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Botanically Based Repellent and Insecticidal Effects Against Horn Flies and Stable Flies (Diptera: Muscidae)

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Abstract

Horn flies, *Haematobia irritans irritans* (L.), and stable flies, *Stomoxys calcitrans* (L.) (Diptera: Muscidae), are economically important blood-feeding ectoparasites of wild and domesticated animals, including cattle, *Bos taurus* L. Conventional insecticides are used for control of biting flies on cattle, but safety concerns and the buildup of insecticide resistance indicate the need for alternative control tactics. Many botanical extracts and oils are composed of more than one bioactive compound that can exert different modes of action, delaying or averting resistance. Plant genera that have shown repellency and toxicity against horn flies and stable flies include *Allium*, *Azadirachta*, *Chrysanthemum*, *Cinnamomum*, *Cymbopogon*, *Derris*, *Eucalyptus*, *Festuca*, *Melaleuca*, *Melinus*, *Mentha*, *Nepeta*, *Nicotiana*, *Pelargonium*, *Pogostemon*, *Ricinus*, *Rosa*, *Syzygium*, *Vitex*, and *Zyloxanthum*. Other botanically based methods for biting fly control have been investigated, such as the use of fatty acids, soybean trypsin inhibitors, and fungal endophytes on forage grasses. Many of the plant-based control methods have been shown to have strong effects against the two biting fly species, but work has only just begun on identifying and, in particular, developing botanically based tactics.

Key words: botanical, essential oil, extract, *Haematobia irritans*, *Stomoxys calcitrans*

The Biting Flies

Biting dipterans of the family Muscidae are chiefly known as irritants and occasionally as vectors of diseases of cattle, *Bos taurus* L., and other livestock animals. Two of the most widespread and arguably economically important of such ectoparasites are the horn fly, *Haematobia irritans irritans* (L.), and the stable fly, *Stomoxys calcitrans* (L.) (James and Harwood 1969, Showler et al. 2014, Showler and Osbrink 2015).

Horn Fly

Haematobia irritans is established throughout much of the Northern Hemisphere, including Europe, North Africa, and Asia Minor (Showler et al. 2014). In the Western Hemisphere, its range extends from southern Canada to temperate parts of Argentina and Uruguay (Depner 1961, Carballo and Martínez 1991, Luzuriaga et al. 1991, Foil and Hogsette 1994). An obligate blood-feeder (Cupp et al. 1998), the pest is hosted almost exclusively by cattle, although it can also parasitize horses, *Equus caballus* L.; water buffalo, *Bubalus bubalis* (L.); sheep, *Ovis aries* L.; goats, and some nondomesticated mammals (Foil and Hogsette 1994, Quiroz 2005, Showler et al. 2014).

Adult horn flies are ~4 mm long, brown-gray with a greenish-yellow sheen, and four dorsal longitudinal thoracic stripes; the outermost two stripes are broken, and the abdomen is blotchy or checkered (James and Harwood 1969). The wings have an iridescent sheen and the mouthparts extend from the anterior of the head, allowing the insect to pierce through the skin. The fly rarely leaves its host, mostly settling on the back, sides, neck, and sometimes the head (Foil and Hogsette 1994, Hawkins 2001). Each fly typically feeds 20–38 times each day (Foil and Hogsette 1994, Cupp et al. 1998, Hawkins 2001), and a bloodmeal is necessary for the initiation of mating and egg production (Foil and Hogsette 1994). Adults start mating 3–5 d after emerging from the pupal stage, and oviposition usually begins in 3–8 d (Lysyk 1992, Foil and Hogsette 1994). Eggs are mostly deposited in fresh dung, and the female returns to the host within 1 min (Foil and Hogsette 1994). A single female can produce up to 400 eggs deposited in clusters of 20–25 eggs and the larvae reside within the dung (Hawkins 2001). Development from egg to pupa requires 4–8 d depending upon temperature (Lysyk and Schaalje 1992), and as many as 14 generations are completed per year in warm regions (Hawkins 2001, Showler et al. 2014).

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The horn fly is problematic because its repeated daily feeding habit stresses livestock, often resulting in reduced body weight gain in beef cattle (Kinzer et al. 1984, Williams et al. 1985). In areas where the pest is particularly abundant, individual cows can be infested by as many as 1,000–4,000 flies (James and Harwood 1969). Under heavy horn fly pressure, cows lose nearly 0.25 kg daily (James and Harwood 1969) and milk production by dairy cows can decline by up to 20% (Williams et al. 1985, Metcalf and Metcalf 1993). In North America, horn fly infestations are responsible for economic losses of ~US$1 billion annually, including costs for insecticides and their application (Foil and Hogsette 1994, Cupp et al. 1998, Hawkins 2001, DeRouen et al. 2010). Although horn flies are not considered to be important disease vectors, they are the intermediate host for *Stephanofilaria stilesi* Chitwood, a filarial nematode that causes skin lesions on cattle (Foil and Hogsette 1994).

Stable Fly

Stomoxys calcitrans is widely distributed in the Old World and the Western Hemisphere (Foil and Hogsette 1994, Showler et al. 2014). This pest is 4–7 mm long, generally gray with a greenish-yellow sheen and four black stripes on the thorax, the outer two of which are broken, and black blotches or checkering on the abdomen (Todd 1964, James and Harwood 1969, Foil and Hogsette 1994). The clear wings are iridescent, and the proboscis is slender, projecting in front of the head to facilitate puncturing skin (Todd 1964, James and Harwood 1969).

Eggs are mostly deposited in clusters, each composed of 20–100 eggs, inside ovipositional substrates which contain decaying vegetation, excluding dung unless it is mixed with, or dropped on, vegetation (Foil and Hogsette 1994). Substrate must be moist and fermentation is favorable to larval development (Todd 1964). Common substrates include decaying hay, alfalfa, silage, sugarcane, beached seaweed, lawn cuttings, compost, piles of waste vegetables (Simmons 1944, Todd 1964, Skoda and Thomas 1993, Foil and Hogsette 1994, Broce et al. 2005, Gilles et al. 2008), and cattle manure on mid-western United States feedlots where summer heat results in a dry surface crust, sealing moisture within and creating an insulated habitat (Skoda and Thomas 1993, Foil and Hogsette 1994). Females lay eggs 4–5 times (up to 20 times has been reported), producing 60–800 eggs per female during a lifetime (James and Harwood 1969, Foil and Hogsette 1994). Larvae burrow into the substrate to feed and third instars enter the drier parts of their habitat and pupate there (James and Harwood 1969, Foil and Hogsette 1994). Most pupae produce an adult in 5–26 d (21–26° C; James and Harwood 1969). Mating begins in 3–5 d and females start laying eggs in 5–8 d (Foil and Hogsette 1994). Adult longevity is <2 wk under field conditions (Killough and McKinstry 1965).

Stable flies attack a wider range of animals than horn flies, including dogs, *Canis lupus familiaris* L.; rats, *Rattus rattus* (L.); guinea pigs, *Cavia porcellus* (L.); rabbits, *Oryctolagus cuniculus* (L.); monkeys; cattle; horses; humans; camels, *Camelus dromedarius* L.; goats, *Capra aegagrus hircus* L.; and even pelicans, *Pelecanus* sp. (Ngeranwa and Kilalo 1994, Hale 2011, Showler et al. 2014). On biting, the pest engorges in 3–4 min, which is sometimes interrupted when the fly changes position and then resumes feeding, often flying from one animal in a herd to another (James and Harwood 1969, Foil and Hogsette 1994). Stable flies feed mostly on the host's lower front legs where shorter hairs provide relatively little protection and where the pest is unlikely to be dislodged by motions of the host's tail (Foil and Hogsette 1994). Biting leads to some blood loss and inflicts pain and, combined with associated stress, this leads to reduced weight gain and lactation (Bishopp 1939; Freeborn et al. 1925; Campbell et al. 1987, 2001). Economic costs from stable flies and control measures are ~US$100 million annually in the United States alone (Campbell 1993), although estimates exceed US$1 billion (Taylor and Berkebile 2006). Numbers of stable flies per cow generally range from 25 to 50, but >2 flies per leg is sufficient to cause economic loss on feeder heifers (Steelman 1976; Campbell et al. 1977, 1987).

The stable fly's habit of interrupting its feeding and moving between host animals contributes toward its efficiency as a vector of pathogens (James and Harwood 1969), which include several species of *Trypanosoma* (e.g., *Trypanosoma evansi*, the cause of surra in horses and camels; Ngeranwa and Kilalo 1994, Coetzer and Tustin 2004). Stable flies might also mechanically transmit equine infectious anemia virus. West Nile virus was found to be ingested by stable flies feeding on infected American white pelicans, *Pelecanus erythrorhynchos* Gmelin (Johnson et al. 2010, Hale 2011), and other disease conditions have been associated with this pest (James and Harwood 1969, Baldacchino et al. 2013b).

Phytochemicals for Pest Management

Conventional insecticides are inherently toxic and can endanger the health of humans and livestock, and they are sometimes undesirable for being highly persistent in the environment, including plants, and animals. Horn fly and stable fly control is primarily conducted using conventional synthetic insecticides, but resistance to organophosphates and pyrethroids has been observed (Cilek and Greene 1994, Kunz et al. 1995, Marçon et al. 1997, Oyarzún et al. 2008). In contrast, plant-derived products, in general, are considered to be minimum-risk pesticides and are often exempt from Environmental Protection Agency registration under section 25(b) of the Federal Insecticide Fungicide and Rodenticide Act (Cloyd et al. 2009). Although the uses of rotenone and nicotine are declining, pyrethrum and neem have become commercially established and other botanical pesticides are being discovered and entering the marketplace for a variety of agricultural applications. Botanically based compounds that are active against agricultural pests can be repellent and toxic against pests (Sharma et al. 1993, Coats 1994, Isman 2006, Krajick 2006, Khater 2012). Plants produce chemical compounds that defend against different pests (Hedin and Hollingworth 1997, Isman and Akhtar 2007, Mann and Kaufman 2012), but these compounds are often easily decomposed by a variety of microbes common in most soils and, as a result, the potential for environmental contamination is reduced (Khater 2012). Broad-spectrum action of many bioactive plant-derived compounds against a wide array of soft-bodied arthropod pests (Isman 1999, Alexenizer and Dorn 2007) occurs chiefly because extracts and essential oils have multiple modes of action, including antifeedant and repellent activity, growth and fecundity reduction, cuticle disruption, and effects on the octopamine pathway in the central nervous system (Saxena 1989, Isman 2000, Enan 2001, Kostyukovsky et al. 2002, Akhtar and Isman 2004, Khater 2011). An advantage of multiple modes of action is the prevention of or a delay in development of resistance by pest populations (Feng and Isman 1995). Also, plant-derived bioactive compounds typically exhibit short residual activity, which reduces field reentry intervals (<12 h) because of detoxification by temperature and UV light (Van Lenteren and Woets 1988, Miresmailli and Isman 2006), but short residual activity can necessitate frequent repeat applications (Showler et al. 2004).

Nearly all of the plant species listed in this review as having repellent or toxic bioactive compounds against horn flies and stable

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flies also express bioactivity against other arthropods and diseases. Many are already in use as traditional arthropod pesticides and repellents, and as traditional medications.

Extracts

Plant extracts are obtained using a variety of solvents and techniques for extracting different, but not all, compounds produced by the plant (Tiwari et al. 2011). Botanical extracts that exhibit bioactivity against pests can be grouped into five major chemical categories: nitrogen compounds (e.g., alkaloids), terpenoids, phenolics, proteinase inhibitors, and growth regulators. Plants with bioactive properties against horn flies and stable flies are grouped as extracts and essential oils (plant genera are listed alphabetically), and other bioactive aspects of plants are described thereafter.

Allium

Garlic, *Allium sativum* L. (Amaryllidaceae), is a widely grown crop that has been reported to have insecticidal and repellent properties against some pests (Showler et al. 2010, 2011). Cattle were fed garlic at 100 g/cow for 3 d, but no repellent or toxic effects against horn flies and stable flies was observed (Massariol et al. 2011).

Azadiracta

Neem is obtained from the neem tree, *Azadirachta indica* A. Juss (Meliaceae), native to the Indian subcontinent but widely grown in tropical and subtropical regions of Africa, the Western Hemisphere, and Australia (Khater 2012). Neem has been applied against pests as extracts, oil, cakes, and leaves (Schmutterer 1988, 1990; Showler et al. 2004). It was first identified as having pest management potential when swarming desert locusts, *Schistocerca gregaria* (Forskål), that had defoliated almost all local flora in Africa did not consume neem leaves (National Research Council 1992). Although neem produces a variety of compounds, extract efficacy is mainly attributed to azadirachtin, a nortriterpenoid (a type of limonoid), that acts as an insect growth regulator against larval insects by disruption of molting, growth inhibition, and malformation that can result in mortality (Kraus et al. 1985, Khater 2012). Other insecticidal compounds found in neem extracts include salannin, salannol, salannolacetate, nimbinen, gedunin, dirachtin, nimbolide, and viselinin derivatives (Jones et al. 1989, Walter 1999, ATS unpublished data). Neem extract effects result from disruption of endocrine activity, including the downregulation of hemolymph ecdysteroid levels that block release of prothoracicotropic hormone or delay ecdysteroid production, an action which inhibits molting in insects (Schluter et al. 1985, Khater 2012), including horn fly larvae (ATS unpublished data). In addition, azadirachtin affects allatropin and juvenile hormone titers (Kraus et al. 1985, Mordue and Blackwell 1993), and its antifeedant and deterrent effects against some herbivorous insects have been reported (Redfern et al. 1981, Rice et al. 1985, Showler et al. 2004, Greenberg et al. 2005).

When serial dilutions of ethanolic extract of ground neem seed were blended into 300 g of cow manure, hatching stable and horn fly larvae reared on the treated manure had LC_{50} of 7.7 and 0.096 ppm azadirachtin, respectively, based on the number of larvae that pupated (Miller and Chamberlain 1989). An ethanolic extract of ground neem seed (2.7 mg azadirachtin/g) blended with cow manure had an LC_{50} and an LC_{90} for horn fly larvae of 0.096 and 0.133 ppm azadirachtin, respectively (Mulla and Su 1999). An emulsifiable concentrate formulation of azadirachtin, Margosan-O (3 mg azadirachtin/ml), similarly mixed into cow dung, had an LC_{50} and an LC_{90} of 0.151 ppm and 0.268 ppm azadirachtin, respectively, against horn fly larvae (Mulla and Su 1999). Ethanolic neem extract administered daily in gelatin capsules at 0.023–0.045 mg azadirachtin/kg cow body weight resulted in nearly complete inhibition of horn fly larval development in the manure, and the same level of inhibition was reported under identical conditions when the emulsifiable concentrate formulation was given as a feed-through at $\geq$ 0.03 mg azadirachtin/kg cow body weight or as ground seeds at $\geq$ 10 mg/kg body weight (Mulla and Su 1999). Against stable fly, however, ground seed mixed into cattle feed at 100–400 mg seed/kg of body weight per day resulted in $<$50% toxicity (Miller and Chamberlain 1989).

Horn flies are more susceptible to the effects of azadirachtin than stable flies and house flies, *Musca domestica* L. (Miller and Chamberlain 1989, Mulla and Su 1999). Nevertheless, when eggs of stable flies were transferred to filter paper treated with 0.01% azadirachtin, 80% ovicidal activity was observed (Mulla and Su 1999). Also, azadirachtin effects against horn flies are not always evident. For example, neem cake mixed in mineral salt in a 2% concentration (azadirachtin was available at 421 mg/kg body weight) failed to reduce horn fly infestations on cows during a 9-wk period (Chagas et al. 2010).

Chrysanthemum and Tanacetum

Pyrethrum is likely the most widely used botanical insecticide. The flowers of chrysanthemum, *Tanacetum cinerariaefolium* Sch. Bip. (Asteraceae), are ground into powder and extracted using hexane or a similar nonpolar solvent (Casida and Quistad 1995). Pyrethrins are two natural organic compounds from *Chrysanthemum cinerariifolium* (Trevir.) that are neurotoxic to insects (Yang et al. 2012), and they exhibit repellency in sublethal amounts. Pyrethrins are widely known as the bases for development of a range of synthetic pesticides called pyrethroids (e.g., bifenthrin, permethrin, cypermethrin). Pyrethrum and pyrethrins are axonic poisons that afflict electrical impulse transmission along axons, affecting both the peripheral and central nervous systems of the insect, instigating repetitive nerve discharges that induce paralysis (Ware and Whitacre 2004). In the absence of a synergist, however, such as piperonyl butoxide, dibutyl succinate, and butoxypolypropylene glycol, most of the paralyzed insects recover from paralytic “knockdown” (synergists also extend the shelf life; Roberts et al. 1963, Khater 2012).

Bruce (1940) reported that pyrethrum at 4 g per hundred weight (cwt) as a bran-based feed-through in cattle was ineffective for controlling horn flies. Home-made pyrethrum–kerosene extract used as a topical spray, however, was reported as being efficacious against horn flies when applied along the backlines of cows (Marlatt 1928). Although numbers of stable flies were reduced, the spray was relatively ineffectual because of its weak effect and short residual activity (Marlatt 1928). The greater bioactivity against horn flies might result from their habit of feeding closer to the dorsal surface of cattle (around the neck and shoulders), whereas stable flies mostly feed around the lower legs (James and Harwood 1969, Foil and Hogsette 1994). Stanbury and Goodhue (1960), however, determined that pyrethrin sprays at 0.025% provided 93% and 94% protection of cows against horn flies and stable flies, respectively. Control of stable flies on dairies sometimes relies on combining sanitary measures with applications of synthetic insecticides (Betke et al. 1986), which can achieve 99.9% control (Patterson et al. 1981).

Cymbopogon

Lemongrass, *Cymbopogon citratus* (D.C.) Stapf. (Poaceae), is a native spice plant of tropical Asia, but it is grown in many temperate

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and tropical areas of the world (Kazembe and Chauruka 2012) for culinary purposes and for its commercially produced essential leaf oil (Paranagama et al. 2002). An ethanol extract of lemongrass in a 1:125 aqueous dilution topically applied to cattle provided 100% repellency for 30 min, 95% for 12 h, 98% for 24 h, 97% for 48 h, 98% for 5 d, 95% for 10 d, and 69% for up to 15 d posttreatment (Milián 2009). Homogenizing dry leaves of Tasmanian blue gum, *Eucalyptus globulus* Labill., with roots and rhizomes of lemongrass, bulbs of garlic, *Allium sativum* L., and animal fat applied along the dorsal line of cattle provided 10.4% and 52.4% repellency against horn flies on the first and second days, respectively (Heimerdinger et al. 2004). On stable flies, Barnard (2000) reported that citronella, a constituent of lemongrass, was as effective as *N*,*N*-diethyl-meta-toluamide (DEET). Agnolin et al. (2010), however, found that citronella from citronella grass, *Cymbopogon nardus* (L.) Rendle, failed to repel stable flies from cattle.

Derris, Lonchocarpus, and Tephrosia

Rotenone is an isoflavonoid broad-spectrum cytotoxin obtained from the roots or rhizomes of tropical legumes in the genera *Derris*, *Lonchocarpus*, and *Tephrosia* (Fabiaceae). A contact and stomach poison, rotenone inhibits the electron transport chain in mitochondria and acts as a respiratory enzyme inhibitor (Ware and Whitacre 2004). The only report of rotenone use against either of the two biting fly species involved bran fed to cattle at a rate of 0.3 g per hundred weight, achieving 100% kill of horn fly larvae in the dung (Bruce 1940). Pyrethrum at 4 g per hundred weight and tobacco at 5 g per hundred weight were considerably less effective (Bruce 1940).

Festuca

Tall fescue, *Festuca arundinacia* Schreb. (Poaceae), is the dominant species on ~20 million ha of grasslands in the humid eastern United States supporting ~25 million beef cattle (Hoveland 1993). The grass contains as many as five bioactive alkaloids (Bush et al. 1993, Siegel and Bush 1996), some of which, when ingested by cattle, are present in the dung (Westendorf et al. 1993, Dougherty et al. 1998). Certain alkaloids in tall fescue, however, have serious adverse effects against livestock and other mammalian herbivores that ingest them (Paterson et al. 1995, Porter 1995). Extract from tall fescue seed, containing *N*-formyl loline, *N*-acetyl loline, and loline (59:21:20 by mass, respectively), caused 100% mortality against first-instar horn flies when dung was supplemented at ≥ 100 μg/g of dung, and the LD_{50} was calculated as being 30 μg/g (Dougherty et al. 1998). In another study, first-instar horn flies were exposed to bovine dung supplemented with up to 50 μM of *N*-formyl loline and of ergotamine tartrate (Dougherty et al. 1999). *N*-formyl loline caused a dose-dependent linear decline in numbers of pupae recovered and its LD_{50} was 36 μM (Dougherty et al. 1999). Ergotamine tartrate showed significant quadratic dose responses by first-instar horn flies with an LD_{50} of 34 μM.

Melinis

Molasses grass, *Melinis minutiflora* P. Beauv. (Poaceae), originally from Africa, has been planted as a pasture grass and is now present in Brazil, Australia, Hawaii, south Florida, and other tropical regions (Wunderlin 1982, Wagner et al. 1999, Hoffman and Haridasan 2008). Aside from its use as a livestock feed, molasses grass is known for its repellency, trapping, and toxic effects against tick larvae (Ruvalcaba et al. 2004). Four concentrations (10%, 20%, 40%, and 80%) of hexanic extract from molasses grass were tested against adult and larval stable flies. The 80% concentration killed 50% of the adults in 3 h compared with 1 h for a conventional dose of cypermethrin (Tobón and Fcéutica 2011). Half of the larvae were killed in response to the same dosage in 8 min, while cypermethrin took 4 min; lower concentrations took significantly longer to induce 50% mortality (Tobón and Fcéutica 2011).

Nicotiana

Nicotine and the related alkaloids nornicotine and anabasine are obtained from aqueous extracts of tobacco, *Nicotiana* spp. (Solanaceae) and *Anabasis aphylla* L. (Chenopodiaceae). All are synaptic poisons that mimic the neurotransmitter acetylcholine, causing symptoms similar to those produced by organophosphate and carbamate insecticides (Hays 1982). Nicotine is in declining use because it is highly toxic to humans through ingestion, dermal exposure, and inhalation (Khater 2012). Fed to cattle at a rate of 5 g per hundred weight, tobacco caused only 30% mortality in horn fly larvae developing in the resulting excreta (Bruce 1940). In a preliminary study where dosages were not reported, *Nicotiana tabacum* L. ethanolic extract that was topically applied to horn flies in vitro was more toxic than extracts of angel's trumpet, *Brugmansia arborea* (L.) Lagerh.; black elderberry, *Sambucus nigra* L.; hairy beggarticks, *Bidens pilosa* L.; and western ragweed, *Ambrosia cumanensis* Kunth (Ramirez et al. 2009).

Ricinus

Castorbean, *Ricinus communis* L. (Euphorbiaceae), is native to Africa and parts of southern Asia, but it has spread into many tropical and temperate areas of both the Western and Eastern hemispheres (Rana et al. 2012). Although ricin is notably toxic and it is present in the castorbean (Wedin et al. 1986), castor oil used at a rate of 473 ml on camels had no effect against stable flies, but 1,892 ml per camel prevented stable flies from landing on and biting camels for 3 d (Cross 1917).

Vitex

Lilac chastetree or monk's pepper, *Vitex agnus castus* L. (Verbenaceae), is a deciduous shrub or tree native to the Mediterranean region, but now it grows in Europe, Asia, North America, and possibly elsewhere (Dogan et al. 2011, United States Department of Agriculture [USDA] 2013). Leaves and flowers of this species have ketosteroid hormones, and the chloroform fraction of extracts contains bioactive compounds casticin, vitexilactone, pinnatasterone, and 17-OH-progesterone, and the ethyl acetate fraction contains vitexcarpan (Ahmad et al. 2010). Dogs sprayed with a CO_2 extract of seeds of the lilac chastetree were protected against stable fly bites for 3 h after treatment when placed in a cage with 200 flies for 3 min (Mehlhorn et al. 2005). Despite that demonstration of efficacy, further experiments using *V. agnus castus* against horn flies and stable flies have not been reported.

Essential Oils

Some botanical compounds have relatively low molecular weights, such as essential oils, obtained through the process of steam distillation (Cseke and Kaufman 1999). Essential oils are complex mixtures of volatile organic compounds produced as defensive secondary metabolites that include various alcohols, terpenes, and aromatic compounds (Khater 2012). There are many examples of essential oils that are bioactive against a variety of arthropod pests of crops (Cloyd et al. 2009). A phenomenon among metabolites of essential oils sometimes results in greater bioactivity. Minor constituents

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reportedly act as synergists, enhancing the effectiveness of the major constituents through a variety of mechanisms (Berenbaum 1985, Khater 2012), consequently reducing the required dose of toxicant substances and the risk of resistance developing in the target pest (Hieu et al. 2010b). Although repellency of essential oils to mosquitoes has been well documented, relatively little testing has been attempted against horn flies and stable flies (Hieu et al. 2010b). The following are botanical sources from which essential oils have been used against either or both species of biting fly.

Carapa

Andiroba, *Carapa guianensis* Aubl. (Sapindales: Meliaceae), is a Neotropical tree often used for timber and seed oil is used for medicinal purposes. Essential andiroba oil at 1% and 5% concentrations used as a contact spray killed all adult horn flies in 1 h and 4 h, respectively (Klauck et al. 2014). An in vitro assay showed that 5% andiroba essential oil had a repellent effect on horn fly adults (Klauck et al. 2015). On naturally infested Holstein cows, 5% tea tree essential oil repelled horn flies such that 12 h after treatment, numbers on the cows were 57.7% lower than on nontreated control animals (Klauck et al. 2014).

Cinnamomum, Mentha, Matricaria, and Allium

Cinnamomum camphora (L.) J. Presl. (Lauraceae) is native to China, Taiwan, and Japan, and it is often grown commercially for its essential oil (Frizzo et al. 2000). When camphor oil was applied at 1.4 ml/kg body weight to the backline of water buffaloes in Egypt, stable flies were repelled for 6 d (Khater et al. 2009). Under the same conditions, dosages of 3.6 ml/kg body weight of peppermint, *Mentha piperita* (Ehrh.) Briq. (Lamiaceae); 3.4 ml/kg body weight of chamomile, *Matricaria chamomilla* (L.) Rydb. (Asteraceae); and 2.9 ml/kg body weight of onion, *Allium cepa* L. (Amaryllidaceae), each repelled stable flies for 6 d (Khater et al. 2009). Essential oil of peppermint at 5% each in sunflower oil repelled horn flies from treated areas on pastured and barn-held cows for >6 and 8 h, respectively (Lachance and Grange 2014).

Cymbopogon

The leaf oil of lemongrass is primarily composed of citral a and b (78%) and myrcene and limonene (10%; Paranagama et al. 2002), but the acyclic monoterpenoid components citronella and citronellol have repellent properties against some insects (Bartlett 1985). Essential oil of lemongrass at 5% each in sunflower oil repelled horn flies from treated areas on pastured and barn-held cows for >6 and 8 h, respectively (Lachance and Grange 2014).

Citronella oil from *C. citratus*, used by Cross (1917) at an unspecified rate on camels, was effective at repelling stable flies for several hours, but repellency was lost after 17 h. In an olfactometer assay, citronella and citronellol oils were associated with dose-dependent repellency against stable flies that was superior to empenthrin (a pyrethroid) and dichlorvos (a volatile organophosphate; Bartlett 1985). Stable flies also avoided feeding on blood mixed with lemongrass oil for 12 h (Baldacchino et al. 2013a). Citronellol was toxic to stable flies after residual contact exposure in jars with LC_{50} values of 40.4, 38.7, and 35.7 μg/cm^2 after 2, 4, and 24 h, respectively; LC_{90} values were 52.8, 50.7, and 39 μg/cm^2, respectively (Mann et al. 2010). Lemongrass and other plants, including *Pelargonium* spp., produce geranyl acetate, which is toxic to stable flies after residual contact exposure in jars with LC_{50} values of 28.1, 27.2, and 25.2 μg/cm^2 after 2, 4, and 24 h, respectively; LC_{90} values were 35.6, 39.3, and 35 μg/cm^2, respectively (Mann et al. 2010).

Eucalyptus

Eucalyptus (Myrtaceae) represents a large genus of trees, with >600 described species native to Australia, Tasmania, and nearby islands, and now eucalyptus trees are extensively grown in tropical, subtropical, and temperate regions worldwide (Moura et al. 2012). Juan et al. (2011) extracted oils from *E. badjensis* Beuzev & Welch, *E. badjenus* × *E. nitens*, *E. benthamii* var. *dorrigeonsii* Maiden & Cambage, *E. botryoides* Smith, *E. dalrympleana* Maiden, *E. fastigoota* Deane & Maiden, *E. nobilis* L.A.S. Johnson & K.D. Hill, *E. polybractea* R. Baker, *E. radiata* ssp. *radiata* Sieber ex Spreng, *E. resinifera* Smith, *E. robertsonii* Blakely, *E. rubida* Deane & Maiden, *E. smithii* R. Baker, *E. elata* Dehnh, *E. fraxinoides* Deane & Maiden, and *E. obliqua* L'Hér and found that component essential oils 1,8-cineole, α-pinene, α-terpineol, 4-terpineol, and *p*-cymene vapors were toxic to horn flies. Essential oil of *E. polybractea* had the highest knockdown activity of 50% at 3.4 min in an enclosed chamber, and a significant correlation was detected between the content of 1,8-cineole in the *Eucalyptus* species and toxicity to horn flies (Juan et al. 2011). Trigg and Hill (1996) determined that 0.5 ml of *p*-menthane-3,8-diol from *Eucalyptus* on the human forearm reduced stable fly biting to 6% compared with the control. Eighty-six percent protection was obtained for 5 h when 0.35 ml of *p*-menthane-3,8-diol was applied to the human forearm, and this improved to 94% protection when the dosage increased to 0.5 ml (Trigg and Hill 1996).

Melaleuca

Tea tree, *Melaleuca alternifolia* (Maiden. and Betche.) Cheel. (Myrtales: Myrtaceae), essential oil is known to exert lethal effects against arthropod pests of crops and humans (Walton et al. 2004, Koul et al. 2008). Using 1% and 5% aqueous concentrations as contact sprays, all adult horn flies were killed within 3 h (Klauck et al. 2014). An in vitro assay showed that 5% tea tree essential oil had a repellent effect on horn fly adults (Klauck et al. 2015), and on naturally infested Holstein cows, 5% tea tree essential oil repelled horn flies such that 12 h after treatment, 61.6% fewer were on the treated cows than the nontreated control animals (Klauck et al. 2014).

Pelargonium

Many medically important attributes have been ascribed to the genus *Pelargonium*, which encompasses many species that are generally referred to as geraniums (Sawaswathi et al. 2011). *Pelargonium* spp. contain the oil geraniol, (2E)-3,7-dimethylocta-2,6-dien-1-ol. In a laboratory experiment, 2 mg of 90% geraniol deterred horn flies from feeding by >85% and the pest was also strongly repelled using only 0.1 μg (Zhu et al. 2015). Feeding deterrence caused by 20 mg geraniol in the laboratory remained >90% during the first 24 h of exposure, >50% at 72 h, and 30% after 4 d (Zhu et al. 2015). Effectiveness of 30% geraniol (in light mineral oil) on cattle was observed to last 3 h (Zhu et al. 2015). Essential oil of geranium at 5% in sunflower oil repelled horn flies from treated areas on pastured and barn-held cows for >6 and 8 h, respectively (Lachance and Grange 2014).

Nepeta

Catnip, *Nepeta cataria* L. (Lamiaceae), is an herbaceous mint native to Eurasia and North Africa, and now it is grown in most of North America (Zhu et al. 2009). A number of plants in the genus *Nepeta* produce an essential oil rich in a class of monoterpenoid compounds known as iridoids (Inouye 1991), specifically methylcyclopentanoid nepetalactones (Regnier et al. 1967, Cavill 1969), that repel some

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insects (Feaster et al. 2009, Zhu 2011). Catnip oil is also bioactive against the horn fly. In a laboratory experiment, 2 mg of catnip oil (85% nepetalactones) deterred horn flies from feeding by >85% and the pest was also strongly repelled using 1 μg (Zhu et al. 2015). Feeding deterrence caused by 20 mg catnip oil in the laboratory was retained during the first 6 h of exposure, >50% antifeedancy at 72 h, and 30% after 4 d (Zhu et al. 2015). Residual effectiveness of 30% catnip oil (in light mineral oil) on cattle was observed to last 24 h (Zhu et al. 2015).

On hydrogenation, nepetalactones yield dihydroneptetalactone diastereoisomers, one of which has been shown to protect humans from stable fly bites (Feaster et al. 2009, Zhu et al. 2010), with twice the efficacy of DEET (Zhu et al. 2012). Catnip essential oil at a dosage of 20 mg resulted in 96% repellency against stable flies in an olfactometer (Zhu et al. 2009), and <15% of stable flies fed in the laboratory when exposed to 0.2 mg of catnip oil in contrast to 93% of stable flies that fed in the control. Knockdown time from exposure to volatiles and contact was 19 min in response to a dosage of 20 mg, and a 50 μg dose per stable fly yielded 100% mortality, respectively (Zhu et al. 2011). In field trials using cattle, two formulations of catnip oil provided >95% protection for an hour, and lesser degrees of repellency were observed for up to 6 h after application (Zhu et al. 2012). Zhu et al. (2012) also reported that catnip oil in egg-laying substrate prevented stable fly oviposition by as much as 98%. Further, wax-based catnip pellets distributed in cattle feedlots resulted in 99% repellency against stable flies for up to 3 h (Zhu et al. 2010). Gelatin caps with 0.1 g of catnip oil provided >85% oviposition deterrence and >98% inhibition of stable fly larval growth in response to 0.5 g of the gelatin microcapsules after 7 d (Zhu et al. 2014). The deterrent effect of 0.5 g microenscapsulated catnip oil against oviposition, however, disappeared within 48 h (Zhu et al. 2014).

Pogostemon

Patchouli, *Pogostemon cablin* (Blanco) Benth (Lamiaceae), is a bushy herb native to tropical Asia with more widespread cultivation. A dosage of 0.5 mg/cm^2 provided 3.67 h of protection from stable flies when treated human hands were used as a biting substrate, but DEET gave 4.47 h of protection (Hieu et al. 2010b). When tamanu nut oil (with a protection time of 0.56 h) was added to patchouli oil, protection increased to match that of DEET (Hieu et al. 2010b).

Rosa

Rose, *Rosa* spp. (Rosaceae), in addition to being used as ornamentals, are also utilized for food and medicinal purposes. The physiological functions may be partly attributed to their abundances of phenolics and flavonoids (Ercisli 2007, Roman et al. 2013). *Rosa* spp. produce the alcohol rosalva (9-decen-1-ol), toxic to stable flies after residual contact exposure in jars with LC_{50} values of 16.3, 15.3, and 13.1 μg/cm^2 after 2, 4, and 24 h, respectively; LC_{90} values were 22.6, 21.7, and 17 μg/cm^2, respectively (Mann et al. 2010).

Syzygium

Clove, *Syzygium aromaticum* (L.) Merrill and Perry (Myrtaceae), is native to the Maluku Islands of Indonesia and cultivation has spread to other parts of Asia and Africa. Essential oil of clove buds and clove leaves at 0.5 mg/cm^2 applied to the human hand provided protection from stable fly bites for 3.25–3.5 h, less than the 4.47 h provided by DEET (Hieu et al. 2010b). At a dose of 0.25 mg/cm^2, essential oils of clove buds and clove leaves protected human hands for up to 2.68 h (Hieu et al. 2010b), but combining the clove oils with tamanu oil gave protection nearly as well as DEET (Hieu et al. 2010b).

Zanthoxylum

Japanese pepper, *Zanthoxylum piperitum* DC (Rutaceae), found in Japan, China, and the Korean peninsula, and winged prickly ash, *Z. armatum* DC, growing throughout Asia, are deciduous shrub-like trees that are also used for ornamental purposes. These plants produce essential oils that meet the criteria of minimum-risk pesticides (Isman 2008). *Zanthoxylum* species have drawn attention for bioactivity against insects such as the cowpea aphid, *Aphis craccivora* Koch (Nissanka et al. 2001), and the maize weevil, *Sitophilus zeamais* Motschulsky (Wang et al. 2011). Steam distillates of seeds contained 28 compounds that were compared against chlorpyrifos and dichlorvos for efficacy against the stable fly. Fumigant toxicity was observed for cuminaldehyde, thymol, (1S)-(-)-verbenone, (-)-myrtinol, carvacrol, (S)-(Z)-verbenol and others, but all of them were <5 orders of magnitude less toxic than the synthetic insecticides, and acetylcholinesterase inhibition did not occur to a substantial degree from any of the *Zanthoxylum*-produced compounds (Hieu et al. 2012). In another study, *Z. piperitum* seed oil was determined to have five major constituents: limonene, cryptone, 1,8-cineole, citronellal, and geranyl acetate (Hieu et al. 2010a). The most active constituents, cuminaldehyde, cuminyl alcohol, limonene, and methyl cinnamate, gave 82%, 74%, 74%, and 64% repellency, respectively, at 30 min using human hands as hosts, but DEET provided 100% repellency (Hieu et al. 2010a). Increased effectiveness and duration of repellency was obtained using binary mixtures of *Z. piperitum* steam distillate, *Z. armatum* seed oil, and bioactive constituents (each 0.01mg/cm^2), with tamanu, *Calophyllum inophyllum* L. (Calophyllaceae), nut oil (0.99 mg/cm^2), which is a synergist (tamanu is native to the tropics of eastern Africa, southern coastal India, parts of southeastern Asia, and Australasia; Hieu et al. 2010a). The repellencies of aerosols containing 2.5% *Z. piperitum* steam distillate or 2.5% *Z. armatum* seed oil and 2.5% *C. inophyllum* nut oil were comparable with that of 5% DEET aerosol (Hieu et al. 2010a). Ten milligrams of cuminaldehyde, citronellal, neral, linalool, linalool oxide, terpinene-4-ol, 1,8-cineole, and piperitone (components of *Z. piperitum* and *Z. armatum* essential oils) in 150 μl of ethanol repelled 86% to 94% of stable flies in a modified static air olfactometer within 15 min (Hieu et al. 2014).

Fatty Acids

Various natural fatty acids have insecticidal properties, some involving action on acetylcholinesterase and octopaminergic receptors (Don-Pedro 1990, Perumalsamy et al. 2015). One milligram of a saturated fatty acid mixture composed of 1:1:1 octanoic acid (also called caprylic acid, found in the milk of some mammals, coconut oil, and palm kernels), nonaoic acid (from *Pelargonium* [geranium] spp.), and decanoic acid (also called capric acid, from coconut oil, palm kernels, and some mammalian milks), collectively called "C8910 acids" (C8, C9, and C10 mixture), repelled horn flies for at least 3 d in a laboratory assay (Mullens et al. 2009). Minimum statistically significant ($P < 0.05$) repellency levels of the C8910 mixture were 0.06–0.12 mg/cm^2 (on filter paper). In a laboratory experiment, 2 mg of 90% C8910 acids deterred horn flies from feeding by >85% and the pest was also strongly repelled using 1 μg of C8910 acids (Zhu et al. 2015). Feeding deterrence caused by 20 mg of C8910 acids in the laboratory remained >90% during the first 24 h of exposure, and >50% antifeedancy was observed at 72 h, but at 96 h horn flies fed with negligible interference (Zhu et al. 2015).

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The same study, however, indicated that residual effectiveness of pure C8910 acids on cattle lasted 24 h (Zhu et al. 2015).

A liquid formulation of 15% C8910 acids in silicon oil applied at 2.8 mg a.i./cm^2 was demonstrated to repel stable flies in a blood-feeding membrane bioassay for ≥8 h (Mullens et al. 2009). The naturally occurring oleic and linoleic acids, and methyl oleate (from *C. inophyllum* nut oil) synergized the repellency of DEET and the monoterpenoids cuminyl alcohol, cuminaldehyde, and α-phellandrene (Hieu et al. 2015).

Soybean Trypsin Inhibitors

Soybean, *Glycine max* (L.) Merr., trypsin inhibitor (SBTI) is a serine-type protease inhibitor (DeLoach and Spates 1980) that is produced by the plant in response to attack by insects and pathogens, contributing to resistance against them (Ryan 1990). Soybean trypsin inhibitors are either constitutive components in various plant tissues or synthesized (Ryan 1990). DeLoach and Spates (1979) found that 3 mg/ml in stable fly bloodmeal diet was effective at reducing proteolytic digestion of blood, and Spates (1979) reported that the same dosage was sufficient to reduce egg production by 90%, slow digestion of hemoglobin, and reduce hemolysis of erythrocytes. When 0.1% (w/v) SBTI was fed to horn flies, fecundity and egg hatch declined by 78% and 50%, respectively (Spates and Harris 1984).

Using a dialysis procedure, 20% of SBTI was encapsulated in bovine erythrocytes and fed to female stable flies, resulting in cessation of egg production, and 50% of adults died (DeLoach and Spates 1980). Protease activity in treated flies was only 20% of nontreated flies and hemolysin activity was reduced by ~86% because encapsulated SBTI decreased either de novo synthesis or activation of the enzymes (DeLoach and Spates 1980). Encapsulation of SBTI might increase its stability, diminishing the amount needed to inhibit egg production, reduce midgut protease activity, and increase mortality (DeLoach and Spates 1980).

Fungal Endophytes and Pasture Grasses

Tall fescue and perennial ryegrass, *Lolium perenne* L., are commonly infected with fungal endophytes such as *Neotyphodium* spp. in a symbiotic relationship (Hoveland 1993, Schardl et al. 2004, Potter et al. 2008). The fungus synthesizes alkaloids that confer resistance to the plant against herbivorous arthropods (Hoveland 1993, Schardl et al. 2004, Potter et al. 2008). Alkaloids extracted from seeds of endophyte-infected tall fescue had a larvicidal effect against horn flies in cow dung (Dougherty et al. 1999). A chloroform extract (reconstituted in ethanol) of cattle dung from endophyte-infected pastures of tall fescue mixed with cattle manure was associated with 80% larval mortality, while cattle dung from noninfected tall fescue did not result in any mortality (Parra et al. 2016). Parra et al. (2013) reported that cows grazing on endophyte-infected tall fescue for 2 wk had 50.5% fewer horn flies on them than cows grazing on noninfected brome grass, *Bromus* sp., and that dung from the cattle grazed on the endophyte-infected tall fescue killed 80% of horn fly larvae (Parra et al. 2013). In a different study, dung from cows grazed on infected tall fescue was associated with 90% mortality of horn fly larvae compared with 55% mortality in dung from cows that grazed on noninfected tall fescue, and only 29% of pupae in the dung from cows that grazed on the endophyte-infected pasture versus 93% in the noninfected pasture produced adults (Parra et al. 2016). Field-collected dung from cattle that grazed on infected tall fescue harbored the same number of horn fly larvae as dung from noninfected tall fescue, but the control dung supported ~3.5-fold more pupae and produced 4.5-fold more adults (Parra et al. 2016).

Sawdust

Relative to straw, pine, shavings, sand, and gravel substrates, pine sawdust reduced larval stable fly populations in livestock bedding by 46–91% (Schmidtmann 1991). Pine sawdust might be useful for managing stable fly populations on dairies, but further testing to assess pine species, grades, and quantities of sawdust is needed to ascertain the best for achieving moderate- to long-term repellency.

Conclusions

Numerous botanically based compounds are bioactive as repellents and toxins against horn flies and stable flies. Both muscid species can sense certain bioactive botanical compounds through olfaction (Bay 1980, Jeanbourquin and Guerin 2009, Oyarzún et al. 2009), which facilitates repellency. Several biochemical mechanisms are responsible for toxic effects among different compounds. Some bioactive extracts, essential oils, and isolated compounds were applied topically, while others were delivered as feed-throughs for reducing fly survival in manure, and other means of application might be possible. Although bioactive botanical compounds are not a panacea for controlling horn flies and stable flies, these chemicals often can offer environmentally friendly alternatives to many synthetic pesticides that can improve upon safety, and delay or avert the buildup of resistance. These advantages, and the variety of available compounds, are potentially valuable tools for integrated pest management strategies that can be tailored against each of the two biting fly species. Discovery and development of bioactive botanical compounds has only begun. Many of the botanically based biting fly control tactics described have not been researched beyond some initial studies demonstrating their efficacy, and none of them are commercially available products for control of horn flies and stable flies. Development of delivery methods and extension of residual activity have yet to be investigated for most botanical compounds and combinations require further assessment.

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