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Ecology and Management of Canyon Flies (Fannia benjamini complex) in California

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Ecology and Management of Canyon Flies (*Fannia benjamini* complex) in California

A Thesis submitted in partial satisfaction of the requirements for the degree of

Master of Science

in

Entomology

by

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June 2012

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Last, but not least, a special thank you to the US – Sri Lanka Fulbright Commission and the Institute of International Education for funding my study program and for providing me with many opportunities to make my stay in USA a very enlightening and memorable time.
Host seeking “canyon flies” (*Fannia benjamini* complex) (Diptera: Muscidae) cause significant nuisance to humans and animals. To determine if a barrier trapping system using attractive traps would reduce the number of canyon flies reaching a human host within a protected area, a barrier of CO$_2$-baited CDC-type suction traps (without light) was evaluated during the peak fly activity season at a location known for high *F.conspicua* Malloch activity in southern California. To select a suitable radius for the barrier, the effect of an operating CO$_2$ baited trap on the fly capture rate of a nearby human was evaluated with traps placed at 10, 20, 30 and 40m away from the human collector. The number of flies captured by the human collector was very significantly reduced by traps placed 10m away, only slightly by traps 20m away and unaffected by traps $\geq$ 30m away. Because of site characteristics and the rapidly decreasing human-trap
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Introduction

The attraction to hosts and near-host activities of nuisance flies can cause significant stress or irritation to humans and other animals. The presence of large numbers of nuisance flies can prevent people from enjoying common out-door activities, because the flies land on their body, on their food, and can cause nuisance simply by their activities in houses and yards. While there is no accepted method of measuring nuisance, flies that cause nuisance can have a considerable influence on humans and animals, and may require extensive control measures. Of these nuisance flies, the species that make direct contact with a host to feed on host body secretions are of greatest concern. They generally do not draw blood from a host, but some species may feed on blood from wounds on hosts caused by other biting flies (Garcia and Rodovsky 1962). The most widely known of these nuisance flies are those associated with animal agriculture, mainly due to their habit of developing in animal feces. Face flies (Musca autumnalis De Geer) and bush flies (M. vetustissima Walker) that feed on tears, saliva, mucous, blood or other excrement from pasture animals, especially cattle, are common examples of nuisance flies associated with animal agriculture. Filth flies such as blow flies (Family Calliphoridae) and house flies (Family Muscidae) that develop in manure or garbage, and flesh flies (Family Sarcophagidae) that develop in animal carcasses are also considered as nuisance flies, but are not obligate feeders on host secretions.
Host-attracted nuisance fly species may also be common in native habitat where their development sites are less well characterized. In the United States, several groups of unrelated nuisance flies are known to attack humans and animals in non-agricultural habitats. These native flies include the widely distributed “eye gnats” (Chloropidae: *Hippelates* spp.), as well as the more focally distributed “canyon flies” (Muscidae: *Fannia benjamini* Malloch complex) and “trail gnats” (Drosophilidae: *Amiota picta* Coquillet). These native nuisance flies are different from filth flies in that they do not associate with humans and animals in search of oviposition sites, but are attracted to hosts to obtain host derived secretions for oogenesis.

**Canyon Flies:** Nuisance flies of the *Fannia benjamini* Malloch complex (Diptera: Muscidae), commonly known as “canyon flies” are significant pests of animals and humans. Female canyon flies are known to feed on animal body secretions such as tears, mucus, sweat and saliva, and blood from open sores or wounds caused by other biting flies (Garcia and Radovsky 1962), from which they acquire proteins needed for their egg development (Mullens and Gerry 2006). Once a suitable animal host has been located, the persistence with which these flies attempt to land on the head and body results in considerable nuisance to both humans and animals. They are also known to cluster around the eyes of deer and horses (Poorbaugh 1969). At geographic locations where these canyon flies are numerous, their host-seeking behavior can severely limit the human use of the outdoor environment during the times that these flies are actively host-seeking.
The *F. benjamini* complex includes 7 species (Turner 1976), namely *F. benjamini* Malloch, the type species of the group, *F. conspicua* Malloch, *F. arizonensis* Chillcott, *F. neotomaria* Chillcott, *F. operta* Chillcott, *F. tescorum* Chillcott and the most recently described species, *F. thelaziae* Turner. The average body size of members of this group ranges from 3.5 to 4.5 mm (Chillcott 1960). Members of the *F. benjamini* complex can be distinguished from other *Fannia* by their yellow basal antennal segments and palpi, hind tibiae bearing one anterodorsal and two anteroventral bristles, and trimaculate abdomen (Turner 1976).

In the early 1960s, *F. benjamini* was considered as the intermediate host of the California eye worm, *Thelazia californiensis* Price (Winkler and Wagner 1961), a nematode that lays eggs in the eyes of mammalian hosts such as cattle, horses, rabbits, coyotes and sometimes, even in the eyes of humans (Weinmann et al. 1974). However, it was later discovered that, even though the developing stages of this nematode eye worm were reported to be present in *F. benjamini* (Burnett et al. 1957), there was no evidence of it being able to transmit the eye worm (Weinmann et al. 1974). Moreover, it was later discovered that the intermediate host of the eye worm was another member of the *F. benjamini* complex, *F. thelaziae* (Weinmann et al. 1974, Turner 1976). This species of canyon flies occurs primarily at the margins of oak woodland areas of the San Francisco Bay area and in adjacent counties (Turner 1976).
Distribution of canyon flies: Members of the *F. benjamiini* complex are mainly distributed in upland coastal mountain and Sierra foothill habitats of California with some distribution in Baja, Mexico and Arizona (Chillcott 1960). The specific geographic distribution of each of the “canyon fly” species is poorly described due to few collection records for the majority of the species in this complex. In southern California, the most commonly collected members of the group are *F. conspicua* and *F. benjamiini* (Figure 1.1), which are frequently encountered in coastal mountain ranges (Gerry and Mullens 2006). Recent studies on this pest complex have been mainly focused on a single species, *F. conspicua* Malloch, which is a significant nuisance pest in some foothill areas of southern California (Gerry and Mullens 2006; Mullens and Gerry 2006; Mohr et al. 2011a, b). It has been documented that *F. benjamiini* is common to dry chaparral and oak woodland areas throughout California (Turner 1976). Both groups can be collected together in many locations and *F. benjamiini* can be separated morphologically from *F. conspicua* by the coloration of the base of the arista, which is mostly orange in *F. conspicua* and black in *F. benjamiini* (Figure 1.2). In general, specimens of *F. conspicua* also tend to be slightly smaller in size than specimens of *F. benjamiini* (Chillcott 1960).

Early studies on the *F. benjamiini* complex were conducted in Lytle creek area, San Bernardino mountain foothill by Winkler and Wagner (1961), while most of the recent studies on this group of emerging pests in the dry canyon areas of coastal and southern California (Mullens and Gerry 2006) have been conducted in La Habra Heights, Los Angeles County, California (Mullens and Gerry 2006, Gerry and Mullens 2006, Mohr et
Larval development of canyon flies: Canyon flies use a wide array of organic substrate for their development, ranging from fungi and rotting plant material to decaying animal manure (Chillcott 1960). Winkler and Wagner (1961) reared *F. benjamini* complex eggs from wild caught females and described their larval development under laboratory conditions. They demonstrated that the culture material that can be used for the most successful development of canyon flies is a damp medium, such as urine-moistened feces, dirt or detritus. Mullens and Gerry (2006) observed that *F. conspicua* developed to maturity when given only vegetation, indicating that soil and feces is not absolutely necessary for their development. Poorbaugh (1969) observed that larvae of *F. benjamini* fed on surface of moist (but not wet) microbial films, on fecal or alfalfa pellets in the laboratory and did not enter the substrate, which agrees with the feeding observations of *F. conspicua* larvae, made by Mullens and Gerry in 2006. Gerry and Mullens in 2006 recorded a common larval development site of *F. conspicua* in southern California. In La Habra Heights, the key development site of canyon flies is where red apple (*Aptenia cordifolia* Aizoaceae) is grown, which is a leaf litter or possibly fungi – mold type habitat (Mullens and Gerry 2006). Red apple is an exotic (South Africa) succulent plant, grown throughout the canyon slopes of La Habra Heights, as a ground cover to prevent soil erosion and also as a fire protection. Since its introduction around the early 1980s, it has become the main developmental site of canyon flies in La Habra Heights.
Female *F. conspicua* deposit eggs singly in depressions of old *Aptenia* leaves in the field and in the laboratory. Live immature stages have been observed in soil, where there is a low humidity level (Mullens and Gerry 2006). “Prepupal diapause” in canyon flies has been discussed by Chillcott (1960) and Poorbaugh (1969), with prolonged larval and pupal stages (extended development) of *F. benjamini* observed in rearing dishes that were allowed to dry out (Poorbaugh 1969). “Stasis” in dry conditions has also been suggested for *F. conspicua* by Mullens and Gerry (2006). The exact number of generations that occur per year is unknown, but about 3–5 generations have been suggested, with some adult activity throughout the year (Mullens and Gerry 2006). They also observed that, under laboratory conditions *F. conspicua* males emerge earlier than females, similar to the observations of Poorbaugh (1969), for *F. benjamini*. In La Habra Heights male swarms and the presence of males near the ground cover of red apple suggest that they are there in search of females that emerge from the soil under the ground cover, or red apple itself (Mullens and Gerry 2006).

**Feeding behavior of canyon flies:** *Fannia* spp. commonly feed on natural plant sugar sources such as honey dew and plant sap (Chillcott 1960). Winkler and Wagner (1961) found that under laboratory conditions, sugar or food of some kind is essential for the adult survival beyond a few days, and that flies that had access to sugar lived longer than those that had access to only protein. Mullens and Gerry (2006) documented that canyon flies that were not given any food, under laboratory conditions, died quickly, whereas canyon flies that were provided access to water, dry milk and sugar survived up to 130
days at laboratory conditions. Mohr et al. (2011a) reported that canyon flies of both sexes commonly fed on fructose sugars with 99.94% of host-seeking female flies and 98.93% of swarming male flies having gut sugar levels exceeding those of starved flies. They also showed that host-seeking female flies had significantly higher overall sugar content than swarming male flies. Mullens and Gerry (2006) noted that female flies with access to sugar only survived longest but laid only a few eggs, while flies with access to raw liver laid many eggs but survived less than 27 days. These findings indicate that a source of protein is necessary for egg development in female *F. conspicua*. Male canyon flies are rarely collected in sweep net collections near a host. This suggests that the vertebrate host possesses a material that is required only by the females and this is most likely for reproduction, specifically for the development of eggs (Gerry and Mullens 2006).

**Seasonal abundance and daily activity patterns of canyon flies:** Canyon flies belong to the subfamily Fanniinae, which is temperate in distribution, but the *F. benjamini* complex has a preponderance of Neotropical members (Chillcott 1960). Mullens and Gerry (2006) documented the seasonal adult activity of *F. conspicua* and *F. benjamini* in southern California and recorded that both species are intolerant of high temperatures, because their numbers are reduced with the onset of very hot summer weather. This is corroborated by the fact that they are more common in coastal mountain range habitats, than in warmer inland areas. They also noted that *F. benjamini* may be more sensitive to high temperatures than *F. conspicua*. While the adult activity of both species peaked in June - July in their main study site in La Habra Heights, at a second study site in a much
hotter inland southern California location (Riverside, CA) *F. conspicua* were active from late April through early June with a second activity period from September-October. Although both species could be collected year round at the La Habra Heights study site, *F. benjamini* was relatively more common in cooler weather between November and April.

The daily activity pattern of most species of insects is limited in the broadest sense to the time of day with a characteristic light level, i.e. day, night, or twilight (Gibson and Torr 1999). Gerry and Mullens (2006) observed that diel host seeking by female *F. conspicua* appeared to be bimodal, with peak activity in early morning and early evening. The peak host-seeking activity was concentrated in the hour after sunrise and the hour before sunset, and with little activity during the hottest hours of the day (Mohr et al. 2011a). During the early summer months, the peak activity hours occurred from 0700 to 0800 in the morning and between 1900 and 2000 in the evening (Mohr et al. 2011a). Host seeking activity tends to be driven predominantly by the position of the sun above the horizon, with host seeking greatest when the sun is near the horizon, and with an increase in solar height resulting in reduced activity (Mohr et al. 2011a). By documenting the daily behavioral patterns, in conjunction with the documentations on their seasonal behavior (Mullens and Gerry 2006), the highest fly activity times have been estimated for *F. conspicua*. This would be very helpful in the establishment of control measures targeted for the times that these flies are most actively host-seeking.
**Trail Gnats:** Similar to canyon flies, trail gnats (*Amiota picta* Coquillett) (Diptera: Drosophilidae) are also nuisance pests, generally attracted to humans, especially to the face and eyes. They have a body length of 2 to 3 mm and are smaller than *F. benjamini* (Powell and Hogue 1979). They are primarily recorded from southern, central and coastal California mountain ranges north to the San Francisco Bay area (Powell and Hogue 1979), but with collections also recorded from southeastern Arizona (specimen in UCR Entomology Museum), indicating a more widespread distribution throughout the southwestern U.S. Both *F. benjamini* and *A. picta* are known to be encountered near streams (Chillcott 1960, Powell and Hogue 1979). The larval development sites of trail gnats have not been documented, but their abundance in native habitats suggests that their larval development sites are in the natural environment.

**Response to Host Odors:** Carbon dioxide (CO$_2$), a nearly universal kairomone for host-seeking Diptera (Gibson and Torr 1999) has been shown to attract hematophagous insects such as culicids (Gillies 1980), glossinids (Vale 1980), tabanids (Thornhill and Hays 1972) and simuliiids (Sutcliffe 1986). Like canyon flies, the face fly *M. autumnalis* De Geer, also requires host-derived proteins such as those present in tears, for egg development. The on-host populations of face flies also tend to be heavily female biased which indicates that female host-seeking is associated with the need for host-derived substances for egg development. There is at least anecdotal evidence that face flies respond to CO$_2$ under field conditions (Pickens and Miller 1980). The attraction to CO$_2$ shows a wide variation among different insect species (Wilson and Richardson 1970,
Gillies and Wilkes 1972) and has been made use of in many vector surveillance programs. While non-attractant traps have also been used in insect surveillance (Bidlingmayer 1974), visual and olfactory cues that attract insects have been the major consideration when designing trapping methods to capture various species of Diptera (Gillies 1974).

Gerry and Mullens (2006) showed that female canyon flies could be captured using CDC light traps (without the light) baited with a source of CO₂. CDC light traps are mostly used for surveillance purposes; especially in mosquito surveillance programs (Moore et al. 1993). This is mainly because they capture a greater number and variety of adult mosquitoes in comparison to other trap types, such as resting boxes, malaise traps, ovitraps etc. (Williams and Gingrich, 2007). CDC light traps have been studied extensively as a human host surrogate for the estimation of mosquito landing / biting rates, especially for malaria vectors (Barnard et al. 2011). Although canyon flies are not typically blood feeders, their powerful attraction to CO₂ baited traps also indicates that vertebrate hosts have something they require; possibly protein rich secretions that are useful for egg development (Gerry and Mullens 2006). The attraction to CO₂ has been utilized in several studies where CDC-type suction traps were used to collect canyon flies. In a study conducted by Gerry and Mullens (2006), in La Habra Heights, CDC light traps (without the light) caught a total of 7112 canyon flies of which the majority were F. conspicua, while only 0.2% of the collection was F. benjamini. This suggests that either F. conspicua is much more abundant during the time of the day and time of the year that
the traps were operated, or that *F. benjamini*, though present in the study site, was not readily collected in these CO$_2$ baited suction traps. Mullens and Gerry (2006) documented that between June–July, in the same study site, the host sweep net collections were also comprised mainly of *F. conspicua* (94 – 98%), with *F. benjamini* being between 2-6% of the sweep net collections. Therefore, comparison of trap catches and host sweep net collections in the above studies suggest that, *F. benjamini* is less likely to be collected in a trap, than by a sweep net collector.

Adult female canyon flies respond positively to mammalian host odors, such as ammonia, when it is presented in addition to carbon dioxide (Mohr et al. 2011a). These authors showed that ammonia works synergistically with CO$_2$ in CDC light traps (without the light), and will capture more *F. conspicua* than the combined collection of canyon flies in the traps baited with CO$_2$ or ammonia alone. Ammonia is also found to work in synergy with other host odors such as the sweat odor L-lactic acid to increase capture of mosquito species such as *Aedes aegypti* L., although ammonia was not attractive when used alone (Geier et. al. 1999). Host-seeking Diptera are also known to respond to other host associated volatiles such as 1-octen-3-ol (octenol) (Vale and Hall 1985, Holloway and Phelps 1991), which were first isolated from the breath of oxen. Octenol, in combination with CO$_2$ is known to increase trap catch in several mosquito species (Takken and Kline 1989).
**Response to color:** The host-seeking behavior of insects involves many factors of which olfaction plays an important role, and is often integrated with other senses, especially vision (Nicolas and Sillans 1989). Long and middle range orientation of many insects attracted to hosts is driven by host specific odors, mainly CO₂ (Bursell 1987). In close range orientation, where most directed visual responses occur, behavior is affected by the physical make-up of the host: color, size, shape and movement (Bradbury and Bennett 1974, Gillies and Wilkes 1982). Most hematophagous Diptera are attracted to black color (Gibson and Torr 1999) presumably because of its similarity to the skin color of a host. Adult sand flies (*Phlebotomus papatasi* Loew) are more attracted to darker surfaces or surfaces contrasted with dark and light colors compared to white surfaces (Kline et al 2011). To date, no studies have been conducted on the color preferences of canyon flies.

**Barrier Trapping For Pest Management:** The use of CDC light traps (without the light) baited with CO₂, and perhaps synergized with additional host odors has been suggested for the use in a barrier trapping system or trap-out program to mitigate nuisance attributable to the *F. benjamini* complex (Mohr et al. 2011b). Barrier trapping is defined as the construction of a protective perimeter barrier consisting of killing stations baited with an attractant, between the area to be protected and the source of the biting insects (Day and Sjogren 1994). It is positioned to collect insects moving upwind in search of hosts. If the wind direction is changing in the area, the area to be protected must be surrounded by such a barrier of baited killing stations. A barrier trapping system is mainly targeted at protecting a small area from nuisance insects. In this way, barrier
trapping contrasts with a removal trapping (or trap-out) program where the goal is to achieve area-wide suppression of a targeted arthropod species through large scale removal of the pest, typically using baited traps or insecticide-treated targets (Day and Sjogren 1994). In a removal trapping program, traps or lethal targets are used to lure large numbers of individual insects to centralized killing stations for the purpose of pest population reduction.

The greatest success story of removal trapping is the control of the medically important disease vector, tsetse fly in different parts of Africa, especially, in West Africa (Vale 1993, Torr 1994). This included the first successful use of animal mimic type tsetse traps, in the late 1920s, in a control campaign directed against Glossina pallidipes (Harris 1938). The traps were designed to imitate the appearance of large ungulates and were deployed over several hundred square kilometers of Africa, in a seven year program that reduced tsetse population by 99.9% (Day and Sjogren 1994). While there has been some success in the control of Hippelates, Stomoxys and tabanids in similar trap-out programs, at present, removal trapping is only considered as a minor part of vector control.

The first documented use of a barrier trapping system was when sticky traps, baited with a source of CO₂ (12.5 lb block of dry ice) was used as a perimeter barrier around a pasture, in Louisiana (Wilson 1968). This sticky trap barrier significantly reduced the number of tabanids on cattle within the pasture. In Cape Cod (1970 – 1971), 300-730 box traps were placed around the perimeters of salt marshes to capture and kill green head
flies (*Tabanus lineola* Fabricius and *T. nigrovittatus* Macquart), which resulted in a noticeable, measurable decrease in their population and nuisance level in the nearby beaches (Wall and Doane 1980). In 1993 – 1995, a project was conducted on Key Island, Collier County, FL (Kline and Lemire 1998) to evaluate the impact of a protective barrier around a designated resort area on attacks by pest species of mosquitoes, primarily *Anopheles atropos* Dyar and Knab, *Culex nigripalpus* Theobald, and *Ochlerotatus taeniorhynchus* Widemann. The efficacy of single line barriers, consisting of traps (1994) and impregnated targets (1995), baited with CO$_2$ and octenol, resulted in a noticeable (but not significant: P > 0.05) reduction in mosquito abundance in the resort area, when the barrier was functional. In this study, the success of the barrier was evaluated by means of baited surveillance traps located on both sides of the barrier. When the trap system was modified to a perimeter CO$_2$ pipeline, with targets that were sprayed with insecticide (permethrin or lambda-cyhalothrin), and with octenol also released near the target, there was a significant difference between the ratio of mosquitoes collected inside and outside the pipeline when the system was on, compared to when it was off.

Barrier trapping has also been used for the control of tsetse populations in Ivory Coast, where biconical traps, each impregnated with 400 mg of Deltamethrin, spaced at intervals of 300m along a 82 km section of gallery forest was used to control *Glossina palpalis* populations involved in the transmission of animal trypanosomiasis (Day and Sjogren 1994). This program achieved a 96.6% reduction in the tsetse population. The use of “Mosquito Magnet Pro” traps along a nature trail on an isolated island (Atsena Otie) in
the Gulf of Mexico resulted in a significant reduction in the annoyance caused by the black salt marsh mosquito *O. taeniorhynchus* Wiedemann (Kline 2007), while in another study where attractive toxic sugar bait solutions were sprayed on to the surrounding vegetation near ponds resulted in a 94% reduction of *C. pipens* (L.) (Muller et al. 2010). In this study, CDC traps have been used to monitor the reduction of mosquitoes inside the barrier compared to the surrounding area.

**Study Objectives:** The present research was conducted as two main studies in two different study sites in California. The first study was conducted in La Habra Heights, Los Angeles County, California, an area where many of the recent canyon fly studies have been conducted. The purpose of the study was to determine if CO₂-baited suction traps, arrayed in a barrier along the perimeter of a hilltop known for high canyon fly activity, will reduce the number of host seeking *F. conspicua* that successfully reach a human host within the protected area. The second study was conducted in the Santa Lucia Preserve, Carmel, California, where *F. benjamiini* and (unexpectedly) *A. picta* have been causing significant nuisance to the residents in the area. Understanding the daily activity patterns of pest species and if they can be collected using methods that have been used to catch similar species is very useful when developing management techniques to control these nuisance pests. The purpose of the second study was to determine the daily activity patterns of *F. benjamiini* and *A. picta*, their preference to hosts wearing light or dark colored clothing, and the relative capture efficiency of CO₂ baited suction traps in collecting these nuisance flies, with or without other secondary host related odors.
References


Figures

Figure 1.1: Female *F. conspicua* (A) and female *F. benjamini* (B).
Figure 1.2: Antenna and arista of female *F. conspicua* (A) and female *F. benjamini* (B).
Chapter 1

Management of Host-seeking Canyon Flies using a Barrier Trapping System

Abstract

Host seeking “canyon flies” (*Fannia benjamini* complex) (Diptera: Muscidae) cause significant nuisance to humans and animals. A circular perimeter barrier of CO$_2$-baited CDC-type suction traps (without the light) was placed in a hilltop location known for high *Fannia conspicua* Malloch activity, in La Habra Heights, Los Angeles County, California, during the peak activity season, to determine if a barrier trapping system using attractive traps would reduce the number of canyon flies that successfully reached a human host within the protected area. To select a suitable radius for the protective barrier, the distance from a human collector, at which an operating CO$_2$ trap would reduce the fly capture rate by the human (the interaction distance) was evaluated by placing CO$_2$ traps along a trap line at 10, 20, 30 and 40 m away from a human collector. The number of flies captured by the human collector was greatly reduced by a CO$_2$ trap operated at 10 m from the human collector, only slightly reduced at 20 m, and unaffected by traps placed $\geq$ 30 m away. Based on these results and study site characteristics, a protective barrier was erected, comprised of 8 CO$_2$ traps placed in a 15 m radius circle encompassing a human collector positioned at the center of the protected area. The protective barrier captured 88% of the flies attracted to the area, but reduced the number of flies reaching the human
collector by only 51% due to an increase in the total number of flies attracted to the area relative to collection periods when the barrier trap was not in operation. A reduced barrier radius of 5 m resulted in a 39% reduction of the number of canyon flies that reached the protected human collector. Implications of using a barrier trapping system to manage canyon flies are further discussed.

Key words: canyon fly, *Fannia*, barrier trap, host-seeking, carbon dioxide
Introduction

Flies of the *Fannia benjamini* Malloch complex (Diptera: Muscidae), commonly known as “canyon flies” are significant pests of humans and animals. This group is represented by several species in the southwestern United States (Chillcott 1960). Female canyon flies feed on animal body secretions such as tears, mucus, sweat and saliva, and blood from open sores or wounds (Garcia and Radovsky 1962), from which they acquire proteins needed for egg development (Mullens and Gerry 2006). Once a suitable animal host has been located, the persistence with which these flies attempt to land on the head and body results in considerable nuisance to both humans and animals. At geographic locations where these flies are numerous, their host-seeking behavior can severely limit human use of the outdoor environment.

*Fannia conspicua* Malloch, a member of the *F. benjamini* complex, is an emerging pest in the dry canyon areas of coastal and southern California (Mullens and Gerry 2006). This species is present year round, with a seasonal activity peak between early June and late July (Mullens and Gerry 2006). During the peak activity months in southern California, *F. conspicua* display distinct diel activities, with host-seeking activity concentrated in the hour after sunrise and the hour before sunset, and with little activity during the hottest hours of the day (Mohr et al. 2011a).
Although not typically blood feeders, *F. conspicua* are readily captured in suction traps baited with carbon dioxide (CO$_2$) (Gerry and Mullens 2006), especially when combined with ammonia as an additional host odor (Mohr et al. 2011b). The use of suction traps baited with CO$_2$ and perhaps synergized with additional host odors has been suggested for use with a barrier trapping system or trap-out program to mitigate nuisance attributable to these flies (Mohr et al. 2011b).

The goal of a trap-out program is to achieve area-wide suppression of a targeted arthropod species through large scale removal of the pest, typically using baited traps or insecticide-treated targets (Day and Sjogren 1994). A more limited, and perhaps achievable, approach would be to protect a small area by intercepting the targeted arthropod using attractive traps placed along a barrier, separating the protected area from the surrounding unprotected habitat (Day and Sjogren 1994).

The purpose of the current study was to determine if CO$_2$-baited suction traps arrayed in a barrier along the perimeter of a hilltop known for high canyon fly activity, will reduce the number of host seeking *F. conspicua* that successfully reach a human host within the protected area.
Materials and Methods

Study site: The study was conducted at a hilltop location in the coastal mountain city of La Habra Heights (33° 57’ N, 117° 57’ W, 280 m elevation), Los Angeles County. This area has been utilized in previous canyon fly studies, due to the presence of a large population of *F. conspicua* and minimal human activity that may disrupt a study (Mullens and Gerry 2006). The study site borders a residential area to one side and is otherwise surrounded by hills and canyons with native vegetation. Slopes near residential homes in this community are commonly planted with the low-growing succulent plant red-apple (*Aptenia cordifolia*), as a ground cover to prevent soil erosion and to serve as a fire break separating homes from the often dry native vegetation in the adjacent canyons. Although red apple is not native to California, it has become a common developmental site for *F. conspicua* in this region following the introduction of this plant in the mid 1980s (Mullens and Gerry 2006). All trials were conducted during June-August 2011, when canyon fly host-seeking activity was at its seasonal peak (Mullens and Gerry 2006).

Human-Trap Interaction Distance: Two trap lines, each consisting of a single human collector and four CDC-type miniature suction traps (Model 512, John W. Hock, Gainesville, FL), with the lights removed, were placed on a hilltop at the study site where canyon fly host-seeking activity was known to be high. A human collector (trap line collector) was positioned at one end of each trap line and used a short handled sweep net (45 cm diameter opening) to catch flies attracted to their immediate vicinity during each
sampling period. The four suction traps in each trap line were separated from the trap line collector by increasing 10 m distances (10-40 m) (Figure 2.1A). Suction traps were suspended 0.7 m above the ground and 10 cm below a black 3.8 L insulated paint can (possible visual cue) with a lid. A 567 g (20 oz.) carbon dioxide (CO₂) tank with a two-stage regulator was positioned beneath each suction trap with vinyl plastic tubing containing a miniature inline flow restrictor (4LR-010 D06, Norgren, Littleton, CO) ensuring constant delivery of 350 ml/min of CO₂ to the area just above the trap fan housing. This CO₂ flow rate is within the range of the resting human CO₂ expiration rate (calculated from Ganong 2003). The delivery of CO₂ to the suction trap was easily turned on or off as desired using the regulator, with proper gas flow confirmed using a flow meter each time it was turned on. Canyon flies attracted to the vicinity of the suction traps were collected in fine mesh catch bags placed below the suction trap fan housing (Figure 2.2).

The two trap lines, each with a trap line collector and 4 CO₂-baited suction traps, were arranged along a north–south axis, perpendicular to the predominantly westerly winds, and separated by a distance of 150 m at the closest points (Figure 2.1A). A third human collector (control collector), was positioned midway between the two trap lines at a distance of 75 m from each trap line. The control collector provided a relative measure of local canyon fly activity during each collection period and was unaffected by the presence of nearby. Trapping began each day when the control collector first captured ≥
10 canyon flies within a 5 min period, and trapping ended when the control collector captured < 10 canyon flies within a 5 min period.

Each sampling period began with a pre-sampling collection, where all human collectors simultaneously captured flies by sweep net for 5 min to remove any flies that had accumulated near them during trap set up prior to the start of the sampling period. Study participants communicated via wireless handheld transceiver to ensure a simultaneous start and end to each collection period. Immediately following the pre-sampling collection, each trap line collector turned on one of the four traps within their trap line, and quickly returned to their terminal trap line position to begin a 5 min “trap on” sweep net collection. During each collection period, only one suction trap was turned on and had a flow of CO$_2$ supplied; all other traps remained off and without attractant. Following the “trap on” collection period, each trap line collector turned off the suction trap and again quickly returned to their terminal trap line position to conduct a 5 min “trap off” sweep net collection. This pattern of “trap on” followed by “trap off” collections was continued until a pair of collections was made for each of the four trap distances within the trap line. A sampling period thus consisted of a pre-sampling collection followed by four paired “trap on” and “trap off” collections. The order in which the CO$_2$-baited suction traps at each distance were turned on within the sampling period was randomly determined and was the same for both trap lines.
Sampling periods were separated by a 15 min rest period when captured insects were killed and placed into labeled collection vials for later identification and counting. Captured flies were identified to species and sex (Chillcott 1960, Turner 1976) with the number of female *F. conspicua* recorded for each collection. Of 16 paired collections (repetitions) for each of the four trap line distances obtained from 15-29 June 2011, only 15 were retained for analysis as one paired collection was discarded due to a trap set-up error (Appendix 1).

**Barrier Trapping:** To evaluate the efficacy of barrier traps to reduce canyon fly activity within a protected zone, 8 CO$_2$-baited CDC-type suction traps (without light) were placed equidistant along the perimeter of a 15 m radius circle to form a protective barrier of attractant traps surrounding a single human collector (barrier collector). The 15 m barrier radius was selected based on sampling site characteristics and the results of the human-trap interaction study (above), and with 8 traps on the perimeter barrier, this resulted in a separation between traps of 11.8 m. Another human collector (control collector) was positioned on the same hilltop, but 150 m away from the barrier trap perimeter, to provide a measure of fly activity, unaffected by the presence of CO$_2$ traps, during each collection period (as in the study above) (Figure 1.1B). All CO$_2$ traps were equipped as in the earlier study, with a 567 g (20 oz.) CO$_2$ tank, two-stage regulator, and flow restrictor so that CO$_2$ gas could be rapidly turned on or off at a constant flow rate of 350 ml/min. Barrier trapping was conducted during the morning canyon fly activity period from 08 July – 04 August 2011.
As in the earlier study, trapping began each morning when the control collector first captured \( \geq 10 \) canyon flies within a 5 min period. Also as before, each sampling period began with the two human collectors simultaneously performing a 5 min pre-sampling sweep net collection to remove flies accumulated near the collectors, prior to the start of the sampling period. The pre-sampling collection was immediately followed by a 5 min “trap off” collection period. The barrier collector then quickly turned on all eight suction traps along the trap barrier and returned to the center of the protected area. At this time, another 5 min pre-sampling collection was performed simultaneously by both human collectors to remove any flies accumulated near them before the collections began and when the barrier collector was turning on the traps. This was followed by a 5 min “trap on” collection period while all 8 traps in the trap barrier remained “on”. Traps were then turned off to end the sampling period.

Sampling periods were separated by a 20 min rest period when captured insects were killed and placed into labeled collection vials for later identification and counting. The rest period allowed the dissipation of CO\(_2\) produced at the sampling site by the CO\(_2\)-baited suction traps. Twenty paired “trap on” and “trap off” barrier trap collections were obtained, but two of these collections were not used in the analysis due to human or animal interference at the study site during a collection period (Appendix 2).

The study was repeated with the 8 CO\(_2\)-baited suction traps in a barrier trap perimeter with a reduced radius of 5 m to evaluate the effect on the attack rate at the human
collector when traps were moved well within the human-trap interaction distance. This also reduced the separation between the eight equidistant barrier traps from 11.8 m to only 3.9 m providing greater overlap of the attraction range for each perimeter trap. An additional 18 paired collections were obtained using the same methodology as described above (Appendix 3).

**Statistical Analysis for Human-Trap Interaction Distance:** Pre-sampling collections were not used in the analysis. All analyses were performed with SAS 9.2 (SAS Institute Inc. 2009) and MINITAB 15 (2012 Minitab Inc. State College, PA) and $P < 0.05$ was considered as statistically significant. As the host-seeking activity of canyon flies changes throughout the day (Gerry and Mullens 2006, Mohr et al. 2011a), the “trap on” and “trap off” sweep net collections at each distance cannot be directly compared. Therefore, the control collector provided a means to standardize each of these collections so that changes in background fly activity could be separated from any treatment effects. To ensure that the fly capture rate of the control collector was sufficiently related to the fly capture rate of the two trap line collectors, the number of flies captured by each human collector when traps were off was log$_{10}$ transformed and subjected to regression analyses. The capture rate for each of the trap line collectors was significantly positively correlated with capture rate for the control collector ($R^2 = 0.42$ and 0.47, $P < 0.0001$). To standardize collection data, the number of flies captured by each trap line collector during each collection period was divided by the number of flies caught by the control collector during the same collection period to give a ratio (proportion) of relative activity. Activity
ratios calculated for collection periods when all suction traps were turned off are “trap off” ratios while ratios calculated for collection periods when a single suction trap was turned on are “trap on” ratios. For each separate trap line, the difference (“diff”) between a “trap off ratio” and the paired “trap on ratio” (diff = trap off ratio – trap on ratio) was subsequently calculated for each paired collection period, as a measure of the effect of a CO₂ trap placed at one of the four distances, on the fly capture by the trap line collector. A diff = 0 would indicate no effect of trap operation on the fly capture rate of the trap line collector.

A multivariate mixed linear model ANOVA (PROC mixed) was used to evaluate significant differences in the “trap on” and “trap off” capture ratios, with “diff” as the response, trap “distance” (10, 20, 30 or 40m) and “time of day” (morning or evening) and their interaction as fixed factors, and “trap line collector” and “collection date” as random (covariate) factors. Lacking an interaction between distance and time of day, variation in “diff” for each trap distance was further examined by least squares means (LSMEANS) to test whether the mean “diff” for each distance was significantly different from zero. The trap capture ratios (Trap catch / Control collection at the same time period) at each of the 4 distances, separated by the trap line (trap line 1 or 2) were also compared by means of a two-way ANOVA (trap line and distance) to determine if there was a difference in the collection of flies when a trap was at 10 m, 20 m, 30 m or 40 m.
**Statistical Analysis for Barrier Trapping:** Similar to the trap line part of the study, presampling collections were discarded and not used in the analysis. All analyses were performed with MINITAB 15 (2012 Minitab Inc. State College, PA). For each collection period, the number of flies caught by the barrier collector was divided by the number of flies caught by the control collector to give a ratio of relative fly capture, thereby adjusting the barrier collector capture rate for changes in background fly activity. Fly capture ratios calculated for collection periods when all barrier traps were turned off are “trap off” ratios while ratios calculated for collection periods when all barrier traps were turned on are “trap on” ratios. Because the study was conducted on 7 days, and because the random variable “day” considered in the PROC mixed analysis in the first part of the study was not significant, a paired t-test was conducted between the “trap off” and “trap on” ratios across all study days to assess variation in fly capture by the protected barrier collector when barrier traps were on relative to when barrier traps were off.

The difference (“diff”) between the “trap off ratio” and the “trap on ratio” (diff = trap off ratio – trap on ratio) was also calculated for each paired collection period. The percentage reduction in the number of canyon flies that reached the barrier collector when the traps were turned on relative to periods when the traps were off (percentage reduction = “diff” / “trap off ratio”) was calculated for the 15 m and 5 m barriers separately. To further compare the effect of trap barrier radius, the number of flies capture by all 8 traps during each collection period was converted to a mean per trap capture and then divided by the number of flies captured by the control collector during the same collection period,
giving a relative mean trap capture for each collection period adjusted for changes in background fly activity. Relative mean trap captures were then compared by t-test with unequal variance for significant differences between barrier trap radii (15 m or 5 m). The trap capture ratios (Trap catch / Control collection at the same time period) for each of the 8 trap positions, separated by “repetition” were also compared, for the 15 m and 5 m barriers separately, by means of two-way ANOVA (trap position and repetition) to determine if there was a difference in the collection of flies in traps at different positions. The 8 positions were compared using Tukey’s multiple comparison method to determine significant differences among positions.
Results

**Human – Trap interaction distance:** The average 5 min sweep net collection for the two trap line collectors when no traps were turned on was 249 female *F. conspicua*. The maximum 5 min sweep net collection by a single human collector was 923 *F. conspicua*, by a trap line collector, at 1930 hrs on 15 June 2011. The maximum CO$_2$ trap catch was 162 flies captured in a trap placed at 10 m from a human trap line collector, at 1810 hrs on 27 June 2011. The collector: trap ratio when a trap was positioned at 10 m, 20 m, 30 m and 40 m were 3.7: 1, 5.6: 1, 5.1: 1 and 5.4: 1 respectively. During this part of the study an impressive total of 82013 female *F. conspicua* was captured and removed from the study site, with no obvious impact on canyon fly host-seeking activity during subsequent days.

There was significant variation in the difference (“diff”) between paired trap on and trap off sweep net capture ratios by time of day (morning vs. evening) (F = 8.51; df = 1, 99; P < 0.05), indicating that the relationship between the fly capture rate of the trap line collector and the control collector varied somewhat between morning and evening, perhaps due to variable shade cover at each location throughout the day. There was no interaction between time of day and trap distance (F = 1.12; df = 3, 99; P > 0.05); consequently, collection data were not separated by time of day for further analyses. The mean “diff” value was not significantly altered by trap distance (F = 1.21; df = 3, 99; P > 0.05), by trap line collector (F = 1.02, df = 1, 99; P > 0.05), or by collection date (F =
1.89; df = 4, 99; P > 0.05). However, when the reduced model was used in the Least Square Mean Test, the mean difference in trap ratios was significant when traps were operated at 10 m (T = 3.34; P < 0.005) and 20m (T = 2.12; P < 0.05) away from the human trap line collector, but not at 30 m or 40 m away (P > 0.05) (Figure 2.3). However, the mean trap capture ratio, corrected for the control was not significantly different among the 4 distances (F= 0.33; df = 3, 104; P > 0.05). The two trap lines were significantly different (F = 6.69; df = 1, 104; P < 0.05), but since there was no interaction between the distance and trap line (F = 1.03; df = 3.104; P > 0.05), the difference between the trap lines were not further analyzed.

Results for Barrier Trapping: When testing the 15 m barrier, the mean number of flies captured by the barrier collector during the 5 min collection period was 187 female *F. conspicua* with the barrier traps off and 55 *F. conspicua* with the barrier traps on; a very significant reduction in the number of flies reaching the protected barrier collector when the barrier traps were on (T = 6.01, P < 0.001) (Figure 2.4). The mean trap catch in a CO2 trap was 52 *F. conspicua*. When traps were on, the barrier collector and the 8 barrier traps collectively captured a mean of 471 *F. conspicua*, considerably more than were captured by the barrier collector alone when the traps were off or by the control collector when the traps were on (Mean collection = 213).

Similarly, when testing the 5 m trap barrier, the mean number of flies captured by the barrier collector during the 5 min collection period was 91 *F. conspicua* with the barrier
traps off and 27 *F. conspicua* with the barrier traps on; a significant reduction in the number of flies reaching the protected barrier collector when the barrier traps were on (T = 2.73, P < 0.05). The mean trap catch in the CO\textsubscript{2} traps was 22 *F. conspicua*. At the time the 5 m trap barrier was turned on, the barrier collector and the 8 traps, on average, caught 203 *F. conspicua*, which is much greater than the collection of the barrier collector (91) when the traps were turned off, or the control collector when the traps were on (Mean collection = 94).

The 8 barrier traps comprising the protective barrier captured 88.32\% or 86.28\% of the total flies captured during a “trap on” collection period with a trap barrier radius of 15 m or 5 m, respectively. The 15 m and 5 m barrier did not differ in the number of flies captured relative to the fly capture of the control collector during the same period (T = 0.75; df = 1,24; P = 0.460). Only one trap caught significantly more canyon flies compared to the other traps (F= 5.88; df = 7, 119; P < 0.001; Tukey’s comparison P < 0.05) when the 15 m radius barrier was turned on. The trap with this higher collection was the only trap in a position shaded by trees. There was no significant difference in the flies collected in the traps in different positions in the 5 m barrier (F = 1.87; df = 7, 119; P > 0.05). Overall, the number of canyon flies reaching the barrier collector was reduced by 51\% and 39\% of the expected number (adjusted by control collector counts) for the 15 m and 5 m perimeter barriers, respectively. A total of 53627 *F. conspicua* were collected and removed from the study site during this part of the study.
Discussion

Canyon flies cause significant nuisance to humans and animals in areas where they are abundant. The present study demonstrated that attractive traps placed in a perimeter barrier surrounding an area can be successful in reducing the number of canyon flies that enter the protected area. In this study, traps arrayed in either a 15 m or 5 m barrier captured 86-88% of the total flies captured in the protected area. However, the presence of the barrier traps with their associated CO2 output increased the overall number of flies attracted to the area by 369 – 404 % relative to collection periods when the traps were not operated. With this increase in the number of flies attracted to the protected area, the 12-14% of flies that reached the protected human collector represented an actual reduction of only ≤ 51% of the flies that would have reached the human collector in the absence of a trap barrier. While this system may work better in at lower attraction rates, this level of protection is not sufficient to consider this method for area protection against canyon flies in areas with very high fly populations. Perhaps the trap barrier system could be improved through optimization of the separation distance between barrier traps and through use of supplemental host odors to increase trap attraction and fly capture (e.g. see Mohr et al. 2011).

According to the results of the human-trap interaction part of the study, the 15 m barrier, which showed a 51 % reduction in canyon flies, was at a distance where the human to trap interaction was comparatively weak. The 5 m barrier was within a high human-trap
interaction range and showed only a 39 % reduction in the flies that reached a host at the center of the barrier. Therefore, in future studies, it would be important to consider the effect on the percentage reduction if the traps were moved beyond 30 m, where no interaction between the trap and the collector was shown. Also, the present study used only 8 CO\textsubscript{2} traps in the barriers with a radius of 15 m and 5 m. It would also be useful to change the number of traps and analyze the outcome with more or less traps in the perimeter barrier.

Gerry and Mullens (2006) noted that \textit{F. conspicua} are attracted to areas of the body where sweat accumulates. A major component of sweat, urine, feces and other body products associated with humans and animals is ammonia (Noble and Somerville 1974; Richards et al. 1975), which is known to attract hematophagous insects like mosquitoes (Rudolfs 1922, Braks et al. 2001) and horseflies (Hribar et al. 1992). Mohr et al. (2011b) showed that when CO\textsubscript{2} and ammonia are offered together, the two compounds act synergistically to increase the trap catch of \textit{F. conspicua} by 1.7-fold over the combined capture (additive effect) of traps baited with CO\textsubscript{2} or ammonia alone. The present study required turning the traps on and off within a short period of time, with a time lag of 15 min -20 min between collections. This time lag was sufficient for the dissipation of CO\textsubscript{2}, as evident by the lack of flies when the collectors returned to the study site for a subsequent sampling period. In addition, the use of CO\textsubscript{2} tanks allowed turning the flow of CO\textsubscript{2} “on” and “off” as needed, which was advantageous over dry ice, the most commonly used CO\textsubscript{2} source in modified CDC traps. Additional host odors were not utilized in this
study because their release could not be readily turned on or off between collection periods. Nevertheless, it is presumed from Mohr et al. (2011b) that the addition of host odors like ammonia would have increased trap capture and would be suitable for use in a protective trap barrier.

A total of 137640 canyon flies was collected and removed from the study site, not including flies from pre-sampling periods. However this large removal did not seem to have a significant impact on the canyon fly population in the area, as flies remained numerous throughout the study period. *F. conspicua* is a native fly, but this fly is known to readily develop within the undergrowth of an exotic succulent groundcover, red apple (*Aptenia cordifolia*), which has been planted on slopes near residential homes throughout this canyon area to prevent soil erosion and for fire protection (Mullens and Gerry 2006). The widespread planting of red apple throughout the region is likely responsible for the high number of *F. conspicua* in the study area. Considering the large population of canyon flies in the area and the cost of a trap-out program, as well as the limited effect on the fly population even after the removal of more than 140,000 *F. conspicua* from the area during the study period, a trap-out program would also be unsuitable. A barrier trapping system, as in the present study, can be used only when flies are host seeking or only when area protection is needed, thus reducing costs and possible environmental impacts relative to a trap out or area-wide fly control program.
The primary concern of the barrier trapping system using CO$_2$-baited suction traps, as used in this study is, whether a 39 – 50 % (depending on the radius of the barrier) reduction of canyon flies within a protected area is sufficient, when considering the large numbers that were collected in the area, and the cost and effort of implementing a barrier trapping system, to a home owner. Instead of using suction traps in the barrier, “Attractive Toxic Sugar Baits” (ATSB), that contain oral insecticides, which can be sprayed on vegetation or around a selected area, can also be considered to protect an area from nuisance flies (Schlein and Muller 2010). While these methods are based on the attraction and removal of a nuisance insect species from the area to be protected, instead of attractants, repellants can also be considered in personal or area protection from nuisance flies. A future consideration for canyon fly control could utilize a “Push – Pull – Strategy”, also known as a “Stimulo deterrent diversionary strategy”, where a combination of behavioral modifying stimuli are used to manipulate insects and deter them away (push) from the area to be protected using a deterrent or repellent, while simultaneously attracting them (pull) using an attractive stimuli to another area, where they are eliminated (Cook et al. 2007). Masking techniques may also be considered to disrupt the host-seeking behavior of these flies and reduce their ability to detect and orient towards a host (e.g. see Turner et al. 2011). Both these methods, as well as a modified barrier trapping system that also utilizes other host attractants should be considered when finding a method to better control these nuisance causing, host-seeking canyon flies.
References


Figures

Figure 2.1: (A) Spatial arrangement of the two trap lines with terminal human trap line collectors and four CO₂-baited traps, separated by a human control collector positioned 75 m from each trap line, and (B) the spatial arrangement of the perimeter trap barrier (15 m radius or 5 m radius) with 8 CO₂–baited suction traps comprising the barrier, and with the barrier collector positioned at the center of the protected area, and the human control collector 150 m away from the barrier (BC = barrier collector, 1-8 = CO₂-baited traps).
Figure 2.2: CO$_2$–baited suction trap with CO$_2$ supplied as compressed gas with a release rate of 350 ml/min controlled by a two stage regulator and an inline flow restrictor. (A- 3.8 L Insulated paint can with lid, B- Suction trap fan housing, C- Fine mesh catch bag, D- Flow regulator, E- 567 g CO$_2$ tank)
Figure 2.3: Average of ratios between sweep net collections made by the trap line collectors and the control collector when CO2-baited suction traps were placed at 10 m, 20 m, 30 m, and 40 m.
Figure 2.4: Average of ratios between the sweep net collections made by the barrier collector and the control collector when a barrier was placed with a radius of 15m and 5m.
Chapter 2

Host-seeking activity of two nuisance fly species, the “canyon fly” *Fannia benjamini* (Diptera: Muscidae) and the “trail gnat” *Amiota picta* (Diptera: Drosophilidae), in Carmel Valley, California

**Abstract**

The diel activity of the host-seeking “canyon fly” *Fannia benjamini* Malloch and the “trail gnat” *Amiota picta* Coquillett was determined by hourly sweep net collections made in September, in the Santa Lucia Preserve, in Carmel, California. Both species showed two daily high activity periods, one in the morning (0830–0930) and another in the evening (1630–1830). Activity of *A. picta* almost ceased during midday hours, while *F. benjamini* maintained low host–seeking activity throughout the day. The total sweep net collection of *F. benjamini* was greater when the collector was standing in the shade, compared to when standing at a sun–exposed position. The sweep net collections made by two collectors when wearing either dark or white colored clothing were also compared. There was no significant difference in the attraction of *F. benjamini* or *A. picta* to collectors wearing dark or white clothing. CDC- type suction traps (without the light) baited with a source of CO$_2$ were used to compare the collection efficiency of a human collector and a trap. The human collector caught significantly more *F. benjamini* and *A. picta* compared to the CO$_2$ trap. Using additional known host attractants, namely,
ammonia and 1-octen-3-ol, in the CO$_2$ traps did not significantly increase the trap collections of _F. benjamini_ or _A. picta_. The inability to use CO$_2$ traps in collecting these species is further discussed.

**Key words: nuisance fly, diel activity, California**
Introduction

Nuisance flies cause stress or irritation to humans and animals by their near-host activities. Of these flies, those species that make direct contact with a host to feed on host body secretions are particularly bothersome. Nuisance flies are typically not equipped to draw blood from a host, though some species may feed on blood from pre-existing host wounds (e.g., see Garcia and Rodovsky 1962). The most widely known of these nuisance flies are those associated with animal agriculture, mainly due to their habit of developing in animal feces. Face flies (*Musca autumnalis* De Geer) and bush flies (*M. vetustissima* Walker) that feed on tears, saliva, mucous, blood or other excrement from pasture animals, especially cattle, are common examples of nuisance flies associated with animal agriculture. Other host-attracted nuisance fly species are associated with native habitat where their development sites are less well characterized. In the United States, several unrelated native fly species are known to attack humans and animals causing considerable nuisance in habitats where they are locally abundant. These native flies include the widely distributed “eye gnats” (Chloropidae: *Hippelates* spp.), as well as the more focally distributed “canyon flies” (Muscidae: *Fannia benjamini* Malloch complex) and “trail gnat” (Drosophilidae: *Amiota picta* Coquillett).

“Canyon flies” are known to be significant human and animal pests throughout the western U.S., particularly in foothill areas of coastal, central and southern California. In areas where they are abundant, these flies cause significant nuisance to humans and
animals by flying around and landing on the head and body to feed on tears, mucus, sweat, and even blood from open sores or wounds caused by other biting flies (Garcia and Rodovsky 1962, Poorbaugh 1969). Canyon flies range from 3.5 to 4.5 mm in length with seven recognized species in the complex (Turner 1976). Flies in this complex were considered intermediate hosts of the California eye worm, *Thelazia californiensis* Price (Winkler and Wagner 1961), but it was later determined that only one member of the species complex, *F. thelaziae* Turner (1976), supported eye worm development and transmission (Weinmann et al. 1974).

Recent studies on this pest complex have focused on a single species, *F. conspicua* Malloch, which is a significant nuisance pest in hillside communities of southern California (Gerry and Mullens 2006; Mullens and Gerry 2006; Mohr et al 2011a, b). Little is known about the remaining species in the complex, with the exception that Winkler and Wagner (1961) noted *F. benjamini* host-seeking activity to be greatest either early or late in the day and that even a slight breeze would result in the cessation of this activity.

*Amiota picta* Coquillett, commonly known as “Trail gnats”, are nuisance pests commonly encountered along nature trails in California (Powell and Hogue 1979). They are primarily recorded from southern, central and coastal California mountain ranges north to the San Francisco bay area (Powell and Hogue 1979), but with collections also recorded from south eastern Arizona (UCR) indicating a more widespread distribution throughout
the southwestern U.S. They have a body length of 2 to 3 mm making them smaller than *F. benjamini*. Like canyon flies, little is known about the biology, ecology, or habits of trail gnats, other than a noted association with wild natural areas near streams (Powell and Hogue 1979).

The host-seeking behavior of insects involves many factors of which olfaction plays an important role and is often integrated with other senses, especially vision (Nicolas and Sillans 1989). Long and middle range orientation of many insects attracted to hosts is driven by host specific odors, mainly carbon dioxide (CO$_2$) (Bursell 1987), which is regarded as a potent and essentially universal kairomone for obligate blood-feeding Diptera (Gibson and Torr 1999). Female *F. conspicua* canyon flies are readily captured using suction traps baited with CO$_2$ (Gerry and Mullens 2006). Ammonia acts synergistically with CO$_2$ to increase the trap catch of female *F. conspicua* (Mohr et al. 2011 b). Many host–seeking Diptera are also known to respond to other host associated volatiles such as 1-octen-3-ol (Vale and Hall 1985, Holloway and Phelps 1991). However, it is not known if traps baited with CO$_2$ or other host associated volatiles can be used to capture *F. benjamini* or *A. picta*.

In close range orientation, where most directed visual responses occur, behavior may also be affected by the physical makeup of the host: the color, size, shape and movement (Bradbury and Bennett 1974, Gillies and Wilkes 1982), with many hematophagous Diptera attracted to black or dark colors (Gibson and Torr 1999), presumably because of
the similarity to the body color of many suitable hosts. The preference for colors has not been examined for flies of the *F. benjamini* complex or for *A. picta*.

The current study was designed to determine the diel activity of *F. benjamini* and *A. picta* during the putative peak activity season at a California coastal foothill location where *F. benjamini* was known to be abundant. Additionally, preference for hosts wearing light or dark color clothing and relative capture efficiency of CO$_2$ baited suction traps with or without other host related odors was examined.
Materials and Methods

Study Site: The study was conducted at the Santa Lucia Preserve (36°26′ N, 121°47′ W, 440.4m elevation) in the upper Carmel Valley along the central coast of California from 07-12 September 2011. The Preserve is comprised of protected native habitat encompassing a small number of sparsely distributed residential homes. It has a large diversity of plant and animal species commonly associated with redwood forests, oak woodlands, savannahs, grasslands, coastal prairies, and streams and wetlands.

A single undeveloped residential lot at the Preserve was utilized for this study. This residential lot was situated along the ecotone between oak woodland and open grassland meadow, and retained its native vegetation including large mature oak trees with an undergrowth of grasses and shrubs. The site was bordered by a similar unoccupied property, a road, an occupied lot with several buildings, a water treatment pond, and a dry stream bed filled with poison ivy (Toxicodendron radicans (L.)). A single gravel driveway bisected the lot leading from the lot entrance, heavily shaded by large oaks, toward the back of the lot which was typically in full sun and contained low to tall grasses and shrubs. Columbian black-tailed deer (Odocoileus hemionus columbianus Richardson) were commonly encountered moving through this study site or bedding in the tall grasses in or adjacent to the site.
Fly Collection Methods: Flies were captured by human collectors using short handled sweep nets (45 cm diameter opening), with the collectors catching all the insects in the vicinity of their head and body during a 5 min collection period (unless noted differently below). CDC light traps without the light (Model 512, John W. Hock, Gainesville, FL) were also used and were suspended 0.7 m above the ground and 10 cm below a black 3.8 L paint can (possible visual cue). The traps were baited with CO$_2$ at a flow rate of 350 ml/min from a 567 g (20 oz.) CO$_2$ tank with a two-stage regulator (4LR-010 D06, Norgren, Littleton, Colorado).

Diel activity of nuisance flies: Flies were captured by sweep net on 09 and 12 September from 0630 (before sunrise) to 1930 (after sunset) on each day. Sunrise occurred at 0643 and 0647 while sunset occurred at 1927 and 1921 on 09 and 12 September, respectively. Two human collectors were positioned 100 m from each other along the gravel driveway, with one collector randomly assigned to a shaded location near the lot entrance, while the other collector was positioned in a sun-exposed location near the back of the lot. Each collector made a simultaneous sweep net collection at half past each hour. The two collectors switched collection positions on the second collection day. Immediately following each collection, temperature, humidity, wind speed and wind direction were measured and recorded (Appendix 4).

Between diel collections, variation between the relative fly capture rates of the two collectors was examined as follows. The two human collectors were positioned in the
shade along the gravel road, separated by 20 m, and simultaneously conducted a sweep net collection. The collectors then switched positions and made another simultaneous collection using a second sweep net. This comparative collection was repeated four times (with each repetition consisting of 4 collections made by the 2 collectors in the 2 different positions), but never more than once between any two diel collections, so that the study site was vacant of collectors for at least 15 min before the start of the next diel collection (Appendix 5).

**Comparison between host clothing colors:** Between diel collections on 12 September, variation in the relative fly capture rate of the collectors when wearing light or dark clothing was examined. Collectors wore dark clothing, cotton pants and long sleeve shirts, or a white Tyvek cover-all over the dark clothing. The two human collectors were positioned in the shade along the gravel road, separated by 50 m, and simultaneously conducted a sweep net collection after being randomly assigned to wear either their dark clothing or the white cover-all. Following this first collection, collectors remained at the same collection site but immediately switched to the alternate clothing color for another simultaneous sweep net collection. This comparative collection was repeated four times (with each repetition consisting of 4 collections made by the 2 collectors wearing the 2 different colored clothing), but never more than once between any two diel collections so that the collection site was vacant of collectors for at least 15 min before the start of the next diel collection (Appendix 6).
Relative collection efficiency of Human collector and CO$_2$-baited trap: The attraction of *F. benjamini* and *A. picta*, to a human collector using a sweep net and to a CO$_2$ baited trap were compared on 8 September, between 0950 and 1810. A single trap and a human collector were randomly assigned to positions 50 m apart, along an east-west direction, in a shaded area of the study site, and 10 min paired sweep net and trap collections were made. After the 10 min collection period and another 15 min to allow any remaining CO$_2$ to dissipate, the position of the human collector and CO$_2$ trap was alternated for an additional 10 min collection period. These two collection periods comprised a single repetition and a total of six repetitions (with each repetition consisting of 2 collections made by the human collector at each of the 2 different positions and 2 trap collections at the 2 different positions) were conducted (Appendix 7).

On 9 September, CO$_2$ traps were placed in three positions, North, East and West, 30m apart from each other, in similar environments, under the shade of oak trees. They were randomly assigned to have one of the following treatments: 1) CO$_2$ only, 2) CO$_2$ and ammonia, or 3) CO$_2$, ammonia and 1-octen-3-ol, with each treatment rotated through the three trap locations. Both ammonia (5 ml) and 1-octen-3-ol (5 ml) were contained in small glass vials with a cotton wick at the vial opening, and attached to the trap near the CO$_2$ out-flow tube (following the methods of Mohr et al. 2011b). This allowed the continuous release of these volatile chemicals (release rate undetermined). At the start of each repetition a human collector was positioned at a site 50 m away from the nearest
trap to make a 5 min sweep net collection. This was followed by a 45 min trapping period during which all three baited traps were turned on (Appendix 8).

**Specimen handling:** Flies captured in the sweep nets and traps were knocked down using CO$_2$ and sorted under a field microscope by species and sex (Chillcott 1960, Turner 1976, McAlpine et al. 1981) prior to being counted and stored in labeled vials containing 70% alcohol.

**Statistical Analysis:** All statistical analyses for this study were performed using either MINITAB 15 (Minitab Inc. State College, PA) or GraphPad InStat (GraphPad Software, San Diego, CA) software packages and $P \leq 0.05$ was considered as statistically significant. The number of *F. benjamini* captured by the two collectors in simultaneous collections under similar conditions was compared using a paired t-test for variation in the mean fly capture by individual collector. This analysis was repeated for *A. picta*. With no variation in the mean fly capture rate between human collectors for either fly species, the number of flies (of each species) captured during each diel sweep net collection was analyzed by General Linear Model (GLM) ANOVA for differences in the mean capture rate by time of day, by collection site (shade or sun), and by collection date. A chi-squared test was also conducted between the mean collections of *F. benjamini* and *A. picta* made in the sun and in the shade.
For clothing color trials, the differences in the number of *F. benjamini* or *A. picta* collected by the 2 collectors wearing either dark or white colored clothing was analyzed by two-way ANOVA (human collector and clothing color). Similarly, the collection efficiency of the human collector and the trap were also compared by two-way ANOVA (Collection method: trap or human collector and Position: East or West). Traps baited with additional host odors were compared to each other and to the human collector, by means of one-way ANOVA and the differences between treatment means were further analyzed using Tukey’s honestly significant test.
**Results**

The collection days were sunny and warm, with clear skies throughout the study period. The average daytime temperature was 21.9°C, with a maximum temperature of 28.6°C and a minimum of 14.3°C. The average day time humidity was 52.6%, with a minimum of 28%. The average wind speed was 0.65 m/s, with a maximum wind speed recorded as 2.7 m/s (Figure 3.1A, 3.1B).

*Comparison of the two human collectors:* The two human collectors were not significantly different in their sweep net capture rate of *F. benjami*ni with a mean ± SE of 13.1 ± 2.1 and 13.1 ± 1.8 flies captured per collector (T = 0.00; df = 1, 8; P = 1.00). The capture rate of *A. picta* also did not differ between human collectors (T = 1.99; df = 1, 8; P = 0.087), but the capture rate was relatively low (0.8 ± 0.5 and 3.0 ± 1.1, respectively) (Figure 3.2).

*Diel Activity of Fannia benjami*ni and *Amiota picta:* The diel activity of *F. benjami*ni and *A. picta* each show two main high activity periods, one in the morning and one in the evening (Figure 3.1A, 3.1B). The morning activity peak for *F. benjami*ni occurred from 0930 - 1030 with a high evening activity period at around 1630 - 1730. The high activity period of *A. picta* in the morning was from 0730 - 0830 and again in the evening at 1830. Following the morning activity peak, *F. benjami*ni continued to actively host seek throughout the day, but at a somewhat lower level. In contrast, activity of *A. picta*
essentially ceased with only very low activity during the inter-peak period throughout the day. Therefore, both species show reduced activity during mid-day, when the environmental temperature was at its highest (Figure 3.1A, 3.1B). Greater numbers of canyon flies were collected when the collector was positioned in the shade relative to when the collector was standing in a sun-exposed position (Figure 3.3).

The maximum *F. benjamini* collected by either collector during a 5 min collection period was 30, whereas the maximum collection of *A. picta* was 32. There was no significant difference in the number of flies captured on the two collection dates for *F. benjamini* (F= 0.29; df= 1, 40; P = 0.593) or *A. picta* (F = 0.27; df= 1, 40; P = 0.607). There were significant differences in the number of *F. benjamini* collected by time of day (F = 2.86; df = 13, 40; P = 0.005), and especially the collections made when the collectors were standing either in the sun or the shade (F = 18.35; df= 1, 40; P < 0.001) (Figure 3.3). The number of *A. picta* collected at different times of the day were different, but this difference was not significant (F = 1.66; df = 13, 40; P = 0.122), nor was the number of *A. picta* captured significantly different in the sun or the shade (F = 0.000; df = 1, 49; P = 0.959). A chi-squared analysis ($\chi^2 = 28.0$; P < 0.0001) indicated that *F. benjamini* was significantly more likely to be captured in the shade relative to *A. picta*. However, definite conclusions cannot be drawn based on this data due to the relatively small sample size.
The data collected on 2 days were not sufficient to draw conclusions regarding the relationship between the activity of *F. benjamini* and *A. picta*, and the environmental parameters. However, a reduction in the number of *A. picta* coincided with a reduction in humidity, suggesting that this species perhaps is more active at higher humidity levels (Figure 3.1A, 3.1B).

**Comparison between host clothing colors:** A mean of 8 and 5 *F. benjamini* were captured when collectors wore dark colored clothing or a white colored suit, respectively (Figure 3.4). However, this difference was not significant (*F* = 2.51; *df* = 1, 12; *P* = 0.139). The capture rate again did not differ by human collector (*F* = 0.75; *df* = 1, 12; *P* = 0.404). The average 5 min collection of *A. picta* was 0.5 during this study, which was insufficient to make any effective comparisons.

**Relative collection efficiency of human collector and CO₂-baited trap:** A significantly greater number of *F. benjamini* and *A. picta* were collected by the human collector (225 *F. benjamini* and 78 *A. picta*) in comparison to the collection in the CO₂ trap (1 *F. benjamini* and 1 *A. picta*) (*F* = 35.93; 7.94, *df* = 1, 44; *P* < 0.001) and the position of the trap relative to the human collector did not affect the capture rate (*F* = 0.02; *df* = 1, 44; *P* = 0.890). Even when additional attractants were used in the traps, the human collector’s total collection of 60 *F. benjamini* was significantly higher than any of the odor treatment collections in the traps (*F* = 7.72; *df* = 3, 6; *P* = 0.018; Tukey’s pair-wise comparison: *P* < 0.05), whereas, there was no significant difference in the *A. picta* collections by the
human collector (37 *A. picta*) or in the traps (F = 1.19; df = 3, 6; P = 0.390). When the
traps were compared without considering the collections made by the human collector,
the total collection of *F. benjamini* and *A. picta* was not significantly different among the
traps with CO₂ only (1 *F. benjamini* and no *A. picta*), CO₂ and ammonia (4 *F. benjamini*
and 3 *A. picta*), or CO₂, ammonia and 1-octen-3-ol (no *F. benjamini* and 2 *A. picta*).
Discussion

Residents and employees of the Santa Lucia Preserve have reported nuisance from flies since the Preserve was founded in 1990. In a previous visit to the Preserve by one of us (ACG) in September 2009, untimed sweep net collections of nuisance flies at three separate locations resulted in the capture of only canyon flies (F. benjamini). Thus, it was quite a surprise when initial sweep net collections during this visit resulted in the capture and identification of a second nuisance fly, the trail gnat (A. picta). Both canyon flies and trail gnats were collected by sweep net from a number of locations throughout the Preserve during the study site selection process at the beginning of the current study. It is likely that previous sampling efforts simply missed the very early or very late activity periods of A. picta resulting in the mid-day capture of only F. benjamini. It was noted by both study participants that A. picta was more persistent in attempts to land on the face than was F. benjamini which could be collected near the face and body.

This study is the first to document the diel activity pattern of F. benjamini and A. picta. Observations of canyon flies in the San Bernardino mountain foothills by Winkler and Wagner (1961) indicated that these flies were mostly active in the early or late hours of the day, in agreement with the activity pattern of F. benjamini in this study and with previous studies on the closely related canyon fly species F. conspicua (Gerry and Mullens 2006, Mohr et al. 2011a). The diel activity of both F. benjamini and A. picta showed two activity peaks, one in the morning and one in the evening, 1-3 hours within
the sunrise and sunset times. During mid-day hours, *A. picta* showed minimal host-seeking activity, while *F. benjamini* remained active, but at a lower level relative to early morning and late afternoon high activity periods. Studies on *F. conspicua* have also shown a similar pattern with reduced host-seeking activity during mid-day hours (Gerry and Mullens 2006, Mohr et al. 2011a). The host-seeking behavior of *F. conspicua* appears to be driven by the position of the sun above the horizon, with an increase in activity when the sun is near the horizon (Mohr et al. 2011a). Similarities in activity patterns suggest that this may be true for *F. benjamini* and *A. picta* as well. However, the limited number of repetitions does not allow well supported conclusions regarding the effects of temperature, humidity, or wind speed and direction, on the activity of these two species.

Flies have the ability to distinguish between different colors (Pichaud et al. 1999). Many hematophagous Diptera are attracted to black color (Gibson and Torr 1999). Female sand flies are significantly attracted to black colored traps or traps with contrasting colors (Kline et al. 2011), while *Chrysops* spp. and *Hybomitra* spp. are attracted to blue and red colors and not attracted to black, yellow or white (Browne and Bennett 1980). In general, attraction to colors by insects is considered to be species or ecotype specific (Burkett et al. 1998, Burkett and Butler 2005). The present study demonstrates that *F. benjamini* and *A. picta* have no significant preference to either dark or light colored clothing worn by human collectors. However, the dark and light clothes worn by the collectors were not
made of the same material. Therefore, this variation in the material of the clothes may have had an impact on the attraction of flies in an unpredictable way.

Carbon dioxide (CO$_2$), a nearly universal kairomone for host-seeking Diptera (Gibson and Torr 1999) has been shown to attract hematophagous insects such as culicids (Gillies 1980), glossinids (Vale 1980), tabanids (Thornhill and Hays 1972) and simulids (Sutcliffe 1986) as well as stable flies (Stomoxys calcitrans L.) in the family Muscidae (Warnes and Finlayson 1985) under which canyon flies are classified. This attraction to CO$_2$ or other animal baits has been used in many trapping methods of different Diptera (Gillies 1974). Gerry and Mullens (2006), in their studies on $F.$ conspicua, showed that female canyon flies could be collected using CDC light traps (without the light) baited with CO$_2$. The present study indicates that these traps are not suitable for capturing $F.$ benjamini or $A.$ picta. Dekker et al. (2001) used electric nets placed in front of trap entry sites to demonstrate that Anopheles gambiae was attracted to CO$_2$, but did not enter CO$_2$-baited traps. Similarly, the lack of capture of $F.$ benjamini and $A.$ picta in the CO$_2$ traps during the present study may be due to near trap behaviors of the flies preventing their capture, rather than a lack of response to CO$_2$.

Ammonia is commonly found in association with humans and animals as a major component of urine, and in feces, sweat and other body products (Richards et al. 1975). Mohr et al. (2011) showed that when CO$_2$ and ammonia are offered together, the two compounds act synergistically to increase the trap catch of $F.$ conspicua by 1.7-fold over
the combined capture of traps baited with CO₂ or ammonia alone. Another host associated odor, octenol (1-octen-3-ol) is known to attract tsetse flies (Hall et al. 1984) and tabanids (Mizell et al. 2002), with CO₂ and octenol together enhancing the trap catches for several species of mosquitoes (Takken and Kline 1989). However, having ammonia or ammonia and octenol, in addition to CO₂, in the suction traps used in the present study did not significantly increase the number of F. benjamini or A. picta collected in the traps. The additional host odors may have contributed in attracting these flies to the vicinity of the traps, but the traps were not efficient in collecting either of the two species.

Most Fannia pass their larval life in wet and decaying plant and animal material, soft damp medium such as urine-moistened feces, dirt or detritus (Chillcott 1960, Winkler and Wagner 1961). In La Habra Heights, California, where most canyon fly studies have been conducted, the dominant member of the F. benjamini complex in southern California, F. conspicua Malloch, develops in decaying organic matter beneath an exotic succulent plant called red apple (Aptenia cordifolia) (Mullens and Gerry 2006). F. benjamini Malloch is also present in this area in low numbers (Gerry and Mullens 2006). Observations in the present study site did not reveal any obvious larval development sites. However, considering the number of canyon flies that were host-seeking at the site and assuming that dispersal by F. benjamini is relatively limited as it is for F. conspicua (Gerry and Mullens 2006), larval development sites were probably very close by. Several Columbian black-tailed deer were observed in or near the study site, especially near the
stream bordering the study area. *F. benjamini* are known to cluster around the eyes of deer and horses (Poorbaugh 1969), and both *F. benjamini* and *A. picta* are known to be encountered near streams (Chilcott 1960, Powell and Hogue 1979). Most stream banks are well shaded by the branches of large trees growing near the streams, and the present study shows a preference by these flies for shaded areas. Therefore, there is a high possibility that the abundance of *F. benjamini* and *A. picta* is related to these deer that are present throughout the study area.

The presence of *F. benjamini* or *A. picta* causes significant nuisance to the residents and others in the area, making it necessary to implement control measures for the control of these host-seeking dipterans. However, because the Carmel Valley Preserve is a nature conservancy, the use of insecticides is not a recommended method of fly control. A barrier system using traps or non-insecticidal repellents to protect residential sites from these flies might seem an ideal alternative. Identifying the diel activity pattern of these nuisance flies is important in this regard, as it will enable the activation of a barrier trapping system during the times that these insects are actively host-seeking. The CO$_2$ baited suction traps used in this study have been suggested for the use in a barrier trapping system for *F. conspicua* (Mohr et al. 2011). The human collector to trap collection ratio for *F. conspicua* was 5:1 (Ekanayake and Gerry, in preparation) in comparison to the 114:1 ratio of *F. benjamini*, as shown in the present study ($\chi^2 = 53.8; P < 0.0001$). Therefore, the inefficiency of these traps in capturing *F. benjamini* or *A. picta* indicates that these traps cannot be considered in the management of *F. benjamini*
or *A. picta* in Carmel, California. Additional research to identify suitable attractants or repellents, and a better suited trapping system is needed to control these host-seeking nuisance flies in northern California.
References


Figures

A.

Average Collection of *F. benjamini* and *A. picta* on Day 1
B.

**Figure 3.1:** The average number of *F. benjamini* and *A. picta* collected by two collectors on Day 1 (A) and Day 2 (B) at the Santa Lucia Preserve, Carmel, California. The daily temperature (°C), humidity (%) and wind speed (m/s) are also included in the graph.
Figure 3.2: Comparison of the mean (± SE) sweep net collections of *F. benjamini* and *A. picta* made by the two collectors. The data include 4 repetitions with each repetition consisting of four 5 min sweep net collections by 2 collectors at 2 different positions.
Figure 3.3: Mean number of *F. benjamini* collected using sweep nets during a 5 min collection period each hour, in mid-September at the Santa Lucia Preserve in Carmel Valley, California. Data are a mean of collections by two human collectors on two collection days, with the collectors standing in the shade of oak trees or in a position exposed to the sun.
**Figure 3.4:** Mean number of *F. benjamini* and *A. picta* collected using sweep nets during 5 min collection periods, wearing either dark or white colored clothing, during mid-September at the Santa Lucia Preserve in Carmel Valley, California. Data are a mean of collections by two human collectors.
Conclusion

Canyon flies, *Fannia benjamini* Malloch complex (Diptera: Muscidae) cause significant nuisance to humans and animals in areas where they are abundant. The present research was conducted as two main studies; the first related to the management of *F. conspicua* in southern California (La Habra Heights, Los Angeles County) and the second assessing host-seeking activity of two nuisance fly species, the canyon fly (*F. benjamini*) and the Trail Gnat (*Amiota picta*), along the California Coast (Santa Lucia Preserve, Carmel Valley, Monterey County).

**Southern California Study Site:** During the first study conducted in La Habra Heights, Los Angeles County, California, the majority of flies collected were *F. conspicua*, with only a few *F. benjamini* collected throughout the study period. A total of 137,640 *F. conspicua* females were collected from the area during the study period, with a maximum single collection of 1,117 flies by an individual collector during a 5 min collection period. The average 5 min collection by an individual collector was 202 *F. conspicua*, a number much greater than the average collection of about 57 flies reported by Mullens and Gerry (2006) in their studies conducted at the same study site in 2004. However, the removal of such a large total number of canyon flies did not seem to result in an obvious reduction in the canyon fly population in this area, because the collectors continued to capture canyon flies at high numbers throughout the expected activity season. Therefore, the fly population in the area must be very high, or canyon flies are recruited in very large
numbers to the study site, perhaps from continuing emergence of adults from nearby larval developmental habitats.

Long term residents of La Habra Heights report that numbers of adult canyon flies have been drastically higher since the early 1990s, which fits with the known increase in planting of red apple (*Aptenia cordifolia*), an exotic (South Africa) succulent plant, which was first introduced to California landscapes in the mid-1980s (Mullens and Gerry 2006). Red apple is planted on the canyon slopes of this coastal mountain community as a ground cover, to protect against soil erosion and for fire protection. However, planting red apple resulted in decaying plant material and organic debris accumulating beneath the growing succulent plant. This decaying plant material serves as an oviposition and development site for *F. conspicua* larvae (Mullens and Gerry 2006). Due to its value as a ground cover for hillside erosion control and fire protection, and perhaps due to a lack of acceptable alternative ground covers, residents appear to be reluctant to remove the red apple. In addition, the removal of the entire red apple ground cover planted throughout the region would also be an expensive task, if the residents were even willing to attempt it. As it takes several years for the formation of a significant organic debris layer (Mullens and Gerry 2006), continuing increase in larval habitat (even in the absence of new planting of red apple) may significantly contribute to an increasing canyon fly population in this area. Therefore, a different method of controlling canyon flies is needed in these coastal mountain areas.
A major challenge in this study site was the presence of an equestrian riding ring, near the area where the trap line and barrier were operated. During some of the afternoons, it was used by young riders and the presence of 8 – 10 horses and people near the study site brought in more canyon flies than expected, to the area. Therefore, collections had to be spaced out and sometimes even cancelled, in order to avoid any interference from the presence of the humans and animals in the area. Periodically, the road adjacent to the study site was also used by joggers, walkers and their animals, mostly dogs, during the evenings. The collections that were interrupted by the presence of humans or animals were discarded and excluded from any of the analysis in the study.

**Source of CO₂:** The most commonly used source of CO₂ in many attractive traps is dry ice, which releases CO₂ as it sublimes from its holding container. CO₂ sachets have also been utilized, but these sachets have been shown to collect significantly fewer host-seeking species, as well as reduced numbers of insects within a species than traps baited with CO₂ from dry ice (Webb and Russell 2004). This perhaps relates to the concentration of CO₂ released from these two sources. For this study, neither of these CO₂ sources was suitable, because CO₂ output (concentration) needed to be the same throughout the study period and release of CO₂ needed to be rapidly turned on and off throughout a sampling period. It would be difficult to control the flow of CO₂ from dry ice as this would be expected to vary with daily temperature and amount of dry ice utilized, and CO₂ output cannot be readily turned on and off within a short period of time when using dry ice (or a CO₂ sachet). Therefore, a 567 g (20 oz.) carbon dioxide tank was
used in the present study, with a two-stage flow regulator and a separate in-line flow restrictor that ensured a constant delivery of 350 ml/min of CO₂ to the trap; measured by a flow meter before each sampling period. The normal resting human CO₂ expiration rate is between 240 - 360 ml/min (calculated from Ganong 2003). As the collectors in the study were using sweep nets to collect the flies in an open area in the field, an upper value within the resting CO₂ expiration rate was selected as the flow in the CO₂ tanks in the traps. Depending on the body weight and level of activity of a human collector to which a trap is being compared, this CO₂ flow would have to be changed; Barnard et al. (2010) selected 250 ml/min as a suitable CO₂ output for comparison of a CO₂-baited trap, and a human collector within the controlled environment of a glass house.

**Human-Trap Interaction:** Before every paired collection, when the traps were either on or off, a 5 min pre-sampling collection was made by each sweep net collector, and this pre-sampling collection was not included in any of the analyses. The pre-sampling ensured that any canyon flies attracted to the collectors during the trap set-up process were removed, and that the analyses could include direct comparisons of the flies collected during the two 5 min collection periods when the traps were either on or off.

When the human-trap interaction distance was analyzed during the first part of the study, there was a highly significant reduction in the number of flies captured by the human collector when a paired CO₂-baited trap placed 10 m away was turned on (operating), relative to when that trap was off; indicating that the trap had a strong influence on the
sweep net collections made by the human collector. A trap placed 20 m away from a human collector had only a weak influence on the human collector’s sweep net collections. Therefore, for a trap to be useful in drawing canyon flies away from a nearby human host, it would be best if the trap was placed within 20 m of the human host. Traps placed at a distance of ≥ 30 m away from the human collector did not affect the sweep net collections of the paired human collector when traps were on or off. When traps are utilized in a trap barrier surrounding a human host, a barrier radius > 10 m might be expected to reduce the interaction between the trap and the protected human, and a distance between barrier traps of approximately 12 m would ensure overlap of the attractive range of the barrier traps.

**Trap Barrier Performance:** The 15m barrier provided a 51% reduction in *F. conspicua* reaching the human collector, whereas, the 5m barrier provided a 39% reduction in the flies that reached a host at the center of the barrier. Although the 5 m barrier appears to have provided a lower level of protection relative to the 15 m barrier, this difference between the two trap barriers was not significant. In future studies, it would be interesting to move the trap barrier beyond 30 m, while keeping the approximately 12 m distance between barrier traps, to see if a greater level of protection could be achieved when the barrier was considerably beyond the human-trap interaction range.

The ratio of flies captured by the human collector to a single CO$_2$ baited trap was 3.7:1 (human: trap) for traps placed at 10 m from the human collector, 5.6: 1 for the trap at 20
m, 5.1: 1 for the trap at 30 m and 5.4:1 for the CO2-baited trap placed 40 m away from the human collector. With 8 traps utilized in the trap barrier simultaneously capturing flies, it might be expected that the human: trap capture ratio (all 8 traps combined and using the more conservative 5.6:1 ratio) to be decreased 8-fold giving a ratio of 1:1.4 (or 5.6:8). However the actual human: trap capture ratio was approximately 1:7 for both the 15 m and the 5 m trap barrier, indicating that the perimeter barrier arrangement was quite efficient at removing flies entering the area before they reached the protected human at the center of the trap barrier. Approximately 86-88% of the total flies captured were captured in the barrier traps. However, the human collector and the 8 traps together attracted a large number of flies to the study site; a number far greater than would have been expected to reach the human collector in the absence of the barrier traps.

The presence of the barrier traps with their associated CO2 output increased the overall number of flies captured by 369–404% relative to the number of flies captured by the human collector alone when traps were not operated. With this increase in the total number of flies attracted to the protected area, the 12-14% of flies that managed to reach the protected human collector represented an actual reduction of only ≤ 51% of the flies that would have reached the human collector in the absence of a trap barrier. Considering the number of canyon flies that were collected, this level of protection is not sufficient to consider this method for an area protection system against canyon flies.
The present study demonstrated that a perimeter barrier of carbon dioxide (CO$_2$) baited CDC light traps (without the light) surrounding an area to be protected is successful in reducing the number of canyon flies that enter the protected area. However, the reduction in fly activity (numbers of flies reaching the human host) that was achieved was not sufficient to ensure that a homeowner would be satisfied with the results of this fly management option. The present study used only 8 CO$_2$ traps in the barriers at 15 m and 5 m. It would be useful to change the number of traps and analyze the outcome with more or less traps in the perimeter barrier. Furthermore, to implement an efficient barrier trapping system in the field or to consider it for commercial purposes, it would be valuable to standardize the number of traps and the difference in distance between two traps, in order to make it more efficient in reducing the number of canyon flies that can penetrate the barrier. Perhaps the trap barrier system could be improved through optimization of the separation distance between barrier traps, and through the use of supplemental host odors to increase trap capture, as suggested by Mohr et al. 2011b.

**Use of additional odor baits in traps:** Gerry and Mullens (2006) showed that *F. conspicua* are attracted to areas in the body where sweat accumulates. A major component of sweat, urine, feces and other body products associated with humans and animals is ammonia (Noble and Somerville 1974; Richards et al. 1975), and ammonia is known to attract hematophagous insects like mosquitoes (Rudolfs 1922, Braks et al. 2001) and horseflies (Hribar et al. 1992). The magnitude of the response to expired breath from a host may either be due to the additive effects of CO$_2$ and other host odors, as
shown for tsetse flies (*Glossina morsitans orientalis* Vanderplank) (Turner 1971) or from a synergistic effect as suggested for the mosquito *Culex nigripalpus* (Vickery et al. 1966) and the stable fly, *Stomoxys calcitrans* (Warnes and Finlayson 1985). Mohr et al. (2011b) showed that when CO$_2$ and ammonia are offered together, the two compounds act synergistically to increase the trap catch of *F. conspicua* by 1.7-fold over the combined capture of traps baited with CO$_2$ or ammonia alone. The synergistic effects of ammonia and CO$_2$ indicate that it could have been used in traps in combination with CO$_2$ in order to increase the trap catch. However, the present study required turning the traps on and off within a short period of time, with a time lag of 15 min - 20 min between collections. The time lag was sufficient for the dissipation of CO$_2$, as evident by the lack of canyon flies when the collectors returned to the study site. In addition, the use of CO$_2$ tanks allowed turning the flow of CO$_2$ “on” and “off” within a short time period, which was necessary for these studies and could not have been achieved with dry ice. Because ammonia could not be similarly released or removed rapidly, ammonia and other host odors that may attract canyon flies were not used in the present study. It would be valuable however for future studies to examine improvements to a barrier trapping system with other host odors used in conjunction with CO$_2$.

Another host associated odor, octenol (1-octen-3-ol) is known to attract tsetse flies (Hall et al. 1984) and tabanids (Mizell et al. 2002), with CO$_2$ and octenol together enhancing the trap catches for several species of mosquitoes (Takken and Kline 1989). French and Kline (1989) investigated the response of tabanids to canopy traps with CO$_2$ plus 1-octen-
3-ol isolated from cattle, and found that octenol alone increased canopy trap catches by 3 fold over traps without attractants, while octenol and CO₂ together enhanced the trap catch in the total specimens and number of species captured. Therefore, octenol is also a suitable chemical that can be considered in improving the attraction of canyon flies to a barrier trapping system. Furthermore, while both ammonia and octenol could be considered for the use in the barrier traps for the control of *F. conspicua*, a more efficient method of supplying these additional host attractants to the traps would be required. A method similar to the use of bottled CO₂ premixed with octenol, being pulsed into traps through flow meters, as suggested by Day and Sjogren (1994) could be considered in this regard.

**IPM for *F. conspicua***: The nuisance caused by *F. conspicua* to the residents of La Habra Heights is in need of major solutions for control in the near future. The primary concern to using a barrier trapping system (as used in the current study), is whether the 39–50% reduction in the number of canyon flies penetrating the protected area (depending on the radius of the barrier) is sufficient to reduce nuisance to a home owner; especially when considering the large numbers of canyon flies that were still captured within the protected area, and the cost and effort of implementing a barrier trapping system. The barrier trap concept is based on the capture of host-seeking flies in attractive CO₂ baited traps prior to their entry into the area to be protected. Instead of using suction traps in the barrier, “Attractive Toxic Sugar Baits” (ATSB), that contain oral insecticides, which can be sprayed on vegetation or around a selected area, can also be considered to protect an area
from nuisance flies (Schlein and Muller 2010). These ATSB contain fruit juices, beer or similar alcoholic beverages, preservatives and oral toxicants (e.g. Boric acid). The insects get attracted by the floral and fruity scent, while the sugar present in the bait induce and stimulate them to feed on the bait containing the oral insecticide. While ATSB can be suggested for the controlling canyon flies, they may also have non-target effects on other beneficial sugar feeding insects that also get attracted to these toxic sugar baits.

While the above mentioned methods are based on the attraction and removal of a nuisance insect species from the area to be protected, instead of attractants, repellants can also be considered in personal or area protection from nuisance flies. A future consideration for canyon fly control could utilize a “Push – Pull – Strategy”, also known as a “Stimulo deterrent diversionary strategy”, where a combination of behavioral modifying stimuli are used to manipulate insects and deter them away (push) from the area to be protected using a deterrent or repellent, while simultaneously attracting them (pull) using an attractive stimuli to another area, where they are eliminated (Cook et al. 2007). By using PPS, even if flies are not eliminated at the attractive location, the nuisance they cause can be reduced as long as they are removed from the protected area during the limited host-seeking period; early morning and late afternoon in southern California.

Masking techniques may also be considered to disrupt the host-seeking behavior of these flies and reduce their ability to detect and orient towards a host (e.g. see Turner et al.
Volatile odorants that can cause ultra-prolonged activation of CO$_2$-detecting neurons and thereby, compromise the ability to detect CO$_2$ for several minutes, and odors that can inhibit CO$_2$-sensitive neurons have been identified for mosquitoes, Anopheles gambiae, Culex quinquefasciatus and A. aegypti (Turner et al. 2011). Such odors can be used to disrupt the host seeking behavior of insects, and prevent them from orienting towards a host. Odors that can evoke a CO$_2$-like activity have also been suggested for the use as lures in trapping devices (Turner et al. 2011). These methods as well as a modified barrier trapping system utilizing other host attractants should be considered in future studies to control these nuisance causing flies in southern California.

Coastal California Study Site: The second part of the research was conducted along the California coast in the Santa Lucia Preserve, Carmel Valley. The study was conducted mainly because residents and employees of the Santa Lucia Preserve had reported nuisance from canyon flies since the Preserve was founded in 1990. In a previous visit to the Preserve (by ACG) in September 2009, untimed sweep net collections of nuisance flies at three separate locations resulted in the capture of only canyon flies (F. benjamini). However, in the present study, initial sweep net collections conducted in the Preserve resulted in the identification of a second nuisance fly, the “trail gnat” (Amiota picta).

The larval development sites of F. benjamini and A. picta could not be determined at the Santa Lucia Preserve, during this study. However, considering the number of canyon flies that were host-seeking at the study site and assuming that the dispersal by F. benjamini is
relatively limited as it is for *F. conspicua* (Gerry and Mullens 2006) larval development sites were probably very close to the study site. Several Columbian black-tailed deer were observed near the stream that bordered the study area. *F. benjamini* are known to cluster around the eyes of deer and horses (Poorbaugh 1969), and both *F. benjamini* and *A. picta* are known to be encountered near streams which may attract deer or other animals as a source of water and shade (Chillcott 1960, Powell and Hogue 1979). It may be that the abundance of *F. benjamini* and *A. picta* is related to the proximity to larval development sites and the presence of these deer throughout the study area.

**Nuisance Fly Activity:** The present study is the first to document the diel activity patterns of *F. benjamini* and *A. picta*. The diel activity of both *F. benjamini* and *A. picta* showed two periods of high activity, one in the morning and one in the evening, 1-3 hours after sunrise and before sunset. During mid-day, *F. benjamini* continued to show host-seeking activity, even if at a lower level, while *A. picta* showed minimal host-seeking activity more similar to the lack of midday activity by *F. conspicua* in southern California (Gerry and Mullens 2006, Mohr et al. 2011a). The host-seeking activity of *F. benjamini* and *A. picta* ceased after sunset, in Carmel Valley, much like that observed for *F. conspicua* in La Habra Heights (Gerry and Mullens 2006, Mohr et al. 2011a). Winkler and Wagner (1961) also had noted that *F. benjamini* were mostly active in the early or late hours of the day.
The host-seeking behavior of *F. conspicua* appears to be driven by the position of the sun above the horizon, with an increase in activity when the sun is near the horizon and reduced activity with an increase in the solar height (Mohr et al. 2011a). Similarities in diel activity suggest that this may be true for *F. benjamini* and *A. picta* as well. Studies conducted in southern California also indicate that temperature, humidity and wind speed have a small, but significant effect on the activity of *F. conspicua* (Mohr et al. 2011a), while studies by Winkler and Wagner (1961) suggested that even a slight breeze would result in the cessation of *F. benjamini* host seeking activity. However, as only 2 repetitions by two collectors were conducted to determine the diel activity patterns during the present study, no conclusions can be drawn for the relationship between environmental parameters and the activity of *F. benjamini* or *A. picta* in Carmel Valley, California. It may be that all members of the canyon fly complex exhibit a similar bimodal diel activity pattern, with temporal variation from day to day, primarily due to changes in environmental conditions such as precipitation, wind speed, or humidity (Mohr et al. 2011a).

Both canyon flies and trail gnats were collected by sweep net from a number of locations throughout the Preserve during the study site selection process at the beginning of the current study. As the activity period of *A. picta* was about an hour after sunrise and an hour before sunset, it is likely that previous sampling efforts simply missed the very early or very late activity periods of *A. picta*, resulting in the mid-day capture of only *F. benjamini*. It was noted by both study participants that *A. picta* was more persistent in
attempts to land on the face than was *F. benjamini*, which could be collected near the face and body, implying that *F. benjamini* may respond to visual stimuli or host odors other than those associated with host breath when near the host.

**Fly Response to Clothing Color:** Flies have the ability to distinguish colors (Pichaud et al. 1999). Many hematophagous Diptera are attracted to black (Gibson and Torr 1999). Female sand flies are significantly attracted to black traps or traps with contrasting colors (Kline et al. 2011), while *Chrysops* spp. and *Hybomitra* spp. are attracted to blue and red, and not to black, yellow or white (Browne and Bennett 1980). Allan and Stoffalano (1986) reported that *Tabanus nigrovittatus* preferred blue, black and red panel traps. In general, attraction to colors by insects is considered to be species or ecotype specific (Burkett et al. 1998, Burkett and Butler 2005). The present study conducted in Carmel, California demonstrates that *F. benjamini* and *A. picta* have no significant preference to either dark or light colored clothing worn by human collectors. However, the dark and light clothes worn by the collectors were not standardized. The dark clothes were mostly cotton while the white Tyvek cover-all are a synthetic fabric and perhaps leading to differences in light reflection unrelated to color, which may have had an influence on the attraction to host-seeking insects.

**Evaluation of Odor-baited Traps:** Carbon dioxide, a nearly universal kairomone for host-seeking Diptera (Gibson and Torr 1999) has been shown to attract hematophagous insects such as culicids (Gillies 1980), glossinids (Vale 1980), tabanids (Thornhill and
Hays 1972) and simulids (Sutcliffe 1986), and this attraction to CO₂ has been used in various trapping methods to capture different species of Diptera (Gillies 1974). Gerry and Mullens (2006) showed that female canyon flies could be captured using CDC light traps (without the light) baited with a source of CO₂. It resulted in a significantly higher capture of female canyon flies (up to about 2000 flies per trap in a 6-h period) relative to traps baited with other materials or no bait (Gerry and Mullens 2006). However, the trap catch in their studies were mainly *F. conspicua*, with only 0.2% of the trap catch (of a total of 7112) being *F. benjamini*. The present study indicates that, a suction trap baited with CO₂ is not a suitable collection method for *F. benjamini*. Dekker et al. (2001) used electric nets placed in front of trap entry sites to demonstrate that *Anopheles gambiae* was attracted to CO₂, but did not enter CO₂ baited traps at a higher rate than un-baited traps. Therefore, the CO₂ may have attracted *F. benjamini* to the vicinity of the baited traps in the present study, but perhaps the traps were simply not efficient at collecting these flies. At least with the currently tested trap design, CO₂-baited traps would not be useful in a barrier trapping program or a trap-out program for the management of *F. benjamini*.

Mohr et al. (2011b) showed that, for *F. conspicua*, CO₂ and ammonia worked synergistically to increase the trap catch by 1.7-fold over the combined capture of traps baited with CO₂ or ammonia alone. In the present study, even the presence of ammonia or ammonia and octenol, in addition to CO₂, in the suction traps did not significantly increase the number of *F. benjamini* collected in the traps. Near trap flight behavior was not evaluated for either the canyon fly or trail gnat in this study.
IPM for *F. benjamini*: The presence of *F. benjamini* and *A. picta* causes nuisance to residents and conservationists in the Carmel Valley area, making it necessary to implement control measures for these host-seeking dipterans. However, as the Carmel Valley Preserve is a nature conservancy, the use of broadly applied insecticides is not recommended. A barrier system using traps or repellents to protect residential sites from these flies might seem ideal. Identifying the diel activity pattern of *F. benjamini* and *A. picta* was important in this regard, to enable the activation of a barrier trapping system only during the times that these insects are actively host-seeking.

The CO\textsubscript{2} baited suction traps used in this study have been suggested for use in a barrier trapping system for *F. conspicua* (Mohr et al. 2011a), but the inefficiency of these traps in capturing *F. benjamini* or *A. picta*, as demonstrated by the present study, indicate that these traps cannot be considered in the management of *F. benjamini* or *A. picta*. Additional research to identify suitable attractants or repellents, and perhaps a new trap design that might improve capture of flies attracted to the trap vicinity is needed to control these nuisance flies. In addition, identifying the larval development sites and determining if larval control methods can be used, may also be important in the control of these flies. Furthermore, the role of the deer, abundant throughout the study area, on the fly population, and if the control of deer may provide a method of controlling the flies in the area could also be considered in the control of these host-seeking nuisance flies.
**SEM Photos:** The majority of the species in the *F. benjamini* complex are not major vectors of animal or human diseases. Only *F. thelaziae* is now considered an intermediate host of the California Eye worm, *Thelazia californiensis* Price (Weinmann et al. 1974, Turner 1976). Canyon flies also are not strictly hematophagous insects, though they are attracted to hosts in order to obtain host secretions and may even feed on blood if present. However, while not being blood feeders, there is the possibility that these flies may in fact be able to make wounds on their hosts. This is because preliminary SEM photos taken on *F. conspicua* and *F. benjamini* collected in La Habra Heights, California, in January 2011 confirmed the presence of “prestomal teeth” in their labellum (Figure 4.1).

Family Muscidae, under which canyon flies are categorized, includes many species that either take blood as their major source of food or else are facultative hematophages (Elzinga and Broce 1986). Many observations have been made on biting muscoid flies earlier, but the most significant observations were by Stephens and Newstead (1907), who described the presence of prestomal teeth with lateral and terminal serrations in the labellum of stable flies, *Stomoxys calcitrans* (L.). They further described the presence of petiolate blades and used the concept of a carpenter’s auger to indicate the mode of beak action by these structures. Comparative SEM studies by Elzinga and Broce (1986) suggested that many Calypteratae possess prestomal teeth, and that the shape, size and number of these structures are associated with their mode of feeding. However, their comparative study did not include members of the *F. benjamini* complex. The presence of prestomal teeth in the preliminary SEM photos of *F. conspicua* and *F. benjamini*
suggests that there is a possibility that *Fannia* may be able to “bite” a host, probably through scraping at thin epithelial coverings over mucous membranes or at a fibrous clot over a recent wound site, in order to obtain blood. However, the present preliminary studies are insufficient to make definite conclusions about the exact feeding mechanisms of canyon flies.

**Research Needs for Canyon Flies:** Given the enormous reproductive potential of insects, it is generally believed that large scale mass trapping programs are only effective against species with low population densities, or when initiated early in the season to remove enough insects to limit the natural build-up of the population (Kydonieus and Beroza 1982). The major difficulties in mass trapping are the large number of traps required, and the high cost of deploying and maintaining them. Despite the acknowledged limitations, mass trapping has been the method most explored for mosquito population reduction to date (Kline 2007). However, considering the large population size, the area wide distribution and the current information on the biology and development of canyon flies, a mass scale trap out program to control them may not be successful in La Habra Heights or in Carmel, California. In addition, as there is no known medical or veterinary diseases transmitted by these flies (other than the previously mentioned eye worm), and because the main problem they cause is mostly nuisance, a trap out program may also be too costly to be used for canyon flies.
The seasonal and daily host-seeking behavior of these nuisance flies have been studied and well documented (Mullens and Gerry 2006; Gerry and Mullens 2006; Mohr et al. 2011a). Therefore, a control measure that can be set-up during the time of the year when the population becomes too numerous, and the time of day when they are actively host-seeking would be the most efficient method of controlling these nuisance flies. Furthermore, even though canyon flies of the *F. benjamini* complex are known to cause significant nuisance to humans and animals in the areas where they are abundant, only a few studies have been conducted on these flies. More research needs to be conducted to understand the morphology, physiology, ecology and life cycle patterns of these nuisance flies in order to develop effective management strategies against these host–seeking canyon flies in California.
References


Figures

**Figure 4.1:** SEM photographs of Prestomal teeth of *F. conspicua* (A) and *F. benjamini* (B).
# APPENDIX 1

## Human Trap Interaction Distance

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**Abbreviations:**

Rep.: Repetition number

Dis.: Distance between the trap line collector and the Trap

Con: Sweep net collections made by the Control Collector at the time a trap was turned on in the trap line.

Cof: Sweep net collections made by the Control Collector when all traps were turned off.

T1on: Sweep net collections made by the Trap Line Collector 1, when a trap in the trap line 1 was turned on.

T1: Number of flies collected in a single trap at 10 m, 20 m, 30 m or 40 m.

T1of: Sweep net collections made by the Trap Line Collector 1, when all traps were turned off.

T2on: Sweep net collections made by the Trap Line Collector 2, when a trap in the trap line 2 was turned on.

T2: Number of flies collected in a single trap at 10 m, 20 m, 30 m or 40 m.

T2of: Sweep net collections made by the Trap Line Collector 2, when all traps were turned off.
APPENDIX 2
Barrier Trapping – 15 m Radius Barrier

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Table 2: Sweep net collections of *Fannia conspicua* made by the Control Collector and Barrier Collector when the traps were “on” or “off”, and the collections in the CO₂-baited Traps, in a trap barrier with 8 traps and a radius of 15 m.
**Abbreviations:**

Rep.: Repetition number

Cof: Sweep net collections made by the Control Collector when the trap barrier was turned off.

Bof: Sweep net collections made by the Barrier Collector when the trap barrier was turned off.

Con: Sweep net collections made by the Control Collector when the trap barrier was turned on.

Bon: Sweep net collections made by the Barrier Collector when the trap barrier was turned on.

T1, T2, T3, T4, T5, T6, T7 & T8: Number of flies collected in trap 1 through trap 8 respectively, when the trap barrier was turned.
### APPENDIX 3

**Barrier Trapping – 5 m Radius Barrier**

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**Table 3:** Sweep net collections of *Fannia conspicua* made by the Control Collector and Barrier Collector when the traps were “on” or “off”, and the collections in the CO₂-baited Traps, in a trap barrier with 8 traps and a radius of 5 m.
Abbreviations:

Rep.: Repetition number

Cof: Sweep net collections made by the Control Collector when the trap barrier was turned off.

Bof: Sweep net collections made by the Barrier Collector when the trap barrier was turned off.

Con: Sweep net collections made by the Control Collector when the trap barrier was turned on.

Bon: Sweep net collections made by the Barrier Collector when the trap barrier was turned on.

T1, T2, T3, T4, T5, T6, T7 & T8: Number of flies collected in trap 1 through trap 8 respectively, when the trap barrier was turned.
APPENDIX 4

Diel Activity of *Fannia benjamini* and *Amiota picta*

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*Table 4:* Number of *F. benjamini* and *A. picta* collected at each hour on 2 collection days and the hourly wind speed, humidity (RH %) and temperature (Temp).
APPENDIX 5

Comparison of Sweep Net Collections of Two Human Collectors

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<th>Amiota picta</th>
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**Table 5:** Sweep net collections of *Fannia benjamini* and *Amiota picta* made by two human collectors.

**Abbreviations:**

Rep.: Repetition number – Each repetition consisted of two collections made by each human collector at two different positions.
### APPENDIX 6

**Comparison of the Effect of Human Clothing Color**

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**Table 6:** Sweep net collections of *Fannia benjamini* and *Amiota picta* made by two human collectors wearing either dark or white colored clothing.

**Abbreviations:**

Rep.: Repetition number – Each repetition consisted of two sweep net collections made by each human collector wearing either dark or light colored clothing.
APPENDIX 7

Relative Collection Efficiency of a Human Collector and a CO\textsubscript{2}-baited Trap

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<th>Amiota picta</th>
<th>Fannia benjamini</th>
<th>Amiota picta</th>
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<td>3</td>
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<tr>
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<td>10</td>
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</table>

Table 7: Sweep net collections and trap collections of *Fannia benjamini* and *Amiota picta* within 10 min collection periods.

Abbreviations:

Rep.: Repetition number – Each repetition consisted of 2 sweep net collections and 2 trap collections at 2 positions.
APPENDIX 8

Relative Collection Efficiency of a Human Collector and CO$_2$-baited Traps

Baited with Additional Host Attractants

<table>
<thead>
<tr>
<th>Rep.</th>
<th>Time</th>
<th>Collection Method</th>
<th>Fannia benjamini</th>
<th>Amiota picta</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0925</td>
<td>Human Collector</td>
<td>8</td>
<td>35</td>
</tr>
<tr>
<td>1</td>
<td>0925</td>
<td>Trap with CO$_2$ only</td>
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<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0925</td>
<td>Trap with CO$_2$ &amp; Ammonia</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>0925</td>
<td>Trap with CO$_2$, Ammonia &amp; Octenol</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>1020</td>
<td>Human Collector</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>1020</td>
<td>Trap with CO$_2$ only</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1020</td>
<td>Trap with CO$_2$ &amp; Ammonia</td>
<td>1</td>
<td>0</td>
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<tr>
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<tr>
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<td>1105</td>
<td>Trap with CO$_2$ &amp; Ammonia</td>
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<td>0</td>
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<tr>
<td>3</td>
<td>1105</td>
<td>Trap with CO$_2$, Ammonia &amp; Octenol</td>
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<td>0</td>
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</tbody>
</table>

Table 8: Collections of *Fannia benjamini* and *Amiota picta* made by a human collector using a sweep net and in traps baited with CO$_2$ only, CO$_2$ and Ammonia and CO$_2$, Ammonia and Octenol.

**Abbreviations:**

Rep.: Repetition number – Each repetition consisted of a sweep net collection and collections in 3 CDC-type suction traps.