



Status of biopesticides for control of house flies

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ABSTRACT

House flies (*Musca domestica* L.) have resisted human attempts to control them since antiquity, and the global problem of fly resistance to conventional insecticides has resulted in renewed interest in biopesticides as alternative management tools. Entomopathogenic nematodes such as *Steinernema* and *Heterorhabditis* spp. and their associated symbionts are virulent for fly larvae in certain substrates (e.g., cow manure mixed with soil), but the harsh environments presented by poultry and swine manure are inimical to their survival. *Entomophthora muscae* is an important natural regulator of fly populations, but constraints imposed by production, storage stability, and slow kill rate have limited its use as an operational control agent. *Beauveria bassiana* has many advantages and has been developed into commercial fly control products. *B. bassiana* is compatible with other biological agents and strains with superior kill rates have been identified. Field tests of this pathogen in poultry houses and calf hutches have been largely positive. New developments in genetic modification of *B. bassiana* could lead to new faster-acting biopesticide products that are competitive with conventional insecticides. Early research with exotoxin-producing strains of *Bacillus thuringiensis* was promising, but the shift in emphasis to endotoxin-only strains with high activity against Lepidoptera limited discovery of fly-active strains. Surveys have suggested that strains with high levels of the Cry1B endotoxin are more virulent than other strains for muscoid flies. Recent successes with *B. thuringiensis* var. *israelensis* on poultry farms suggest that *Bti* warrants further study. House fly salivary gland hypertrophy virus (MdSGHV) has the appealing property of shutting down reproductive development in adult flies but attempts to develop infective baits have been hampered by the refractoriness of older flies to oral infection. Space sprays to treat flies directly may have more potential for delivering MdSGHV into fly populations. Essential oils with substantial amounts of 1,8-cineole, pulegone, limonene, and menthol have high toxicity against fly adults. Combinations of house fly-active oils (e.g., rosemary, peppermint, pennyroyal mint, blue gum, bay laurel) could be more effective than products that focus on single active constituents. New formulations and possible use of synergists could increase the efficacy of botanicals for fly control.

Key words: Biopesticides, essential oils, house fly, microbial control, *Musca domestica*

INTRODUCTION

The house fly, *Musca domestica* L., is a worldwide pest of agricultural and public health importance that has plagued humans throughout recorded history (West, 1951). The ability of the fly to develop in a vast array of patchily distributed and ephemeral organic larval substrates has enabled it to exploit virtually any area inhabited by humans and their associated animals. Adult flies pose nuisance problems to farm workers and neighboring residents. More importantly, the habit of adult flies to defecate and regurgitate on animal and human food led to the early recognition of their role as vectors of human and animal pathogens, especially those responsible for enteric diseases (Howard, 1911). Recent concerns about food-borne human illnesses have led to increased documentation of the role of flies in spreading

disease-causing organisms, especially *Escherichia coli*, *Shigella* spp., and *Salmonella* spp. (Nayduch and Stutzenberger, 2001; Ahmad *et al.*, 2007; Macovei *et al.*, 2008; Holt *et al.*, 2007). Pathogen-carrying flies are commonly found around human and animal waste and landfills, from which they disperse to areas of human habitation and activity (Moriya *et al.*, 1999; Sulaiman *et al.*, 2000; Mian *et al.*, 2002).

House flies are notorious for their ability to develop behavioral and metabolic mechanisms to avoid and detoxify chemical insecticides. Resistance to DDT was noticed within a few years of its introduction (Varzandeh *et al.*, 1954; Perry, 1958). New chemistries were introduced over the years, and the flies responded by developing resistance to organophosphate, carbamate, and pyrethroid insecticides (Boxler and Campbell, 1983; Plapp, 1984; Scott and Georgi, 1986; Scott *et al.*,

1989; Kaufman *et al.*, 2001b; Butler *et al.*, 2007; Kozaki *et al.*, 2009; Memmi, 2010) as well as to growth regulators such as diflubenzuron and cyromazine (Bloomcamp *et al.*, 1987; Shen and Plapp, 1990). The use of continuous delivery systems such as feed-throughs and frequent use of residual premise treatments has exacerbated the problem, resulting in efficacy losses of once-effective materials such as cyromazine and permethrin, respectively (Sheppard *et al.*, 1990). Cross-resistance also poses challenges and can shorten the effective life of what were thought to be novel chemistries. For example, the avermectins were thought to have a novel mode of action, and initial tests against house fly were encouraging (Geden *et al.*, 1990; Geden *et al.*, 1992b), but flies with high levels of pyrethroid (e.g. Ectiban and related products) resistance showed high levels of cross-resistance to abamectin (Scott, 1989).

During the past 10 years, several promising new insecticides were introduced in bait form that, at least for a time, provided control of populations that were resistant to older chemistries. Spinosad (Elector) and neonicotinoids, such as imidacloprid (QuickBayt) and nithiazine (Quick Strike), were highly effective at the time of their introduction to the market. However, resistance to spinosad was documented almost immediately after market entry (Shono and Scott, 2003; Deacutis *et al.*, 2006). Imidacloprid has essentially become a victim of its own success. At the time of its introduction in 2004, the QuickBayt products containing imidacloprid provided such superior performance and such a rapid fly knockdown that it quickly dominated the fly control market. Sprayable formulations of this sugar bait product followed soon afterwards. Early warning signs of resistance appeared within 2 years of product rollout (Kaufman *et al.*, 2006) and have now reached levels where product failure is imminent (Kaufman *et al.*, 2010a,b; Memmi, 2010).

There are a number of alternatives to conventional chemical insecticides for fly control. Histerid beetles and macrochelid mites feed ravenously on fly eggs and young larvae and have been studied extensively (reviewed by Geden, 1990; Kaufman *et al.*, 2000, 2001a, 2002; Achiano and Giliomee, 2005). Larvae of some species of the fly genus *Hydrotaea* (*Ophyra*) are facultative predators of house fly larvae that have been used for fly control (Nolan and Kissam, 1987; Geden *et al.*, 1988; Turner and Carter, 1990; Turner *et al.*, 1992; Hogsette and Washington, 1995; Farkas *et al.*, 1998; Hogsette *et al.*, 2002). Pteromalid parasitoids that attack the fly in the pupal stage are the best known fly biocontrol agents and have been used extensively for operational fly control for decades (Morgan *et al.*, 1975; Rutz and Axtell, 1979; Morgan and Patterson, 1990; Geden *et al.*, 1992a; Skovgaard and Nachman, 2004; Geden and Hogsette, 2006). Biopesticides in the broad sense

including microbial agents and botanicals have received less attention and are the subject of this review.

Nematodes

Steinernematids and Heterorhabditids. Steinernematid and heterorhabditid nematodes have been studied extensively for control of filth flies, with mixed results. In laboratory studies using substrates that are favorable for nematode survival, fly larvae are highly susceptible to most of the entomogenous nematodes that have been tested (Renn *et al.*, 1985; Geden *et al.*, 1986; Mullens *et al.*, 1987a; Taylor *et al.*, 1998). However, results on more natural substrates have generally been disappointing. An early report suggested that nematodes were effective for controlling fly populations in British Columbia poultry houses (Belton *et al.*, 1987), but several other studies have demonstrated that the nematodes perform poorly in poultry and pig manure (Geden *et al.*, 1986; Georgis *et al.*, 1987; Mullens *et al.*, 1987a; Renn, 1995, 1998). Cow manure, especially when mixed with soil or bedding, may be a more suitable habitat for nematode use (Taylor *et al.*, 1998). Adult flies are less susceptible to parasitism than larvae on treated filter paper but can be infected by visiting bait stations with parasites (Renn *et al.*, 1985; Renn, 1998). In spite of these mixed results, nematodes are widely available from commercial sources that promote their effectiveness for control of fly larvae.

***Paraiotonchium muscadomesticae*.** The life cycle of *P. muscadomesticae* is similar to that of *P. (Heterotylenchus) autumnalis* in the face fly, *Musca autumnalis* (Coler and Nguyen, 1994; Geden, 1997). Young adult nematodes are deposited from the ovaries of infected female flies into fly breeding habitats, where they mate and females seek mature fly larvae. Mated female nematodes penetrate the larval cuticle and enter the haemocoel. As the fly develops into the adult stage the nematodes go through first a parthenogenetic and then a gametogenetic generation resulting in the production of ca. 30,000 nematodes per fly. The nematodes then move into the ovaries of the fly where they are deposited during “mock oviposition” events. Infected flies live about half as long as uninfected flies and do not produce any eggs. The parasite, which has only been found in Brazil, appears to be fairly specific for house flies. So far, two attempts to get *P. muscadomesticae* established outside its home range have been unsuccessful, but it may have potential as a biopesticide if appropriate production, formulation, and storage methods can be developed.

Fungi

***Entomophthora muscae* complex.** Adult house flies are susceptible to infection with the fungal pathogens

Entomophthora muscae and *E. schizophorae*, which typically kill the flies 4-6 days after exposure to conidia. Flies become infected when exposed to conidia discharged from cadavers of infected flies. The intensity and duration of conidial discharge and the survival of conidia depends on temperature and relative humidity (Mullens and Rodriguez, 1985; Krasnoff *et al.*, 1995; Six and Mullens, 1996; Madeira, 1998; Kalsbeek *et al.*, 2001a). Natural epizootics are common in the fall months in temperate regions, with infection rates commonly exceeding 50% (Mullens *et al.*, 1987b; Watson and Petersen, 1993; Steinkraus *et al.*, 1993; Six and Mullens, 1996). Although *E. muscae* is an important natural regulator of fly populations it remains unclear whether this pathogen can be manipulated as a biopesticide. Mass-rearing methods have been developed to produce large numbers of infected flies, and field releases of *E. muscae* and *E. schizophorae* have resulted in increased disease prevalence (Kramer and Steinkraus, 1987; Steinkraus *et al.*, 1993; Geden *et al.*, 1993; Six and Mullens, 1996). The impact of releases on fly control may be dampened by the need for high fly populations to sustain epizootics (Geden *et al.*, 1993) and by the ability of the flies to mitigate the effects of infection by resting in warm areas to raise their body temperature (behavioral fever) (Kalsbeek *et al.*, 2001b; Watson *et al.*, 1993).

***Beauveria* and *Metarhizium*.** Field populations of house flies and stable flies usually have low rates of infection with *B. bassiana* and *M. anisopliae* (Steinkraus *et al.*, 1990; Skovgaard and Steenberg, 2002). In laboratory bioassays, larval and adult flies are highly susceptible to these entomopathogens. Virulence varies widely depending on strain and formulation, and adult house flies are particularly susceptible to sugar baits with *B. bassiana* conidia (Kuramoto and Shimazu, 1992; Geden *et al.*, 1995; Watson *et al.*, 1995; Darwish and Zayed, 2002; Lecuona *et al.*, 2005). Laboratory and field data indicate that use of entomopathogenic fungi is compatible with other natural enemies including *C. pumilio* and the parasitoids *Spalangia cameroni* and *Muscidifurax raptor* (Geden *et al.*, 1995; Kaufman *et al.*, 2005; Nielsen *et al.*, 2005). Although much of the research in this area has concentrated on *B. bassiana*, some strains of *M. anisopliae* have been demonstrated to have superior performance against both adult and larval house flies (Mishra *et al.*, 2011).

Data on efficacy under field conditions are limited but encouraging. Watson *et al.* (1996) applied *B. bassiana* to the inside walls of calf hutches and observed up to 47% infection among house flies in the treated hutches. Kaufman *et al.* (2005) found that space sprays with *B. bassiana* in poultry houses provided fly control comparable to that observed in houses treated with pyrethrin. Three weekly aerosol conidial applications in Venezuelan poultry sheds provided 100%

control of adult flies, although fly populations recovered quickly once treatments were stopped (Cova *et al.*, 2009a,b). *B. bassiana* is commercially produced for fly control in the US under the trade name BalEnce (<http://www.terrege.com>).

One disadvantage of *B. bassiana* and *M. anisopliae* is the rather long time that is required to kill the host, typically 4-6 days. However, a recent comparison of 34 strains identified several with LT_{50} 's of less than 24 hours (Mwamburi *et al.*, 2011a). Such rapid kill rates would place *B. bassiana* biopesticides in a more competitive position relative to conventional chemical insecticides. Further increases in kill rates could be achieved by genetic modification of the pathogen to accelerate cuticular penetration. The potential for this approach was demonstrated by Fan *et al.* (2010), who fused a *Bombyx mori* chitinase to a protease in *B. bassiana*. The chimeric pathogen was substantially more virulent than the wild-type, presumably because of improved binding and delivery of proteases to the target cuticle. This is an exciting development that could lead to significant improvements in efficacy of biopesticides based on this agent.

Bacteria

Early work with *Bacillus thuringiensis* against filth flies was encouraging. For instance, considerable maggot control was achieved by feeding *Bt* spore formulations to cattle and chickens and thereby delivering the bacteria to fly breeding sites in manure (Burns *et al.*, 1961; Miller *et al.*, 1971). Promising results were also obtained by mixing *Bt* directly with fly breeding substrates (Rupes *et al.*, 1987). In these early studies, exotoxin-producing *Bt* strains were used, and flies were more susceptible than most other insect orders to the exotoxin (Carlberg, 1986). However, resistance to exotoxins developed quickly in house flies that were already resistant to chemical insecticides (Harvey and Howell, 1965; Wilson and Burns, 1968). Moreover, safety concerns over vertebrate toxicity led to a prohibition on the use of exotoxin-containing *Bt* products in the US in the mid 1980's (McClintock *et al.*, 1995; Tsai *et al.*, 2003).

When the focus shifted to *Bt* strains that do not produce beta-exotoxins the results with flies were often disappointing, possibly because the discovery process favored strains producing delta-endotoxins with high activity against Lepidoptera (Al-Azawi and Jabbar, 1989; Lonc *et al.*, 1991; Sims, 1997). Indrasith *et al.* (1992) identified several strains with good activity against adult house flies. Subsequently, Johnson *et al.* (1998) identified other strains with activity against house flies and determined that the endotoxin Cry1B was found in all the *Musca domestica*-active strains. The Cry1B endotoxin may be the key item in the activity of these strains for higher flies (Zhong *et al.*, 2000; Lysyk *et al.*, 2010).

The Cry1B producing YBT-226 strain, for instance, is a proprietary strain owned by Dupont with high fly activity (Zhong *et al.*, 2000). The *cry1B* gene is also present in the fly active strains HD2 and HD-290, of which there is a mutant (HD-290-1 a.k.a. HD-290-I) that produces only Cry1B (Brizzard *et al.*, 1991). These results suggest that a screen of *Bt* isolates with the Cry1B toxin could reveal strains with superior activity against house fly.

Lately there has been increased interest in the discovery and use of *Bt* strains for fly control. Promising new strains have been identified in Korea (Choi *et al.*, 2000; Oh *et al.*, 2004). Labib and Rady (2001) reported that *Bt* was more effective for fly control on Egyptian poultry farms when it was added to the birds' food than when it was applied directly to the manure. Similar results were noted on South African poultry farms using a locally obtained strain of *B. thuringiensis* subsp. *israelensis* (*Bti*) (Mwamburi *et al.*, 2009, 2011b). Earlier testing with *Bti* indicated that this subspecies had little effect on flies, either because of gut pH conditions or lack of receptors for the endotoxins in most strains (Vankova, 1981; Wilton and Klowden, 1985). The recent successes in South Africa suggest that *Bti* should be reexamined for use against house flies.

MdSGHV virus

Salivary gland hypertrophy virus of house flies (MdSGHV) is one of three members of the Hytrosaviridae, a recently described family that includes pathogens of adult house flies, tsetse flies (*Glossina* spp.), and the narcissus bulb fly (*Merodon equestris* Fabr.) (Abd-Alla *et al.*, 2009; Lietze *et al.*, 2011a). The virus infecting house flies is an enveloped, double stranded, circular DNA virus with a 124,279 bp genome (Garcia-Maruniak *et al.*, 2008, 2009). MdSGHV was first discovered infecting flies at a dairy farm in central Florida in the early 1990's (Coler *et al.*, 1993). Infected flies do not exhibit any external disease symptoms. The most conspicuous feature of infection is the presence of greatly enlarged (hypertrophied) salivary glands with a blue-whitish appearance that often dominate the abdominal cavity of the fly when dissected. Both sexes can be infected, with somewhat higher prevalence rates in males. Viral replication and morphogenesis is restricted to salivary gland cells, although complete virions are also found in asymptomatic tissues such as midgut, ovaries, fat body and brain (Lietze *et al.*, 2010). The ovaries of females that are infected as young flies do not develop, probably because infection blocks hexamerin and yolk protein gene transcription (Lietze *et al.*, 2007). Infected flies of both sexes have reduced mating success and shorter life spans than healthy flies (Lietze *et al.*, 2007). Stable fly (*Stomoxys calcitrans*), which occurs sympatrically with house fly,

supports viral replication in the laboratory but does not show the classic symptoms of salivary gland hypertrophy (Geden *et al.*, 2011b).

Because of its recent discovery, little is known about the ecology and epizootiology of MdSGHV in the field. Coler *et al.* (1993) found infection rates on a Florida dairy farm to be generally low. In a subsequent field survey, Geden *et al.* (2008) observed that infections were positively correlated with fly abundance, with highest infection rates from June through August. Survey results from the US and Denmark (Geden *et al.*, 2011a) indicate that prevalence is typically low, 0.5-5% but occasional spikes of over 30% infection have been observed. Although most of the research on the virus has been conducted in Florida, infected flies have been collected throughout the world (Prompiboon *et al.*, 2010).

The mechanisms of MdSGHV transmission are still not completely understood. There is no vertical transmission from mothers to progeny, no venereal transmission, and no evidence that flies acquire the infection as immatures (Lietze *et al.*, 2007; Geden *et al.*, 2008). Infected females deposit ca. one million virus particles each over a period of seconds when they feed on solid foods (Lietze *et al.*, 2009). Healthy flies can become infected when they are given food or water from cages of infected flies, and even when they are housed in cages from infected flies and given clean food and water supplies (Geden *et al.*, 2008). Viable virus particles pass through the alimentary tract of infected flies and are deposited with feces, albeit at low rates (Lietze *et al.*, 2009). These elements are all suggestive of oral acquisition by healthy flies when they co-feed with infected flies. However, flies are only susceptible to oral infection during a narrow window after adult emergence; the peritrophic matrix of older flies renders them largely refractory to oral transmission (Lietze *et al.*, 2009). When viremic flies are introduced into a population of healthy flies and monitored over time, the result is decreasing infection levels until a stasis level of ca. 10% is reached (Lietze *et al.*, 2011b). The opportunities for using MdSGHV as a biopesticide in a food bait appears to be limited. However, it has been discovered recently that flies are surprisingly susceptible to infection when they make direct contact with low-dose aqueous virus suspensions (Geden *et al.*, 2011a). Large numbers of infected flies can be produced easily with this method and virus suspensions can be applied as space sprays with conventional mist blowers and other equipment. Further research with new formulations to improve stability, shelf life and adherence to target flies could greatly improve prospects for use of MdSGHV as an operational biopesticide.

Botanicals

Essential oils are generally known to have fumigant insecticidal properties, and the mode of action may involve elements of acetylcholinesterase inhibition and octopaminergic effects (Isman, 2000). Additional effects can be seen in behavior modification (attraction/repellency) and contact toxicity for different life stages (Koul *et al.*, 2008). Natural oils are complexes of many biologically active constituents including terpenes, acyclic monoterpene alcohols, monocyclic alcohols, aliphatic aldehydes, aromatic phenols, monocyclic ketones, bicyclic monoterpene ketones, acids, and esters (Koul *et al.*, 2008). The composition of oils from a particular plant species can be affected by the plant tissues extracted, cultivar variation, climatic and growth conditions, and the methods used for extraction and analysis. For this reason, there have been considerable efforts to examine the effects of individual components that are common to those essential oils known to have insecticidal properties (Isman, 2000; Koul *et al.*, 2008).

Preparations of plant materials have long been used to kill or repel flies. Over 100 years ago, Howard (1911) described a method for making a fly adulticide from quassia (*Quassia amara*) wood that he had seen in “old dispensaries”. He also pointed out that “the butchers in Geneva have from time immemorial prevented flies from approaching... by the use of laurel oil”. Essential oils of bay laurel (*Laurus nobilis*) include large 1,8-cineol (eucalyptus) and linalool fractions (Palacios *et al.*, 2009b). With the advent of synthetic chemical insecticides there was little research on botanical extracts until resistance problems in house flies became acute in the 1970's.

Sharma and Saxena (1974) evaluated the effects of a range of individual terpenoids on house flies and found a wide variety of effects. Some acted as attractants but had inhibitory effects on embryonic or larval development (eugenol and fernesol) whereas others repelled gravid females and inhibited embryonic/larval development. Fly responses to terpenoids were highly dose-dependent, and some were attractive at low concentrations but repellent at high ones. Larvicidal effects of the tested materials were modest at all doses. In another study, neem extracts and refined azadirachtin were moderately toxic to larvae of the horn fly (*Haematobia irritans*), but doses required to control house fly larvae were deemed too high to be practical at the time (Miller and Chamberlain, 1989). Khan and Ahmed (2000) later observed up to 85% mortality of adult house flies after exposure to neem extract, which suggests that this product warrants further study. Ezeonu *et al.* (2001) found that extracts of sweet orange peels (*Citrus sinensis*) were effective as fumigants against adult flies. Malik *et al.*

(2007) provided an excellent review of the status of botanicals against house fly at that time.

In the past few years there has been renewed interest in the topic of essential oils for fly control. Palacios *et al.* (2009a,b) examined the efficacy of essential oils of 21 medicinal and edible plants against house fly. Of the edible plants, essential oils from orange peel and eucalyptus leaves were the most toxic to flies; the principal components of these oils were limonene (92.5%) and 1,8-cineole (56.9%), respectively. Of the medicinal plants, the most toxic to house flies were those whose essential oils were high in pulegone, menthone, limonene, and 1,8-cineole. In a survey of 34 plants conducted by Pavela (2008), essential oils of rosemary (*Rosemarinus officinalis*) and pennyroyal mint (*Mentha pulegium*) had high activity against adult flies in both fumigant and contact toxicity assays. Pennyroyal mint was the most effective overall, and GC/MS analysis of the extract indicated that pulegone made up 83.3% of the extract. Pulegone is also highly toxic to larvae of *Aedes aegypti* L. (Waliwitiya *et al.*, 2008). Oil of rosemary is high in pinene and 1,8-cineole (Jamshidi *et al.*, 2009). Essential oils of peppermint (*Mentha piperita*) and blue gum (*Eucalyptus globulus*) were the most effective of 6 plant extracts examined by Kumar *et al.* (2011) and had both insecticidal and repellent properties. Application of an emulsifiable concentrate formulation of peppermint oil in field tests resulted in over 95% control of house flies (Kumar *et al.*, 2011). The principal components of peppermint oil are menthone (20.9%) and menthol (41.5%) (Palacios *et al.*, 2009b). As part of an assessment of plants native to Chile, Urzua *et al.* (2010) recently found that essential oils from *Haplopappus foliosus* (Asteraceae) had high activity against adult house flies; limonene was the most abundant component in the extract.

Taken together, these results do not point to any single component of essential oils that stand out as the critical element that accounts for activity against house flies. Complex interactions may occur among major and minor constituents in an unforeseen manner that affect insecticidal activity. Similarly, mixtures of essential oils from different plants may have higher activity than individual extracts in ways that are difficult to predict. A new product on the US market, EcoExempt IC, is a combination of essential oils of rosemary and peppermint. Unpublished results in our laboratory indicate that this combination is effective as a space spray and a residual surface treatment for house fly adults. Judicious use of synergists could improve efficacy further. Addition of piperonyl butoxide can reduce the LC₅₀ of essential oils and their individual constituents by several orders of magnitude (Waliwitiya *et al.*, 2008). Further research on blends of essential oils and improved formulations and delivery systems

could lead to substantial improvements in the performance of botanicals for house fly control.

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