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Chemical attractants of Philornis downsi [Diptera: Muscidae], an invasive parasite of birds in the Galapagos Islands

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by

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Abstract

Since its discovery in 1997, *Philornis downsi* has been shown to significantly reduce fledgling success in many bird species within the Galapagos Islands, including endemic and critically endangered species of Darwin's finches. Despite the level of impact on such highly celebrated birds, there are currently no practical methods of controlling or monitoring *P. downsi* populations. This study was designed to explore the potential of chemical ecology to remedy these needs. During the month of February 2012 on the Galapagos island of Santa Cruz, we measured the attractiveness of two prospective *P. downsi* olfactory cues using different designs of baited traps. We evaluated three trap designs for effectiveness. The results indicated cylindrical sticky traps are less effective than McPhail traps in catching *P. downsi* and horizontal flat sticky traps are not effective at all. While simultaneously testing trap designs, the attractiveness of nest-related odors and fly-produced pheromones were evaluated using lures of dichloromethane extracted host nest headspace volatiles and male/female deceased flies, respectively. Traps baited with nest odors were not attractive in comparison to positive or negative controls (n=7). The testing of pheromonal sources of *P. downsi* attraction showed that neither male baited traps, female baited traps, nor unbaited traps were able to catch any specimens (n=5). While the ultimate goal of this study, to support the potential for an efficient olfactory-based method of monitoring and controlling *P. downsi*, was not met, experimental techniques and design details were refined for future investigation of *P. downsi* chemical ecology.

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Advice to Future Honors Students

To those distinct few students who wish to pursue research as an undergraduate, I offer some recommendations:

Be proactive— While taking on a research project may seem like a daunting task, just getting started can result in clarity. At the beginning, a project may feel insignificant and directionless. Getting familiar with the literature, talking to peers and setting aside time to write down ideas can take a broad and underdeveloped curiosity and turn it into a solid research question with potential solutions.

Stay motivated— To quote Albert Einstein, "If we knew what we were doing, it wouldn't be called research, would it?" Especially in the case of undergraduate research, field and laboratory work is frustrating. It appears as though no amount of preparation or forethought can make an experiment go smoothly. While these things are critical to prevent many avoidable mistakes, the most important aspect of research is perseverance. As Einstein pointed out, there is no way to predict what can happen during a project, but taking a problem-solving approach and never abandoning an idea with potential can turn an impenetrable roadblock into a barely noticeable speed bump.

Challenge yourself— Coming from years of homework assignment due dates and final exam schedules, the open-endedness of research can be confusing and overwhelming. It is important to remember that placing yourself out of your comfort zone is the only way to grow as a scientist and as a person. Do what you think you are incapable of and try what seems impossible.

Acknowledgments

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Introduction

PHILORNIS DOWNSI, AN AVIAN ECTOPARASITE

Discovered in the Galapagos Islands in 1997, *Philornis downsi* Dodge & Aitken, 1968, (Diptera: Muscidae) has since become considered a "highly invasive" species and a threat to the island's native, endemic, and critically endangered avifauna (Causton et al., 2006; Fessl & Tebbich, 2002). *Philornis downsi* adult females oviposit in the nest material (O'Connor, Robertson, & Kleindorfer, 2010a) of at least 18 different species of Galapagos birds according to Fessl, Sinclair and Kleindorfer (2006b). Once hatched, the first, second and third instar larvae feed externally on the blood of nesting chicks at night, occasionally becoming semi-subcutaneous and feeding on internal tissue as well (Fessl, Sinclair, & Kleindorfer, 2006b). *Philornis downsi* may also exhibit saprophagous behavior on chicks which have died due to heavy parasitism (O' Connor et al., 2010a). Antibody immunity studies suggest larvae are also able to feed on brooding adult female birds (Huber et al., 2010). After feeding for 4-6 days (or less if their host has prematurely died from parasitism or fledged) (Dudaniec & Kleindorfer, 2006), larvae embed themselves in the bottom of the nest material, secreting a milky substance to cement the debris around them (Fessl et al., 2006b). In this cemented cocoon they pupate for approximately two weeks (Dudaniec & Kleindorfer, 2006).

Post-eclosion behavior has not been studied, as adults are only known from reared immature specimens collected from bird nests or adults captured in insect traps. Video evidence has recorded adult females ovipositing in nests but they only remained walking along inner nest surfaces for a maximum of 10 minutes (O' Connor et al., 2010a). It is generally accepted that the adults are non-parasitic and feed on decaying organic matter,

supported by high survival of reared adults on a papaya/protein-based diet developed by Lincango and Causton (2008).

IMPACTS ON HOSTS IN THE GALAPAGOS

Due to the parasite's generalist tendencies, a wide variety of terrestrial avian species have been observed as hosts including several endemic and endangered passerines. In several species studied, including the small ground finch (*Geospiza fuliginosa*), medium ground finch (*Geospiza fortis*), small tree finch (*Camarhynchus parvulus*), large tree finch (*Camarhynchus psittacula*), warbler finch (*Certhidea olivacea*), woodpecker finch (*Cactospiza pallida*) and medium tree finch (*Camarhynchus pauper*), 100% of nests inspected were parasitized by *P. downsi* (Dudaniec, Fessl, & Kleindorfer, 2007; Fessl et al., 2006a; O'Connor, Sulloway, Robertson, & Kleindorfer, 2010b). Fessl and Tebbich (2002) found a 97% *P. downsi* prevalence rate across 12 species of birds, including eight endemic, three native, and one introduced. To date, the largest number of *P. downsi* parasites found in a single nest is 182, belonging to the endemic Galapagos mockingbird (*Nesomimus parvulus*) (Fessl & Tebbich, 2002). In the extensively studied small ground finch, the species was found to have the least intensely parasitized nests of the five finch species examined with 33 ± 3 parasites per nest (Kleindorfer & Dudaniec, 2009). However, in a single year of another small ground finch study the mean number of parasites per nests was found to be 55.3, equating to 22.4 larvae per nestling (Fessl & Tebbich, 2002). Other Darwin's finches, including the woodpecker finch, small tree finch, warbler finch, medium ground finch, and large tree

finch, were found to have 31.6, 28.8, 21.2, 18.8, and 18.5 *P. downsi* larvae per chick, respectively (Dudaniec et al., 2007; Fessl & Tebbich, 2002).

Such intense nest infestations of the larval obligate *P. downsi* parasite decrease fitness of fledglings and increase nestling mortality in the previously mentioned and other host species. Galligan and Kleindorfer (2009) studied physical deformation and found beak malformation due to *P. downsi* early instar activity in the nares of small ground finch fledglings, evidenced by longer nares, as well as shorter and shallower beaks. Although affected birds were not observed to have lower foraging rates, non-hereditary beak changes due to *P. downsi* could interfere with the normal progression of the archetypal natural selection of finch populations. In addition to physical deformation, Fessl et al. (2006a) found blood loss among chicks can be substantial in cases of *P. downsi* nest parasitism. In small and medium ground finches, nestlings in parasite-reduced nests (via 1% permethrin application) as opposed to untreated nests gained body mass faster, had higher haemoglobin concentrations, and had over a two-fold increase in fledgling success (from 33.93% to 86.58%). In another impact study of the small ground finch, there was a direct correlation between higher haemoglobin level, higher fledgling success rate and lower parasite intensity (Dudaniec, Kleindorfer, & Fessl, 2006). Fessl et al. (2006b) used figures for conversion efficiency for blood to parasite biomass, nestling mass to blood ratio, larval and nestling mass per nest as well as other factors to estimate percent blood loss per nestling for four species of Darwin's finches. Calculations indicated a range of 32-55% of nestling blood was lost to *P. downsi* larvae. In 2005, parasitism accounted for 95.2% of the 90.0% fledgling mortality in small ground finch, medium ground finch, and cactus finch (*Geospiza scandens*) nests. Other years in the

same study, 2000 and 2004, found 32.4% and 60.0% mortality due to parasitism for 61.5% and 71.4% fledgling mortality rates, respectively. In all three years, 72.7-100% of nestlings had damaged nasal cavities and in 2004 and 2005, 26.7- 50.0% of nestlings had infected auditory canals, 22.0-33.0% had wounds or contusions and 22.0-33.0 % had larval-induced openings under the wings, legs and on their backs (Fessl et al., 2006b).

The previously mentioned impacts are severe and pose a great threat to bird population stability. However, several other avian species may be more vulnerable to *P. downsi* infestation. On one Galapagos island, Floreana, the endemic warbler finch has not been found for nearly half a century (Grant, Grant, Petren, & Keller, 2005). While discovery of *P. downsi* was only in 1997, entomology collections indicate the species was in the Galapagos since at least 1964 (Causton et al., 2006). It is speculated that *P. downsi* may have contributed to the presumed extinction of the warbler finch on Floreana. While the warbler finch exists on other islands in the archipelago, each island population of the species, and other endemic birds, is genetically distinct. Loss of such island populations can be harmful to the habitat and the scientific community (Grant et al., 2005).

Because of habitat destruction and predator introduction in its small range on Floreana Island, the medium tree finch is listed as a critically endangered species on the 2009 International Union for Conservation of Nature Red List. In addition to these factors, O'Connor et al (2010b) determined that *P. downsi* is the primary cause of mortality among nestlings in this species. Based on body size studies from other Darwin's finches, the medium tree finch experiences a higher than expected parasite intensity. With a 100% nest infestation rate, 41% of fledgling failure can be attributed to parasitism, contributing to 75% overall mortality (O'Connor et al., 2010b).

The mangrove finch (*Camarhynchus heliobates*), however, is a species that could arguably be most affected by *P. downsi*. Critically endangered and with only 100-110 individuals estimated to be remaining, threats such as rat predation and habitat destruction has wreaked havoc upon breeding success (Fessl et al., 2010). With rat control, fledgling success in the mangrove finch's restricted habitat can increase by 28% but *P. downsi* infestation has been shown to decrease fledgling success by at least an additional 14%. Population viability analyses suggest increasing breeding success of the mangrove finch to a rate that can protect the species will require intensified rat control and also the initiation of a *P. downsi* control program (Fessl et al., 2010).

PHILORNIS DOWNSI CONTROL METHODS

Thus far, attempts to find an effective management technique for *P. downsi* have been unsuccessful as research since the species' recent discovery has focused primarily on investigating its effect on bird populations. Some rudimentary procedures for immediate control have been studied, such as non-pheromonal trapping (Lancango & Causton, 2009; Muth, 2007), physical barriers to parasitism (Koop, Huber, Laverty, & Clayton, 2011), and manual application of pesticide treatments to nests (Fessl et al., 2006a). None of these methods can be implemented in a *P. downsi* management program with the exception of the most urgent and critical cases such as temporarily mitigating impacts on the mangrove finch. Moreover, these attempted control methods display a limited ability to reduce *P. downsi* populations, impractically small scale applications or short-term restrictions which are characteristics that have prevented them from being employed.

Other, more long-term, solutions are being researched, but the lack of knowledge of *P. downsi* biology has prevented these potential solutions from coming to fruition. For example, sterile-insect technique (SIT), where sterilized males are repeatedly introduced to a population to reduce pest reproduction, would be a viable option for *P. downsi* control as the infestations are on a large scale but in a restricted environment (Hellman & Fierke, 2009). Also, the fly would be released as an adult, a life stage considered innocuous. Additionally, because the Galapagos is a unique and fragile island ecosystem, SIT would acceptable because of its benign environmental nature. It has no non-target effects because it is species-specific and is non-polluting, chemically or genetically (Alphey, 2002). Molecular evidence indicates *P. downsi* populations of different islands are genetically similar enough that one sterile male strain can be used for all of the Galapagos (Dudaniec, Gardner, Donnellan, & Kleindorfer, 2008). Another genetic study indicated that female *P. downsi* often mate multiple times (Dudaniec, Gardner, & Kleindorfer, 2010), which may reduce the effectiveness of SIT if there is pre-or post-copulatory selection of mates or sperm by females (Barclay, 2005). To date, such patterns of mating behavior of adult *P. downsi* are unknown and many other gaps in the knowledge of *P. downsi* reproduction still exist.

One of the greatest obstacles to implementing a successful SIT program is this lack of knowledge of *P. downsi* behavioral ecology as well as *P. downsi* population dynamics. SIT programs often fail without explanation despite a high sterile male to wild male ratio. These programs usually lack prior estimation of wild female populations or evaluation of mating competitiveness of the sterile males (Ito & Yamamura, 2005).

Currently, there is no effective way to quantify *P. downsi* wild male or female populations and in order for SIT to be successful this must be rectified.

There has also been extreme difficulty in rearing the insect as adult mating, female oviposition, and first instar larva survival have not been successfully replicated in laboratory conditions (Hellman & Fierke, 2009). In fact, all attempts to raise a single fly from an egg to adult have failed (Lincango & Causton, 2008; Muth, 2007). These complex life history traits must be studied so that captive breeding can be possible and then implemented on the mass scale required of SIT.

Biological control, in which a predator or parasitoid of the pest is introduced, is another promising method of *P. downsi* control. It is a long-term solution that, if executed correctly, is the only management program that is species-specific, self-sustaining and able to work on a very large scale. Biocontrol has already been successfully implemented on the Galapagos using a coccinellid beetle and a pest scale insect, *Icerya pushasi* (Causton et al., 2006), so many infrastructural and administrational details are already in existence. However, choosing a biocontrol agent that will achieve the desired, risk-free result is a long, analytical process that requires extensive biological and ecological knowledge of both the pest and the potential control organism. Biocontrol agents work best when they reduce the pest population at the damaging life stage (in this case, the larvae) and when the control agent is host-specific. It is also important, though not necessary, for natural population dynamics of the agent to self-regulate itself at high enough densities to reduce pest populations without human interference (Hoddle, 2003).

Only four natural enemies of *P. downsi* are currently known and they are all generalist hymenopteran parasitoids of puparia collected from the nests in the Galapagos

(Lincango & Causton, 2008). Because these four parasitoids affect an innocuous life stage, are muscoid generalists, and are already in the Galapagos at low parasitism rates (Lincango & Causton, 2008), they are not ideal candidates for biocontrol. Like SIT, biocontrol requires captive breeding on a sufficiently large scale to allow for an experimental laboratory pest population, as many tests ensuring host-specificity are crucial to a successful program (Hoddle, 2003). Rearing capabilities are not yet at the capacity needed for biocontrol experiments. In addition to resolving the lack of scientific understanding of *P. downsi* and eliminating the barriers to large-scale rearing, any long-term management techniques require a method of monitoring efficacy. Presently, the only way to assess population levels of *P. downsi* is though examination of bird nests for parasite load which is a laborious task and limited to a small spatial scale (Dudaniec et al., 2007).

With more research, *P. downsi* chemical ecology could be a source of effective, long-term, environmentally-safe methods of control or population monitoring. As reviewed by Witzgall, Kirsch and Cork (2010), chemical ecology works on the principal that olfactory cues, such as pheromones, host-derived odors or food-related volatiles, can elicit a behavioral response in a particular insect. Chemical ecology can be used in combination with other management techniques or on its own to reduce pest populations. Several widely used management techniques utilize olfactory cues in order to lower pest populations: attract-and-kill, where insects are attracted to a baited trap and exterminated; mass trapping, where insects are trapped in large enough numbers to lower the population; or mating disruption, where the wide-spread release of species-specific pheromones prevent the sexes from finding each other and mating. If using

semiochemicals to lower insect populations is unnecessary due to the presence of another control mechanism, chemical ecology can be used to assess effectiveness of the control. Spread delineation with traps, where a pest's movement and establishment is delineated in order to detect incipient populations, is a method often used to slow the inevitable spread of a species or to identify a new population that can be eradicated if quickly detected. Population assessment via standardized trapping systems offers a beneficial service in a variety of pest management scenarios. Very effective population delineation and monitoring can be achieved via pheromone-baited traps as they work at all population densities, often optimally when densities are very low (Witzgall, Kirch, & Cork, 2010).

Population monitoring can be very difficult for pests such as *P. downsi* as both the adults and larvae are difficult to find visually without video monitoring or nest destruction. Furthermore, indicators of impact, such as fledgling success and beak deformation rates, are highly variable between species, locations and years (Dudaniec, Fessl, & Kleindorfer, 2007; Fessl & Tebbich, 2002; Galligan & Kleindorfer, 2009). Trapping offers the only feasible way to monitor *P. downsi* populations, which is important in the short-term to assess high-risk bird populations and critical in the long-term, to help assess the value of other control programs such as SIT or biocontrol (Witzgall, Kirsch, & Cork, 2010).

The severity of *P. downsi* impacts makes it critical to find an immediate control technique that is efficient in impact, scale, and longevity. Compared to plans like SIT and biocontrol, developing an appropriate lure requires minimal knowledge of insect population dynamics and ecology and can be developed in less time than multi-phase

programs. Methods of control or monitoring that utilize chemical ecology can be instituted without extensive non-target effect tests or background investigation of insect behavior, and because lures can be inexpensively manufactured once developed, they can be cost-effective as well (Witzgall et al., 2010).

CHEMICAL ECOLOGY OF *PHILORNIS DOWNSI*

Several other muscid pests have had significant enough impacts to warrant investigation of their pheromonal attractants, though results of laboratory bioassays and field experiments for these insects have been meager due to a focus on cuticlar chemistry. Most extensively studied thus far is the female sex pheromone for the common house fly, *Musca domestica* Linnaeus, which has been identified by Carlson et al. (1971) as (Z)-9-tricosene, a twenty-three carbon straight chain alkene. The amount of this chemical found on females is dependent on temperature, humidity, population density and possibly genetic drift (Noorman & Den Otter, 2002) and can sometimes be undetectable (Darbro, Millar, McElfresh, & Mullens, 2005). Olfactometer bioassays, in which live flies are given a choice between chambers containing different odors carried by streams of humidified air, indicated only a third of tested male *M. domestica* were attracted by (Z)-9-tricosene (Carlson et al., 1971). However, later studies indicate it may be an aggregation pheromone, as it attracts equal numbers of males and females in field tests and can also attract gravid females in laboratory tests (Carlson & Beroza, 1973; Chapman, Knapp, Howse, & Groulson, 1998; Jiang et al., 2002). Three other compounds, (Z)-14-tricosen-10-one, cis-9, 10-epoxytricosane and 9-hexadecenyl-9-octadecenoate, have been isolated from female *M. domestica* cuticular extracts, though they are not hydrocarbons like (Z)-9-tricosene (Uebel, Schwarz, Lusby, & Miller, 1978a).

Further tests involving the four identified compounds performed by Adams, Holt and Blomquist (1984), Uebel, Sonnet and Miller (1976), and Uebel (1978a) provide conflicting information, partially due to the difference in bioassay design and compound levels (Adams & Holt, 1987). Generally, however, it is concluded that in *M. domestica* (Z)-9-tricosene is a mild long-range pheromone attractant, while the three non-hydrocarbon compounds are short-range sex-stimulants.

Musca autumnalis DeGeer, a pest and disease vector of livestock, has also been studied in pursuit of its sex pheromones. Several alkenes have been extracted from the surface waxes (also known as cuticular hydrocarbons) of male and mature virgin females with slight differences in ratios of specific chemicals. Each compound, non-synergistically, somewhat stimulates mating attempts in males, indicating they are weak short-range sex-stimulants in *M. autumnalis* (Uebel, Sonnet, Miller, & Beroza, 1975a).

Several compounds have been isolated from male and female cuticular extracts of *Stomoxys calcitrans* Linnaeus, another pest of cattle. Female compounds weakly increase male mating attempts when combined (Sonnet, Uebel, Lusby, Schwarz, & Miller, 1979), and like other studied muscid compounds, are likely mild short-range sex-stimulants. Similar compounds were isolated from *Stomoxys nigra* Macquart and despite weak behavioral results, studies of *S. calcitrans* compounds contributed to our baseline knowledge of fly age and mating status and their influence on sex pheromones. Results from that research indicate hydrocarbon composition diverged between adult males and virgin females after three days and males transferred two chemicals to females during mating. This indicates chemically-based sexual attractiveness of adult female flies depends on age and mating status, as immature or already mated females have surface

chemicals that are more similar to, and possibly less attractive to, male adult flies (Harris, Oehler, & Berry, 1976).

Other female muscid pheromones have been studied in three members of the genus *Fannia* as well as the horn fly, *Haematobia irritans* Linnaeus. Exposure to all isolated compounds elicited a similar response in their respective species in that they increased the number of male mating attempts in laboratory assays (Uebel, Sonnet, Menzer, Miller, & Lusby, 1977; Uebel, Schwarz, Menzer, & Miller, 1978b; Uebel, Schwarz, Miller, & Menzer, 1978c; Bolton, Butler, & Carlson, 1980).

Besides the *M. domestica* sex pheromone, (Z)-9-tricosene, all chemical compounds isolated from muscid flies have been short-range sex-stimulants. While these semiochemicals are not the type useful for trapping, these studies have concentrated on cuticular solvent washes which would isolate non-volatile chemicals found on the surface of the insect. The collection of headspace volatiles, which would contain chemicals released by the insect into open air, would be more likely to contain long-range pheromone attractants which could be used in mass-trapping. Incorporation of cuticular compound photo-oxidation, where the interaction of ultraviolet light and a hydrocarbon results in an oxidation reaction and a more volatile product, may result in a chemical extraction technique leading to the isolation of long-range muscid semiochemicals. This type of chemical reaction has been observed in cuticular hydrocarbons of members of Hymenoptera (Bartelt & Jones, 1983, Swedenborg & Jones, 1992) but has only recently been looked for in Diptera (Collignon, 2011).

Pheromones of *P. downsi* have been investigated by Collignon (2011). This study found cuticular hydrocarbon content was sexually dimorphic, but only between females

and aged males. Until male *P. downsi* adults have aged four to five days, cuticular hydrocarbons between the sexes are identical. However, male cuticular hydrocarbons then begin to change until males are more than eight days old, at which point males and females only share 6% of their chemical compounds. This is similar to the condition in *S. calcitrans*, suggesting the change in male hydrocarbon profiles in both *P. downsi* and *S. calcitrans* may be for the same purpose: male-specific compounds are transferred to females during mating and act as antiaphrodisiacs. Comparison of chemical compositions between headspace volatiles collected from live flies and *P. downsi* cuticular extracts exposed to ultraviolet light revealed chemical differences between the two. However, these compounds were not evaluated to determine if they elicited physiological response in adult *P. downsi* and attempted behavioral assays were inconclusive. Collignon (2011) suggests three possible mechanisms by which *P. downsi* uses olfactory cues to mate: (1) males emit long-range sex or aggregation pheromones while females emit sex-stimulants, (2) both sexes use long-range sex or aggregation pheromones via photo-oxidation or (3) long-range aggregation cues come from environmental sources and both sexes use sex-stimulants. This study is the only research that has been done to examine *P. downsi* chemical ecology, but the results must be explored further in order for a pheromonal cue to be discovered.

While control and monitoring using baited traps often focus on sex pheromones, the addition of other olfactory cues, including food-, oviposition-, and host-based, can make lures more attractive. These odors can occasionally function as the only attractant in a lure if a pheromonal attractant has not been developed (Witzgall et al., 2010). Many investigations of host-volatiles have related to insect pests of agricultural or forestry

concern. Plant-based chemicals, such as the triterpenes released by squash blossoms (Cucurbitaceae) or the sesquiterpenes released by stressed green ash trees (*Fraxinus pennsylvanica*) can elicit behavioral responses in pests such as the chrysomelid western corn root worm and the buprestid emerald ash borer (Crook et al., 2008; Metcalf, Metcalf, & Rhodes,1980). Additionally, several parasitizing dipterans have also been tested for attraction to host volatiles. Most notably, disease-vectoring mosquitoes, such as those from the genera *Aedes, Anopheles,* and *Culex,* have been found to be attracted to CO_2, lactic-acid and other skin, breath and urine-derived odors released by hosts (Takken, 1991). These compounds are often used in combination with light as trap lures in high-priority areas of mosquito control (Burkett et al., 2002). Virgin females of the blood-feeding sand fly, *Lutzomyia longipalpis* Lutz and Neiva (Diptera: Psychodidae), show a strong increase in attraction and a sharp decrease in the proportion of non-responders to male sex pheromone when host odors from a hamster are added (Bray & Hamilton, 2007). These are two examples of how host odors can either work on their own or increase the efficacy of pheromone lures.

Host-derived odor studies of other Diptera indicate it may be possible to attract *P. downsi* with bird or nest olfactory cues. Several species of *Culex,* including *C. quinquefasciatus* Say (which is thought to have a preference for avian rather than human hosts) showed a strong attraction to the odor of bird feathers, especially in combination with CO_2 (Allan, Bernier, & Kline, 2006). *Fannia conspicua* Malloch, a higher fly in the same family as *P. downsi,* also exhibits host-associated odor attraction in the form of CO_2, ammonia, and the combination of the two, as *F. conspicua* is a pest of humans, deer, and cattle (Mohr, Mullens, & Gerry, 2010). Finally, while the Caribbean fruit fly,

Anastrepha suspensa Loew is a non-parasitic tephritid, one study has shown that crude avian feces, due to the food-related odor of protein decomposition, is more attractive than the release of ammonia from feces alone should indicate. The authors argue that other chemicals in the feces that remain to be indentified are responsible for the increase in attraction (Epsky, Dueben, Heath, Lauzon, & Prokopy, 1997). While the fruit fly would be attracted to avian feces as a food odor and not a host odor, as in *P. downsi*, this study indicates odors of avian feces contain chemical compounds bioactive in other species of Diptera.

In addition to fecal material, a source of host-related olfactory attraction could include semiochemicals released by birds. Volatiles released by brooding females, assisting males or nestlings could act as point-sources of odors attracting *P. downsi* to bird nests. The uropygial gland is responsible for secreting preening materials in most orders of birds and can produce a large amount of volatile compounds (Campagna, Mardon, Celerier, & Bonadonna, 2012). In birds of the family Upupidae, an odorous secretion containing several classes of volatile compounds is only produced by breeding females and nestlings (Martin-Vivaldi et al., 2009). In the dark-eyed junco, in the same order as Darwin's finches (Passeriformes), a change in the volatile fraction of uropygial secretions is observed in both males and females when they enter the breeding season (Soini et al., 2007). Volatile extracts containing gender-specific proportions of straight-chain alkanols were found to be similar between four members of the order Passeriformes, indicating these chemicals are phylogenetically related sex pheromones (Zhang, Zuo, & Sun, 2009). In the grey catbird, which shares the family Mimidae with the Floreana mockingbird and others, season-dependent changes in uropygial-derived

volatiles have been found in males (Whelan, Levin, Owen, & Garvin, 2010). Furthermore, a switch in ester composition has been observed in several bird species during the breeding season. This change is found only in the brooding sex in species with uniparental incubation, but in both sexes in biparental incubation (Reneerkens et al., 2007). These studies show that birds inhabiting nests may release unique chemicals according to sex and reproductive stage which is particularly promising for investigating sources of nest attractiveness for *P. downsi*. However, a brooding- or nesting-specific volatile composition would likely not be necessary for parasite attraction, as the concentration of odors created by constant nest inhabitation alone would likely result in a chemical profile unique to nests.

BEHAVIORAL ASSAYS OF *PHILORNIS DOWNSI* CHEMICAL ECOLOGY

Several researchers have attempted to use potential food-related or host-related odors to trap *P. downsi*. Nest debris, bird feces, egg shells, bananas, urine, beer, vinegar, sugar, powdered milk, papaya, fruit fly lures, blow fly lures, ethanol, fruits of native shrubs, and several protein decomposition compounds, as well as various combinations of these, have all been tested (Hellman & Fierke, 2010; Lincango & Causton, 2008; Muth, 2007). Additionally, the female *Musca domestica* sex pheromone, (Z)-9-tricosene, was used a lure as well (Muth, 2007). Despite the number of experiments, small samples sizes, inappropriate lure sources, variable temporal and spatial scale, and trap type inconsistencies have led to inadequate results. The most attractive lures for *P. downsi* involve decomposing fruit (especially papaya) or chemical compounds that are byproducts of decomposition. The number of non-target muscoid flies, however,

indicates that these lures are not specific to *P. downsi* and are likely general adult muscoid food odors (Lincango & Causton, 2008; Muth, 2007). In previous trapping trials, a variety of traps were used in an attempt to find a trap design with the highest efficacy. Lincango and Causton (2008) tested yellow McPhail traps, green McPhail traps, yellow sticky traps and white sticky traps. It was indicated that yellow McPhail traps caught the most *P. downsi* followed by green McPhail traps and then yellow sticky traps.

Using knowledge of previous trapping experiments and an understanding of chemical ecology, I performed several preliminary experiments on the Galapagos Island of Santa Cruz during the month of February, 2012. The first experiment was designed to select the location of highest *P. downsi* population density as measured by the number of adults caught in papaya-baited positive control traps. The same experiment was also used to determine if a novel sticky trap design would be as effective as McPhail traps, as the former would be preferable to use in later field tests. The results of this experiment were used to develop subsequent experiments. The second field assay was performed in order to test nest odor attractiveness. This was done by collecting headspace volatiles from nests with actively incubating or brooding finches and using dichloromethane extractions of these volatiles as trap lures. Nest odors were tested against blank negative controls and positive papaya controls in the location (El Barranco) and trap type (McPhail) that was found most suitable in the first experiment. Finally, a field test was performed using a second novel sticky trap design baited with male *P. downsi* adults, female *P. downsi* adults, and a negative control blank. The purpose of this last experiment was to lay the groundwork for further pheromonal research in *P. downsi* by establishing which sex is responsible for long-range attraction. Sticky traps were used to ensure exposure of

cuticular hydrocarbons to ultraviolet light in case the role of photo-oxidation is important in *P. downsi* pheromone volatization. It was predicted that these experiments would reveal an attraction of *P. downsi* adults to traps baited with nest odors and to traps baited with other adult *P. downsi* specimens. Such results would reveal the potential of a host-odor derived lure for control and also supplement the chemical *P. downsi* pheromone work done by Collignon (2011) with behavioral assessments.

Methods

TRAP TYPE AND LOCATION

Preliminary trapping using (1) self-constructed sticky traps (Fig. 1) made from 20 x 30 sheets of adhesive clear plastic and (2) commercial McPhail fruit fly traps (Fig. 2) were performed for a duration of seven days between 8-Feb to 17-Feb, 2012. Ten of each trap type were placed at two locations on Santa Cruz Island: Los Gemelos and El Barranco (Fig. 3). Los Gemelos is a heavily vegetated, moist upland area with a high canopy while El Barranco is an arid lowland area with densely dispersed but less foliated shrubs and cacti. Traps were hung approximately 2 m high ~20 m apart along available paths with trap types alternating. The vegetation type traps were hung from was not noted. All traps were filled with a portion of a blended mixture of one whole papaya without seeds, ~250 mL water and ~200 g sugar. Each McPhail trap was filled with enough papaya mixture to cover the bottom, and the homemade sticky traps were filled with enough papaya mixture to ensure a similar surface area for both trap types. Traps were checked daily and on day 5 and day 7, captured specimens were removed from the traps for identification and sexing.

After specimens were identified and sexed, data were analyzed using Minitab 16.2.0 (Minitab, Inc). Two 2-proportion z-tests ($\alpha = 0.05$) analyzed target specificity between trap types at both Los Gemelos and El Barranco. A 1-sample t-test and two 2-sample t-tests ($\alpha = 0.05$) analyzed *P. downsi* capture rates between trap types and locations. Two 2-proportion z-tests ($\alpha = 0.05$) analyzed the sex ratio of captured *P. downsi.*

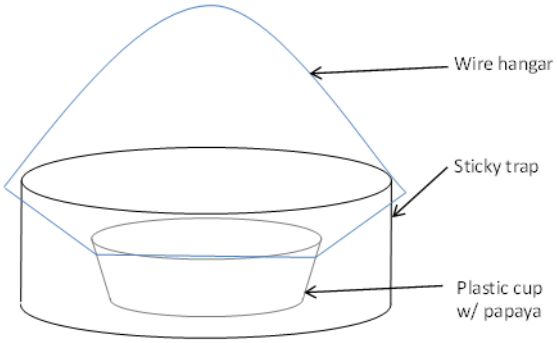

Figure 1. Schematic 10 x 20 cm sticky trap used for testing effectiveness against commercial McPhail traps.

Figure 2. Commercial McPhail trap used for trapping *P. downsi*.

Figure 3. Map of Santa Cruz Island. (Courtesy of Google Maps)

NEST ODOR ATTRACTION

McPhail traps were used to capture insect specimens at El Barranco due to the results from the experiment testing trap types and locations. Traps were placed ~2 m above the ground in various unidentified species of vegetation along an available path ~20 m apart. Traps were placed in the field for five days from 20-Feb to 25-Feb. Treatments were assigned using a random number generator (www.random.org) and consisted of (1) nest volatile extractions, (2) papaya mixture positive control and (3) blank negative control. Each treatment was delivered by slow-release polyethylene sachets (2 ml, 5.1 x 7.6 cm) that were suspended from wire within the McPhail traps. Polyethylene sachets containing 2.5 mL of collected nest volatile extractions were placed

in McPhail traps as the nest odor treatment. The papaya positive control consisted of sachets containing 20 mL per trap of a papaya blend similar to that used for the trap type and location experiment, while the blank negative control consisted of an empty sachet. Traps were checked every other day for condition and specimens were removed on day 5 for identification. This experiment was performed using seven replicates, limited by the number of nests available for volatile collection. Ethylene glycol was used as the preservative. To assess difference between *P. downsi* trapping efficiency for each treatment, a chi-squared goodness of fit test was used at $\alpha = 0.05$.

Nest volatiles used for nest odor lures were collected between 9-Feb and 14-Feb, 2012 from bird nests in Los Gemelos. The nests belonged to warbler finch (*Certhidea olivacea*) and small tree finch (*Geospiza parvula*) mating pairs and contained either late-stage incubating eggs or hatchlings ≤3 days old. Volatiles were obtained by drawing air from within a nest through a pipette containing 370 mg of Porapak Q (80-100 mesh; Sigma-Aldrich Co., St. Louis, MO, USA) for ~7 hr at a 1000 ml/min flow rate. Compounds were then eluted using 3 mL of dichloromethane, 2.5 mL of which were placed in a polyethylene sachet with the remaining 0.5 mL kept for gas chromatography analysis upon return to SUNY ESF in Syracuse, NY.

MALE/FEMALE ATTRACTION

For five days from 21-Feb to 26-Feb, 2012, a different sticky trap design (Fig. 4) was used to test the level of attraction to different sexes of *P. downsi* due to the unsuitability of McPhail traps for this experiment. The sticky trap design, unlike McPhail traps, allows unfiltered UV light to interact with potential *P. downsi* cuticular

hydrocarbons causing photooxidation and subsequent volatization of chemical compounds. The sticky traps were baited with three treatments: (1) male flies, (2) female flies and (3) a blank negative control. For the male fly and female fly trap treatments, two deceased flies of a particular sex were pinned and placed in the center of a 20 x 25 cm rectangle of adhesive clear plastic. The blank trap contained pins on plastic but no flies. Traps were placed on well-illuminated large shrubs or cacti ~ 20 m apart along an available path with treatments assigned using a random number generator. The species of shrub or cactus was not noted. Traps were placed ~2 m above the ground but height was variable based on the plant to which they were tied. Five traps of each treatment were placed at El Barranco, limited by the number of female fly specimens available. Traps were checked every other day for condition and captured specimens were removed on day 5 for identification.

Male and female *P. downsi* were collected as third instar larvae or puparia from warbler finch and small tree finch nests located at Los Gemelos. Specimens were separated pre-eclosion and reared in natural light on a mixture of papaya, water, sugar and milk powder. The sex ratio of eclosed adults was tested using a hypothesis z-test of 2-proportions ($\alpha = 0.05$). Due to a high mortality rate among eclosed adults, deceased flies were frozen within a day post-mortem and later used for male/female attraction tests. Flies were between three and nine days old at the time of death with average female age of 5.3 days and average male age of 5.5 days. Five female flies captured live in McPhail traps from the first experiment were kept for lures as well.

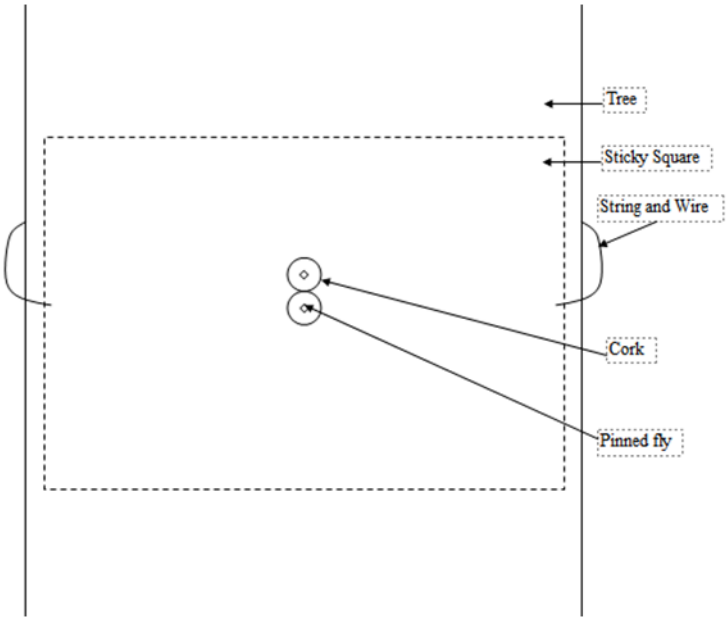

Figure 4. Schematic of 20 x 25 cm sticky trap design used in testing male and female *P. downsi* attractiveness.

Results

TRAP TYPE AND LOCATION

At Los Gemelos, McPhail traps (Fig. 2) were more specific for *P. downsi* ($z = 3.67$, $p = 0.000$) and able to catch more *P. downsi* ($t = 5.01$, d.f. = 9, $p = 0.000$) than sticky traps (Fig. 1). Trapping at El Barranco also showed greater specificity ($z = 9.10$, $p = 0.000$) and higher capture rate ($t = 2.19$, d.f. = 10, $p = 0.027$) for *P. downsi* in McPhail traps than sticky traps. Traps of both types placed at El Barranco caught more *P. downsi* than traps of either type at Los Gemelos ($t = 2.63$, d.f. = 20, $p = 0.008$). These results are summarized in Figure 4. Overall, more females (37) were captured than males (16) regardless of trap type at both Los Gemelos ($z = 2.83$, $p = 0.002$) and El Barranco ($z = 3.66$, $p = 0.000$).

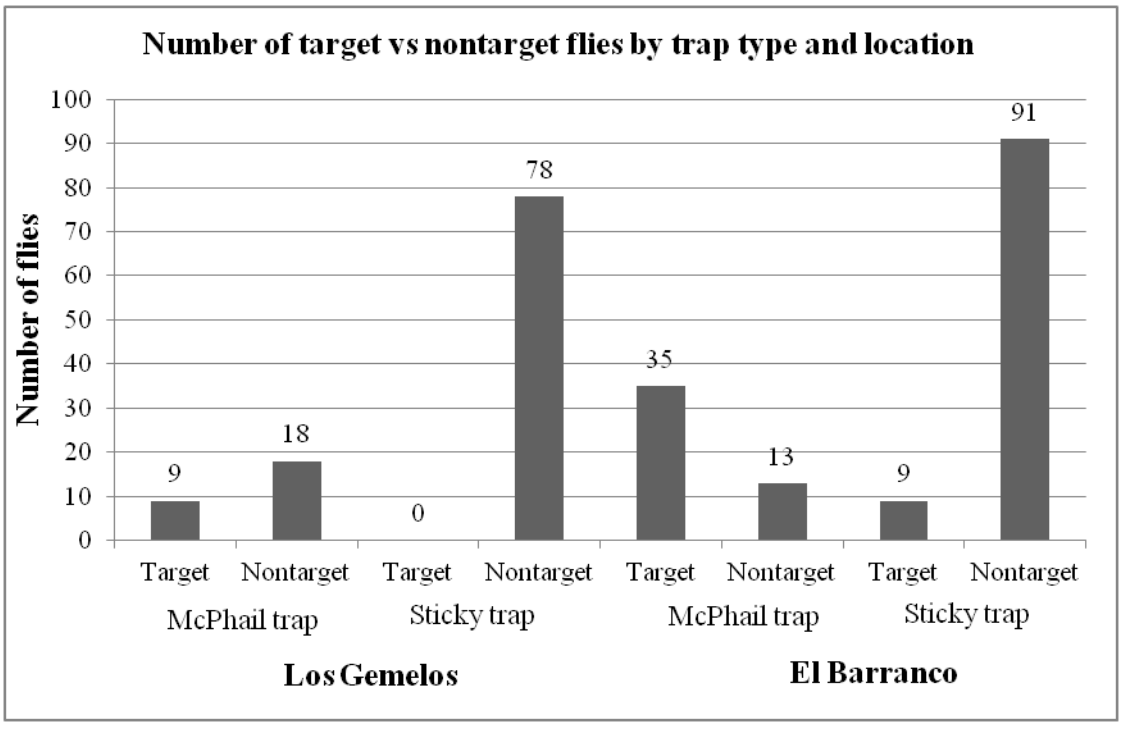

Figure 5. Number of P. downsi target flies and other muscoid nontarget flies caught by McPhail and sticky traps at Los Gemelos and El Barranco.

NEST ODOR ATTRACTION

Nest odor traps and blank traps captured no *P. downsi* adults, statistically fewer than the four flies captured by the papaya mixture positive control ($\chi^2 = 8.000$, d.f.= 2, p = 0.018). The four adult *P. downsi* from the positive control traps consisted of two males and two females. Seven nontarget muscoid flies were captured in all traps, one captured in a nest odor trap and the rest in the papaya mixture positive control traps

MALE/FEMALE ATTRACTION

No *P. downsi* adults were found on any of the male-baited, female-baited or blank control sticky traps. Five nontarget muscoid flies were captured including one on a blank control trap and two on both male- and female-baited traps. Throughout the duration of the trapping experiment, it was observed that lure *P. downsi* adults were damaged or missing from the pins. By the end of the five day trial, 33% of the traps had one or both of the flies entirely missing. One trap had partial insect bodies left on the sticky material, indicating that both lure specimens and captured specimens were removed from the traps by birds or reptiles.

A total of 41 flies were successfully reared to eclosion from larvae and pupae taken from nests at Los Gemelos. More males (33) eclosed than females (8) (z = 6.97, p = 0.000).

SEX RATIO OF *PHILORNIS DOWNSI*

The proportion of females caught in the McPhail traps from the trap types and location experiment (0.698) was significantly larger than the proportion of females reared from nests (0.195) (z = 5.69, p = 0.000).

Discussion

In searching for the most suitable trap design and trapping location, McPhail traps at El Barranco were shown to yield the highest *P. downsi* capture rate and target specificity. The difference in location suitability may be attributed to a difference in weather and vegetation structure, as El Barranco experiences less rain and has a lower canopy than Los Gemelos. In conditions where a large proportion of the day and night receive rain, adult *P. downsi* would have fewer flying opportunities. Birds nest 6 to 10 m above the ground in the higher canopy at Los Gemelos while birds at El Barranco nest 2 to 6 m above the ground. It may be that *P. downsi* remains at the height at which the birds nest, so traps placed at nest height may catch more flies than those not placed at nest height.

One explanation for the greater suitability of the McPhail traps involves the method of entry. McPhail traps are constructed with a hole on the bottom that is about 6 cm in diameter and the lure is placed inside. Similarly, Darwin's finches build dome nests with a comparable sized entrance hole on the side. *Philornis downsi* may be adapted to respond to entrance holes, such as that in the McPhail trap, due to their avian hosts. The increased target-specificity of McPhail traps, despite the lower overall muscoid capture rate, supports that McPhail traps are particularly well-suited for *P. downsi*. While many other explanations for McPhail trap efficacy are possible, this behavioral response is also supported by the comparison of sex ratios. Only females would have acquired this pattern due to oviposition and in this experiment proportionately more females were found in McPhail traps than were reared from nests. In a preliminary study, using the same McPhail traps and papaya-mixture bait, Hellman and Fierke (2009) also found more

females than males in two of their *P. downsi* trapping experiments, but the study did not have an alternative assessment of population sex ratio.

Trapping results from the first experiment indicate a substantial population of *P. downsi* existed at El Barranco around the time of the nest odors and male/female attraction trials. However, the nest odors experiment indicated there was no *P. downsi* attraction to nest volatile extractions and the male/female attraction experiment indicated there was no *P. downsi* attraction to deceased flies of either sex.

While these results could indicate that nests or adult flies do not emit olfactory cues, many experimental design constraints likely influenced the outcomes of the trapping experiments. Both the nest odor and male/female attraction experiments had a fewer than ideal number of replicates. Nests that could be used for volatile collection at Los Gemelos had to be within a feasible height (<6 m) and at a stage in development at which *P. downsi* would oviposit (with eggs close to hatching or freshly hatched chicks) according to O' Connor et al. (2010a). A late breeding season and a large number of failed incubating nests further limited this pool. For these reasons only seven extraction samples could be used as lures. For the male/female attraction experiment, the number of immature fly specimens collected for rearing was limited as only naturally failed nests could be examined and only third instar larvae or puparia successfully eclosed. The two-week period of specimen collection put a temporal constraint on the number of flies that could be collected and reared to maturity in time for trapping experiments. Furthermore, while 21 flies were successfully reared, a skewed sex ratio caused the number of replicates for the male/female attraction test to be limited to five.

The nest odor experiment lacked the background knowledge needed to ensure proper lure development. Because gas chromatography analysis was unavailable in the Galapagos, it could not be confirmed that collection of headspace volatiles and subsequent extraction resulted in actual collection of odors from the nest. If odor collection failed, the nest odor lures would not have contained any potential attractant.

Release rate and concentration of potential host-related cues could have also been an issue. Volatiles were collected for 6-8 hours, but released over five days in an area with nesting birds which were likely more attractive. The polyethylene sachets were not established to be appropriate in release rate prior to the trial, possibly causing volatiles to be released in too low concentrations (where *P. downsi* would not detect it) or in too high concentrations (where odors would be released quickly, leaving a prematurely empty lure).

The third experiment, in which sex-based attraction was tested, would have been more informative if there were not fly mortality among reared specimens. Ideally, trapping would have been performed with live flies as they would continually release long-range pheromonal cues. Dead flies would only release volatiles that were already present on the cuticle at the time of death, resulting in a shorter time for dispersal and less overall volatile quantity. Additionally, fly specimens, particularly the males, should have been aged at least eight days before trapping as Collignon (2011) found there is change in male *P. downsi* cuticular hydrocarbons 8 days after eclosion. The high fly mortality rate was a result of the inability to obtain hydrolyzed protein powder for a suitable *P. downsi* adult diet.

Another issue with the male/female attraction experiment was removal of flies from the pins and also loss of captured specimens. The prevalence of insectivorious birds, e.g., the Galapagos flycatcher (*Myiarchus magnirostris*), observed during field work suggests specimens were removed by predators. This may also be a partial explanation for the inefficiency of other sticky trap designs, both the cylindrical design tested in the nest odor experiment and other forms tested by Lincango and Causton (2008). A method of placing a positive control should have been developed to confirm the trap design itself was ineffective.

In addition to repeating these experiments with corrected replicates, lures, and trap designs, other areas of research should be pursued due to the data gathered in this investigation. Based on the success of McPhail traps, noting their female *P. downsi* specificity, olfactometer behavioral assays utilizing nest-like entrance holes should be investigated. Also, the possibility of male protandry, in which males eclose before females, should be studied due to the skewed sex ratio found in *P. downsi* specimens collected in the beginning of finch breeding season. If protandry exists, this could improve the effectiveness of pheromonal mass trapping if a male-targeted lure is developed (Witzgall et al., 2010).

Other areas of *P. downsi* chemical ecology must be pursued if a control or monitoring program is to be established. Volatiles collected from nests, adult *P. downsi* and also food sources must be collected and identified so bioactivity and behavioral responses can then be assessed. Due to the difficultly in rearing *P. downsi* it may advantageous to work with other species of *Philornis* in pursuit of pheromonal cues, as a

phylogenetic relationship of chemical ecology may be apparent within the genus as it is in *Stomoxys* and *Fannia* (Harris et al., 1976; Uebel et al., 1978b, Uebel et al., 1978c).

Conclusion

While the trapping experiments performed in this study did not indicate that a nest odor or sex-based olfactory cue exists for *P. downsi*, novel methods for trapping the avian parasite were explored. With logistical improvements, these methods can be used to test behavioral responses to potential host or pheromone attractants especially if chemical analysis, such as compound identification and bioactivity assessment, are completed in advance. Observed data involving McPhail trap specificity and collected specimen sex ratio has also revealed new avenues for potential research.

The most crucial step in finding a solution to the negative impacts of *P. downsi* is to explicate the parasite's biology. This study focused on investigating *P. downsi*'s chemical ecology, but many other behavioral and ecological uncertainties remain to be resolved. In order for any control technique to be developed to the point of high efficacy, more research must focus on the entomological aspect of the problem and not just the ornithology.

References

Adams, T.S., & Holt, G.G. (1987). Effect of pheromone components when applied to different models on male sexual behavior in the housefly, *Musca domestica*. *Journal of Insect Physiology, 33*(1), 9-18.

Adams, T.S., Holt, G.G., & Blomquist, G.J. (1984). Endocrine control of pheromone biosynthesis and mating behavior in the housefly, *Musca domestica*. In W. Engel, W.H. Clark, & A. Fischer (Eds.), *Advances in Invertebrate Reproduction 3* (pp. 441-457). New York: Elsevier Science.

Allan, S.A., Bernier, U.R., & Kline, D.L. (2006). Laboratory evaluation of avian odors for mosquito (Diptera: Culicidae) attraction. *Journal of Medical Entomology, 43*(2), 225-231.

Alphey, L. (2002) Re-engineering the sterile insect technique. *Insect Biochemistry and Molecular Biology, 32*(10), 1242-1247.

Barclay, H.J. (2005). Mathematical models for the use of sterile insects. In V.A. Dyck, J. Hendrichs, & A. S. Robinson (Eds.), *Principles and Practice in Area-Wide Integrated Pest Management* (pp. 147-174). The Netherlands: Springer.

Bartelt, R.J., & Jones, R.L. (1983). (*Z*)-10-Nonadecenal: A pheromonally active air oxidation product of (*Z,Z*)-9,19 dienes in yellowheaded spruce sawfly. *Journal of Chemical Ecology 9*(9), 1333- 1341.

Bolton, H.T., Butler, J.F., & Carlson, D.A. (1980). A mating stimulant pheromone of the horn fly, *Haematobia irritans* (L.): demonstration of biological activity in separated cuticular components. *Journal of Chemical Ecology, 6*(5), 951-964.

Bray, D.P., & Hamilton, J.G.C. (2007). Host odor synergizes attraction of virgin female *Lutzomyia longipalpis* (Diptera: Psychodidae). *Journal of Medical Entomology, 44*(5), 779-787.

Burkett, D.A., Lee, W.J., Lee, K.W., Kim, H.C., Lee, H.I., Lee, J.S.,…Klein, T.A. (2002). Late season commercial mosquito trap and host seeking activity evaluation against mosquitoes in a malarious area of the Republic of Korea. *The Korean Journal of Parasitology, 40*(1), 45-54.

Campagna, S., Mardon, J., Celerier, A., & Bonadonna, F. (2012). Potential semiochemical molecules from birds: A practical and comprehensive compilation of the last 20 years studies. *Chemical Senses, 37*(1), 3-25.

Carlson, D.A., & Beroza, M. (1973). Field evaluations of (Z)-9-tricosene, a sex attractant pheromone of the housefly. *Environmental Entomology, 2*(4), 555-59.

Carlson, D.A., Mayer M.S., Silhacek, D.L, James, J.D., Beroza, M., & Bieri, B.A. (1971). Sex attractant pheromone of the housefly: Isolation, identification and synthesis. *Science, 174*(4004), 76-77.

Causton, C.E., Peck, S.B., Sinclair, B.J., Roque-Albedo, L., Hodgson, C.J., & Landry, B. (2006). Alien insects: threats and implication for the conservation of the Galapagos Islands. *Annals of the Entomological Society of America, 99*(1), 121-143.

Chapman, J.W., Knapp, J.J., Howse, P.E., & Groulson, D. (1998). An evaluation of Z-9-tricosene and food odours for attracting house flies, *Musca domestica*, to baited targets in deep-pit poultry units. *Entomologia Experimentalis et Applicata, 89(2),*183-192.

Collignon, R.M. (2011). Semiochemicals of *Philornis downsi* (Diptera:Muscidae), an invasive avian parasite of the Galapagos islands. (Unpublished master's thesis). State University of New York College of Environmental Science and Forestry, Syracuse, NY.

Crook, D.J., Khrimian, A., Francese, J.A., Fraser, I., Poland, T.M., Sawyer, A.J., & Mastro, V.C. (2008). Development of a host-based semiochemical lure for trapping emerald ash borer, *Agrilus planipennis* (Coleoptera: Busprestidae). *Environmental Entomology, 37*(2), 356-365.

Epsky, N.D., Dueben, B.D., Heath, R.R., Lauzon, C.R., & Prokopy, R.J. (1997). Attraction of *Anastrepha suspense* (Diptera:Tephritidae) to volatiles from avian fecal material. *The Florida Entomologist, 80*(2), 270-277.

Darbro, J.M., Millar, J.G., McElfresh, J.S., & Mullens, R.A. (2005). Survey of Muscalure (Z-9-tricosene) on house flies (Diptera: Muscidae) from field populations in California. *Environmental Entomology, 34*(6), 1418-1425.

Dudaniec, R.Y., Fessl, B., & Kleindorfer, S. (2007). Interannual and interspecific variation in intensity of the parasitic fly, *Philornis downsi*, in Darwin's finches. *Biological Conservation, 139*(3-4), 325-332.

Dudaniec, R.Y., Gardner, M.G., Donnellan, S., & Kleindorfer, S. (2008). Genetic variation in the invasive avian parasite, *Philornis downsi* (Diptera, Muscidae) on the Galápagos archipelago. *BMC Ecology, 8*, 13-25.

Dudaniec, R.Y., Gardner, M.G., & Kleindorfer, S. (2010). Offspring genetic structure reveals mating and nest infestation behavior of an invasive parasitic fly *(Philornis downsi)* of Galápagos birds. *Biological Invasions, 12*(3), 581-592.

Dudaniec, R.Y., & Kleindorfer S. (2006). The effects of the parasitic flies *Philornis* (Diptera: Muscidae) on birds. *Emu, 106*(1), 13-20.

Dudaniec, R.Y., Kleindorfer, S., & Fessl, B. (2006). Effects of the introduced ectoparasite *Philornis downsi* on haemoglobin level and nestling survival in Darwin's small ground finch (*Geospiza fuliginosa*). *Austral Ecology, 31*(1), 88-94.

Fessl, B., Kleindorfer, S., & Tebbich, S. (2006a). An experimental study on the effects of an introduced parasite in Darwin's finches. *Biological Conservation, 127*(1), 55-61.

Fessl, B., Sinclair, B.J., & Kleindorfer, S. (2006b). The life-cycle of *Philornis downsi* (Diptera: Muscidae) parasitizing Darwin's finches and its impacts on nestling survival. *Parasitology, 133*(6), 739-747.

Fessl, B., & Tebbich, S. (2002). *Philornis downsi*- a recently discovered parasite on the Galapagos archipelago- a threat for Darwin's finches? *Ibis, 144*(3), 445-451.

Fessl, B., Young, G.H., Young, R.P., Rodgríguez-Matamoros, J., Dvorak, M., Tebbich, S., & Fa, J.E. (2010). *Philosophical Transactions of The Royal Society, 365*(1543), 1019-1030.

Galligan T.H., & Kleindorfer, S. (2009). Naris and beak malformation caused by the parasitic fly, *Philornis downsi* (Diptera: Muscidae), in Darwin's small ground finch, *Geospiza fuliginosa* (Passeriformes: Emberizidae). *Biological Journal of the Linnean Society, 98*(3), 577-585.

Grant, P.R., Grant, B.R., Petren, K., & Keller, L.F. (2005). Extinction behind our backs: the possible fate of one of the Darwin's finch species in Isla Floreana, Galapagos. *Biological Conservation*, *122*(3), 499-503.

Harris, R.L., Oehler, D.D., & Berry, I.L. (1976). Sex pheromone of the stable fly: affect on cuticular hydrocarbons of age, sex, species, and mating. *Environmental Entomology, 5*(5) 973-977.

Hellman, W., & Fierke, M. (2009). *2009 Philornis downsi trapping and rearing* (Technical Report). Charles Darwin Foundation. 17pp.

Hoddle, M.S. (2003). Classical biological control of arthropods in the 21st century. In R.G. Van Driesche (Ed.), *Proceedings of the International Symposium on Biological Control of Arthropods. Honolulu, Hawaii, 14-18 January 2002* (pp. 3-16). Morgantown, West Virginia: USDA Forest Service.

Huber, S.K., Owen, J.P., Koop, J.A.H., King, M.O., Grant, P.R., Grant, B.R., & Clayton, D.H. (2010). Ecoimmunity in Darwin's finches: invasive parasites trigger acquired immunity in the medium ground finch (*Geospiza fortis*). *PLoS ONE, 5*(1), e8605.

Ito, Y., & Yamamura, K. (2005). Role of population and behavioral ecology in the sterile insect technique. In V.A. Dyck, J. Hendrichs, & A. S. Robinson (Eds.), *Principles and Practice in Area-Wide Integrated Pest Management* (pp. 147-174). The Netherlands: Springer.

Jiang, Y., Lei, C., Nui, C., Fang, Y., Xiao, C., & Zhang, Z. (2002). Semiochemicals from ovaries of gravid females attract ovipositing female houseflies, *Musca domestica*. *Journal of Insect Physiology, 48*(10), 945-950.

Kleindorfer, S., & Dudaniec, R.Y. (2009). Love thy neighbour? Social nesting pattern, host mass and nest size affect ectoparasite intensity in Darwin's tree finches. *Behavioral Ecology and Sociobology, 63*(5), 731-739.

Koop, J.A.H., Huber, S.K., Laverty, S.M., & Clayton, D.H. (2011). Experimental demonstration of the fitness consequences of an introduced parasite of Darwin's finches. *PLoS ONE, 6*(5), e19706.

Lincango, P., & Causton, C. (2008). *Crianza en cautiverio de Philornis downsi, en las islas Galapagos* (Technical Report). Fundación Charles Darwin. 28pp.

Lincango, P., & Causton, C. (2009). *Ensayos de atrayentes para la captura de la mosca parásito, Philornis downsi (Diptera: Muscidae) en las Islas Galápagos* (Technical Report). Fundación Charles Darwin. 18pp.

Martin-Vivaldi, M., Ruiz-Rodrigues, M., Soler, J.J., Peralta-Sanchez, J.M., Mendez, M., Valdivia, E., … Martinez-Bueno, M. (2009). Seasonal, sexual, and developmental differences in hoopoe *Upupa epops* preen gland morphology and secretions: evidence for a role of bacteria. *Journal of Avian Biology, 40*(2), 191-205.

Metcalf, R.L., Metcalf, R.A., & Rhodes, A.M. (1980). Cucurbitacins as kairomones for diabroticite beetles. *Proceedings of the National Academy of Sciences of the United States of America, 77*(7), 3769-3772.

Mohr, R.M., Mullens, B.A., & Gerry, A.C. (2010). Evaluation of ammonia, human sweat, and bovine blood as attractants for the female canyon fly, *Fannia conspicua* (Diptera:Muscidae), in southern California. *Journal of Vector Ecology, 36*(1), 55-58.

Muth, A. (2007). *Control of Philornis downsi, bird parasite* (Technical Report). Charles Darwin Research Station. 26pp.

Noorman, N., & Den Otter, C.J. (2002). Effects of relative humidity, temperature, and population density in production of cuticular hydrocarbons in housefly, *Musca domestica*. *Journal of Chemical Ecology, 28*(9), 1819-1829.

O' Connor, J.A., Robertson, J., & Kleindorfer S. (2010a). Video analysis of host-parasite interactions in nests of Darwin's finches. *Oryx, 44*(4), 588-584.

O' Connor, J.A., Sulloway, F.J., Robertson, J., & Kleindorfer, S. (2010b). *Philornis downsi* parasitism is the primary cause of nestling mortality in the critically endangered Darwin's medium tree finch (*Camarhynchus pauper*). *Biodiversity and Conservation, 19*(3), 853-856.

Reneerkens, J., Almeida, J.B., Lank, D.B., Jukema, J., Lanctot, R.B., Morrison, R.I.G., …Piersma, T. (2007). Parental role division predicts avian preen wax cycles. *Ibis, 149*(4), 721-729.

Soini, H.A., Schrock, S.E., Bruce, K.E., Wiesler, D., Ketterson, E.D., & Novotny, M.V. (2007). Seasonal variation in volatile compound profiles of preen gland secretions of the dark-eyed junco (*Junco hyemalis*). *Journal of Chemical Ecology, 33*(1), 183-198.

Sonnet, P.E., Uebel, E.C., Lusby, W.R., Schwarz, M., & Miller, R.W. (1979). Sex pheromone of the stable fly. *Journal of Chemical Ecology,* 5(3), 353-61.

Swedenborg, P.D., & Jones, R.L. (1992). (*Z*)-4-Tridecenal, a pheromonally active air oxidation product from a series of (*Z,Z*)-9,13 dienes in *Macrocentrus grandii* (Hymenoptera: Braconidae). *Journal of Chemical Ecology, 18*(11), 1913-1931.

Takken, W. (2001). The role of olfaction in host-seeking of mosquitoes: A review. *International Journal of Tropical Insect Science, 12*(1-2-3), 287-295.

Uebel, E.C., Schwarz, M., Lusby, W.R., Miller, R.W., & Sonnet, P.E. (1978a). Cuticular non-hydrocarbons of the female housefly and their evaluation as mating stimulants. *Lloydia, 41*, 63-67.

Uebel, E.C., Schwarz, M., Menzer, R.E., & Miller, R.W. (1978b). Mating stimulant pheromone and cuticular lipid constituents of *Fannia pusio* (Wiedemann) (Diptera: Muscidae). *Journal of Chemical Ecology, 4*(1), 73-81.

Uebel, E.C., Schwarz, M., Miller, R.W., & Menzer, R.E. (1978c). Mating stimulant pheromone and cuticular lipid constituents of *Fannia femoralis* (Stein) (Diptera: Muscidae). *Journal of Chemical Ecology 4*(1), 83-93.

Uebel, E.C., Sonnet, P.E., Menzer, R.E., Miller, R.W., & Lusby, W.R. (1977). Mating-stimulant pheromone and cuticular lipid constituents of the little house fly, *Fannia canicularis* (L.). *Journal of Chemical Ecology*, 3(3), 269-278.

Uebel, E.C., Sonnet, P.E., & Miller, R.W. (1976). Housefly sex pheromone: Enhancement of mating strike activity by combination of (Z)-9-tricosene with branched saturated hydrocarbons. *Environmental Entomology, 5*(5), 905-8.

Uebel, E.C., Sonnet, P.E., Miller, R.W., & Beroza, M. (1975a). Sex pheromone of the face fly, *Musca autumnalis* De Geer (Diptera: Muscidae). *Journal of Chemical Ecology, 1*(2), 195-202.

Whelan, R.J., Levin, T.C., Owen, J.C., & Garvin, M.C. (2010). Short chain carboxylic acids from gray catbird (*Dumetella carolinesis*) uropygial secretions vary with

testosterone levels and photoperiod. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 156*(3), 183-188.
Witzgall, P., Kirsch, P., & Cork, A. (2010). Sex pheromones and their impact on pest management. *Journal of Chemical Ecology*. *36*(1), 80-100.
Zhang, J.X., Zuo, M.X., & Sun, L. (2009). The volatile composition of uropygial glands contains information about sex, individual, and species in Bengalese finches, *Lonchura striata*. *Current Zoology*, *55*(5), 357-365.

Appendix A

Trap types and location experiment raw data

LOS GEMELOS							
Trap		**Day 5**		**Day 7**		**Total**	
		Target	Nontarget	Target	Nontarget	Target	Nontarget
Sticky 1		0	3	0	2	0	5
S2		0	0	0	1	0	1
S3		0	2	0	6	0	8
S4		0	2	0	2	0	4
S5		0	3	0	4	0	7
S6		0	2	0	0	0	2
S7		0	0	0	0	0	0
S8		0	1	0	0	0	1
S9		0	28	0	19	0	47
S10		0	1	0	2	0	3
McPhail 1		0	1	1	2	1	3
M2		0	1	1	0	1	1
M3		0	0	0	2	0	2
M4		0	0	0	4	0	4
M5		1	0	0	1	1	1
M6		0	0	1	0	1	0
M7		0	0	1	1	1	1
M8		2	0	0	2	2	2
M9		1	0	0	3	1	3
M10		1	0	0	1	1	1
TOTAL:	S	0	42	0	36	**0**	**78**
	M	5	0	4	16	**9**	**18**

EL BARRANCO							
Trap		**Day 5**		**Day 7**		**Total**	
		Target	Nontarget	Target	Nontarget	Target	Nontarget
Sticky 1		0	4	1	8	1	12
S2		1	8	0	10	1	18
S3		2	3	1	8	3	11
S4		0	3	1	3	1	6
S5		0	11	1	0	1	11
S6		0	2	0	5	0	7
S7		1	6	1	0	2	6
S8		0	2	0	1	0	3
S9		0	5	0	2	0	7
S10		0	7	0	3	0	10
McPhail 1		1	0	0	0	1	0
M2		0	1	4	2	4	3
M3		0	5	3	1	3	6
M4		2	0	9	2	11	2
M5		0	0	6	0	6	0
M6		0	0	0	0	0	0
M7		0	0	0	0	0	0
M8		0	1	3	0	3	1
M9		0	0	0	0	0	0
M10		0	0	7	1	7	1
TOTAL:	S	4	51	5	40	**9**	**91**
	M	3	7	32	6	**35**	**13**

Appendix B

Nest odor attraction experiment raw data

Trap	**Treatment**	**Target**	**Nontarget**
N1	Papaya	0	1
N2	Nest	0	0
N3	Papaya	0	0
N4	Papaya	2	1
N5	Blank	0	0
N6	Nest	0	0
N7	Papaya	0	1
N8	Papaya	0	2
N9	Papaya	1	0
N10	Nest	0	0
N11	Nest	0	1
N12	Blank	0	0
N13	Nest	0	0
N14	Blank	0	0
N15	Blank	0	0
N16	Blank	0	0
N17	Nest	0	0
N18	Nest	0	0
N19	Papaya	1	1
N20	Blank	0	0
N21	Blank	0	0
TOTAL:		**4**	**7**

Appendix C

Male/female attraction experiment raw data

Trap	Treatment			Target	Nontarget	Notes
	Sex	Age 1	Age 2			
P1	Blank	-	-	0	0	
P2	Female	T3	5	0	0	1 missing fly
P3	Male	7	3	0	1	
P4	Female	3	3	0	0	
P5	Blank	-	-	0	0	
P6	Blank	-	-	0	0	
P7	Blank	-	-	0	0	
P8	Female	3	8	0	1	1 missing fly
P9	Blank	-	-	0	1	
P10	Female	8	8	0	1	
P11	Male	8	3	0	0	2 missing flies
P12	Male	6	3	0	0	2 missing flies
P13	Female	9	3	0	0	1 missing fly
P14	Male	6	8	0	1	
P15	Male	5	6	0	0	
TOTAL:				**0**	**5**	**5 traps missing flies**

Appendix D

*P. downsi r*eared adult eclosion records

Date	**Male**	**Female**	**From Puparia**	**From Larvae**
2/4/2012	4	1	-	-
2/5/2012	1	0	1	0
2/6/2012	0	0	0	0
2/7/2012	0	0	0	0
2/8/2012	2	0	2	0
2/9/2012	1	0	1	0
2/10/2012	0	0	0	0
2/11/2012	3	1	4	0
2/12/2012	1	0	0	1
2/13/2012	5	2	4	3
2/14/2012	2	3	2	3
2/15/2012	14	1	1	14
2/16/2012	0	0	0	0
2/17/2012	0	0	0	0
2/18/2012	0	0	0	0
TOTAL:	**33**	**8**	**15**	**21**