructor (Acari: Varroidae) in honey bee colonies in Texas and Florida. Experimental and Applied Acarology 40, 249-258.

Kaufman, P.E., Reasor, C., Rutz, D.A., Ketzis, J.K., Arends, J.J., 2005. Evaluation of *Beauveria bassiana* applications against adult house fly, *Musca domestica*, in commercial caged-layer poultry facilities in New York state. Biological Control 33, 360-367.

Kaur, G., Padmaja, V., 2009. Relationships among activities of extracellular enzyme production and virulence against *Helicoverpa armigera* in *Beauveria bassiana*. Journal of Basic Microbiology 49, 264-274.

Kebede, B., Wrigley, S.K., Prashar, A., Rahlff, J., Wolf, M., Reinshagen, J., Gribbon, P., Imhoff, J.F., Silber, J., Labes, A., Ellinger, B., 2017. Establishing the secondary metabolite profile of the marine fungus: *Tolypocladium geodes* sp. MF458 and subsequent optimisation of bioactive secondary metabolite production. Marine Drugs 15. pii: E84. doi: 10.3390/md15040084.

Kerwin, J.L., 1984. Fatty acid regulation of the germination of *Erynia variabilis* conidia on adults and puparia of the lesser housefly, *Fannia canicularis*. Canadian Journal of Microbiology 30, 158–161.

Khachatourians, G.G., 1986. Production and use of biological pest control agents. Trends in Biotechnology 4, 120-124.

Koch, S., Epp, A., Lohmann, M., Böl, G-F., 2017. Pesticide residues in food: attitudes, beliefs, and misconceptions among conventional and organic consumers. Journal of Food Protection 80, 2083-2089.

Köhler, H-R., Triebskorn, R., 2013. Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond? Science 341,759-765.

Kongsuwan, K., Gough, J., Kemp, D., McDevitt, A., Akhurst, R., 2005. Characterisation of a new *Bacillus thruringiensis* endotoxin, CRY47Aa, from strains that are toxic to the Australian sheep blowfly, *Lucilia cuprina*. FEMS Microbiology Letters 252, 127-136.

Krasnoff, S.B., Gupta, S., 1991. Identification and directed biosynthesis of efrapeptins in the fungus Tolypocladium geodes gams (Deuteromycotina: Hyphomycetes. Journal of Chemical Ecology 17, 1953-1962.

Lee, S.J., Lee, M.R., Kim, S., Kim, J.C., Park, S.E., Li, D., Shin, T.Y., Nai, Y-S., Kim, J.S., 2018. Genomic Analysis of the insect-killing fungus *Beauveria bassiana* JEF-007 as a biopesticide. Scientific Reports 8, 12388.

Leemon, D.M., Jonsson, N.N., 2012. Comparison of bioassay responses to the potential fungal biopesticide *Metarhizium anisopliae* in *Rhipicephalus* (*Boophilus*) *microplus* and *Lucilia cuprina*. Veterinary Parasitology 185, 236-247.

Li, J., Ying, S.H., Shan, L.T., Feng, M.G., 2010. A new non-hydrophobic cell wall protein (CWP10) of *Metarhizium anisopliae* enhances conidial hydrophobicity when expressed in *Beauveria bassiana*. Applied Microbiology and Biotechnology 85, 975-984.

Liu, J., Sun, H.H, Ying, S.H., Feng, M.G., 2017. The Hog1-like MAPK Mpk3 collaborates with Hog1 in response to heat shock and functions in sustaining the biological control potential of a fungal insect pathogen. Applied Microbiolial Biotechnology 101, 6941-6949.

Lyness, E.W., Pinnock, D.E., Cooper, D.J., 1994. Microbial ecology of sheep fleece. Agriculture, Ecosystems and Environment 49, 103-112.

Lysyk, T.J., Kalischuk-Tymensen, L.D., Rochon, K., Selinger, L.B., 2010. Activity of *Bacillus thuringiensis* isolates against immature horn fly and stable fly (Diptera: Muscidae). Journal of Economic Entomology 103, 1019-1029.

Machtinger, E.T., Weeks, E.N.I., Geden, C.J., 2016. Oviposition deterrence and immature survival of filth flies (Diptera: Muscidae) when exposed to commercial fungal products. Journal of Insect Science 54, 1-6.

Mazet, I., Vey, A., 1995. Hirsutellin A, a toxic protein produced in vitro by *Hirsutella thompsonii*. Microbiology 141, 1343-1348.

Morris, M.C., Morrison, L., Joyce, M.A., Rabel, B., 1998. Trapping sheep blowflies with lures based on bacterial cultures. Australian Journal of Experimental Agriculture 38, 125-130.

Merdan, A.B., 2012. *Bacillus thuringiensis* as a feed additive to control *Musca domestica* associated with poultry houses. The Journal of Basic and Applied Zoology 65, 83-87.

Mohanty, S.S., Raghavendra, K., Dash, A.P., 2008. Induction of chymoelastase (Pr1) of *Metarhizium anisopliae* and its role in causing mortality to mosquito larvae. World Journal of Microbiology and Biotechnology 24, 2283-2288

Morris, M.C., Morrison, L., Joyce, M.A., Rabel, B., 1998. Trapping sheep blowflies with lures based on bacterial cultures. Australian Journal of Experimental Agriculture 38, 125-130.

Mwamburi, L.A., Laing, M.D., Miller, R., 2009. Interaction between *Beauveria bassiana* and *Bacillus thuringensis* var. *israelensis* for the control of house fly larvae and adults in poultry houses. Poultry Science 88, 2307-2314.

O'Callaghan, M., Garnham, M.L., Nelson, T.L., Baird, D., Jackson, T.A., 1996 The pathogenicity of *Serratia* strains to *Lucilia sericata* (Diptera: Calliphoridae). Journal of Invertebrate Pathology 68, 22-27.

Omoto, C., McCoy, C.W., 1998. Toxicity of purified fungal toxin hirsutellin A to the citrus rust mite phyllocoptruta oleivora (Ash.). Journal of Invertebrate Pathology 72, 319-322.

Ortiz-Urquiza, A., Keyhani, N.O., 2013. Action on the surface: entomopathogenic fungi versus the insect cuticle. Insects 4, 357-374.

Opota, O., Vallet-Gély, Vincentelli, R., Kellenberger, C., Iacovache, L., Gonzalez, M.R., Roussel, A., van der Goot, F-G., Lemaitre, B., 2011. Monalysin, a Novel ß-Pore-Forming Toxin from the *Drosophila* Pathogen *Pseudomonas entomophila*, Contributes to Host Intestinal Damage and Lethality PLoS Pathogens 7(9): e1002259.

Pedras, M.S., Irina Zaharia L, Ward, D.E., 2002. The destruxins: synthesis, biosynthesis, biotransformation, and biological activity. Phytochemistry 59, 579-596.

Pedrini, N., Crespo, M.R. Juáre, P., 2007. Biochemistry of insect epicuticle degradation by entomopathogenic fungi. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 146, 124-137.

Pereira, L.D.A., Junqueira, R.M., Carramaschi, I.N., Queiroz, M.M.C., 2018. Bioactivity under laboratory conditions of *Brevibacillus laterosporus* towards larvae and adults of *Chrysomya putoria* (Diptera: Calliphoridae). Journal of Invertebrate Pathology 158, 52-54.

Pessanha, R.R., Carramaschi, I.N., dos Santos Mallet, J.R., Queiroz, M.M.C., Zahner, V., 2015. Evaluation of larvicidal activity and effects on post embrionary development of laboratory reared *Lucilia cuprina* (Wiedemann, 1830) (Diptera:Calliphoridae), treated with *Brevibacillus laterosporus*. Journal of Invertebrate Pathology 128, 44-46.

Phillips, C.J.C., 2009. A review of mulesing and other methods to control flystrike (cutaneous myiasis) in sheep. Animal Welfare 18, 113-121.

Quandt, C.A., Bushley, K.E., Spatafora, J.W., 2015. The genome of the truffle-parasite *Tolypocladium ophioglossoides* and the evolution of antifungal peptaibiotics. BMC Genomics DOI:10.1186/s12864-015-1777-9

Ravallec, M., Vey, A., Riba, G., 1989. Infection of *Aedes albopictus* by *Tolypocladium cylindrosporum*. Journal of Invertebrate Pathology 53, 7-11.

Robinson, P.W., Scott, R.R., 1995. The toxicity of cyromazine to *Chironomus zealandicus* (Chironomidae) and *Deleatidium* sp. (Leptophlebiidae). Pesticide Science 44, 283-292.

Ruiu, L., 2015. Insect pathogenic bacteria in integrated pest management. Insects 6, 352-367.

Ruiu, L., Delria, G., Ellar, D.J., Floris, I., Paglietti, B., Rubino, S., Satta, A., 2006. Lethal and sublethal effects of *Brevibacillus laterosporus* on the housefly (*Musca domestica*). Entomologia Experimentalis et Applicata 118, 137-144.

Sandeman, R.M., Levot, G.W., Heath, A.C.G., James, P.J., Greef, J.C., Scott, M.J., Batterham, P., Bowles, V.M., 2014. Control of the sheep blowfly in Australia and New Zealand – are we there yet? International Journal for Parasitology 44, 879-891.

Savage G., 1998. The residue implications of sheep ectoparasiticides. A report for the Woolmark Company, The National Registration Authority for Agricultural and Veterinary Chemicals, Canberra Australia, November 1998.

Sayed, A.M.M., Behle, R.W., 2017. Evaluating a dual microbial agent biopesticide with *Bacillus thuringiensis* var. *kurstaki* and *Beauveria bassiana* blastospores. Biocontrol Science and Technology 27, 461-474.

Sbaraini. N., Guedes, R.L.M., Andreis, F.C., Junges, A., de Morais, G.L., Vainstein, M.H., de Vasconcelos, A.T.R., Schrank, A., 2016. Secondary metabolite gene clusters in the entomopathogen fungus *Metarhizium anisopliae*: genome identification and patterns of expression in a cuticle infection model. BMC Genomics 17 (Suppl 8), 736 https://doi.org/10.1186/s12864-016-3067-6.

Schrank, A., Vainstein, M.H., 2010. *Metarhizium anisopliae* enzymes and toxins. Toxicon 56, 1267-1274.

Seleena, P., Lee, H.L., Chiang, Y.F., 1999. Compatibility of *Bacillus thuringiensis* serovar *israelensis* and chemical insecticides for the control of *Aedes* mosquitoes. Journal of Vector Ecology 24, 216-223.

Senthil-Nathan, S., 2015. A review of biopesticides and their mode of action against insect pests. Pp. 49-63 In. Thangavel, P., Sridevi, G., (Eds) *Environmental Sustainability* Springer India.

Sneddon, J., Rollin, B., 2010. Mulesing and animal ethics. Journal of Agricultural and Environmental ethics 23, 371-386.

Soarés, G.G., 1982. Pathogenesis of infection by the hyphomycetous fungus, *Tolypocladium cylindrosporum* in *Aedes sierrensis* and *Culex tarsalis* [Dip.: Culicidae] Entomophaga 27, 283-299.

St. Leger R.J., 1995. The role of cuticle-degrading proteases in fungal pathogenesis of insects. Canadian Journal of Botany, 73(S1), 1119-1125.

St Leger, R.J., Charnley, A.K., Cooper, R.M., 1986. Cuticle-degrading enzymes of entomopathogenic fungi: synthesis in culture on cuticle. Journal of Invertebrate Pathology 48, 85-95.

Wall, R., Green, C.H., French, N., Morgan, K.L., 1992. Development of an attractive target for the sheep blowfly *Lucilia sericata*. Medical and Veterinary Entomology 6, 67-74.

Wall, R., 2012. Ovine cutaneous myiasis: Effects on production and control. Veterinary Parasitology 189, 44-51.

Wall, R., Smith, K.E., 1997. The potential for control of the blowfly *Lucilia sericata* using odour-baited targets. Medical and Veterinary Entomology 11, 335-341.

Wang, C., St Leger, R.J., 2007. The MAD1 adhesin of *Metarhizium anisopliae* links adhesion with blastospore production and virulence to insects, and the MAD2 adhesin enables attachment to plants. Eukaryotic Cell 6, 808-816.

Wang, B., Kang, Q., Lu, Y., Bai, L., Wang, C., 2012. Unveiling the biosynthetic puzzle of destruxins in *Metarhizium* species. Proceedings of the National Academy of Science U.S.A. 109, 1287-1292.

Wang, C., Wang, S., 2017. Insect pathogenic fungi: genomics, molecular interactions, and genetic improvements. Annual Review of Entomology 62, 73-90.

Wang, J.J., Bai, W.W., Zhou, W., Liu, J., Chen, J., Liu, X.Y., Xiang, T.T., Liu, R.H., Wang, W.H., Zhang, B.L., Wan, Y.J., 2017. Transcriptomic analysis of two *Beauveria bassiana* strains grown on cuticle extracts of the silkworm uncovers their different metabolic response at early infection stage. Journal of Invertebrate Pathology 145, 45-54.

Weeks, E.N.I., Machtinger, E.T., Gezan, S.A., Kaufman, P.E., Gedan, C.J., 2017. Effects of four commercial fungal formulations on mortality and sporulation in house flies (*Musca domestica*) and stable flies (*Stomoxys calcitrans*). Medical and Veterinary Entomology 31, 15-22.

Weiser, J., Maťha, V., 1988. Tolypin, a new insecticidal metabolite of fungi of the genus *Tolypocladium*. Journal of Invertebrate Pathology 51, 94-96.

Werren, J.H., Baldo, L., Clark, M.E., 2008. Wolbachia: master manipulators of invertebrate biology. Nature Reviews in Microbiology 6, 741-751.

Wraight, S.P., Ramos, M.E., 2005. Synergistic interaction between *Beauveria bassiana* and *Bacillus thuringiensis tenebrionis*-based biopesticides applied against field populations of Colorado potato beetle larvae. Journal of Invertebrate Pathology 90, 139-150.

Wright, C., Brooks, A., Wall, R., 2004. Toxicity of the entomopathogenic fungus *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) to adult females of the blowfly *Lucilia sericata* (Diptera: Calliphoridae). Pest Management Science 60, 639-644.

Wright, D.A., Cummings, N.J., Haack, N.A., Jackson, T.A., 2009. *Tolypocladium cylindrosporum*, a novel pathogen for sheep blowflies. New Zealand Journal of Agricultural Research 52, 315-321.

Zhang, Y., Zhao, J., Fang, W., Zhang, J., Luo, Z., Zhang, M., Fan, Y., Pei, Y., 2009. Mitogen-activated protein kinase hog1 in the entomopathogenic fungus *Beauveria bassiana* regulates environmental stress responses and virulence to insects. Applied and Environmental Microbiology 75, 3787-795.

Zhang, L., Yan, C., Guo, Q., Zhang, J., Ruiz-Menjivar, J., 2018. The impact of agricultural chemical inputs on environment: global evidence from informetrics analysis and visualization. International Journal of Low-Carbon Technologies 13, 338-352.