Pollinator Study on Lakeside Ceanothus (*Ceanothus cyaneus*) and San Diego Thorn-mint (*Acanthomintha ilicifolia*)

Prepared for the
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Section 6 Project Final Report
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Project Background

Lakeside Ceanothus (*Ceanothus cyaneus*) was originally described by Alice Eastwood in 1927. It is a narrow endemic shrub in the Buckthorn Family (*Rhamnaceae*) that occurs in the San Diego Region and extreme northern Baja California. As with most Ceanothus species, Lakeside ceanothus is found within mixed chaparral and chaparral communities. This species can grow quite tall, up to five meters. It is subject to hybridization with other species of Ceanothus (Reiser, 1994).

Lakeside ceanothus flowers between April and June (Beauchamp, 1986; Munz, 1974; Reiser, 1994; and Skinner & Pavlik, 2001). This time line may shift due to location, elevation and seasonal rainfall. This is one of the unique features of this shrub since most of the Ceanothus in San Diego is known to flower two to three months earlier. One of the other distinguishing features is its intense deep cyan blue flowers. Flowers are small and form clusters with many clusters occurring on each stem and branch. When flowering Lakeside Ceanothus may appear nearly solid blue with a smattering of green leaves peeking through.

Figure 1: Lakeside Ceanothus (*C. cyaneus*) shrub in flower

Figure 2: Lakeside Ceanothus (*C. cyaneus*) flower cluster

Figure 3: Lakeside Ceanothus (*C. Cyaneus*) close up of flowers within a cluster

San Diego thorn-mint (*Acanthomintha illicifolia*) (Beauchamp, 1986) often cited with an alternate spelling as San Diego thornmint (Bauder and Sakrison, 1997; Gray, 1872; Hickman, 1993; Munz, 1974; Reiser, 1994; and U. S. Fish and Wildlife Service (USFWS) 1998, 2007, 2008) was listed as federally threatened October 13, 1998 (Federal Register Vol. 63) and state endangered in January 1982. San Diego thorn-mint was first described by Asa Gray in 1872 as *Calamintha illicifolia* (Gray, 1872) and later renamed *Acanthomintha illicifolia* in 1878 by Gray. It is an annual aromatic herb in the mint family (Lamiaceae). Plants within this genus have paired leaves and several (7-10) spined bracts (modified leaves) (Hickman 1993 ranging in length from 4-8 mm. The flower possesses a calyx of about 5 mm in length with a tubular two-lipped corolla (fused petals) of 12 mm. The upper lip is smaller than the lower lip. Also the lower lip, at times, has a rose or lavender tinge. As it begins to flower to a more lavender or purple-tinge, purple spots will appear on the lower lip. The upper two stamens are sterile and the anthers and style are hairless which is unique to this species and differentiate it from others in Acanthomintha.

San Diego thorn-mint flowers between April and June (Beauchamp, 1986; Munz, 1974; Reiser, 1994; and Skinner & Pavlik, 2001). This time line may shift due to location, elevation and seasonal rainfall. Populations nearer to the coast can begin to flower as early as mid-March (personal observation). Seasonal rainfall can cause longer

flowering seasons or shortened seasons depending on the amount and timing of those rains.

San Diego thorn-mint occurs in clay soils within openings within coastal sage scrub or chaparral where native grasses may also occur (Beauchamp, 1986; Reiser, 1994; and Skinner & Pavlik, 2001). Reiser describes the soil type noting it is within 'friable or broken clay soils. Clay lenses may be associated with Las Posas or San Miguel-Exchequer soils' (Reiser, 1994). The broken clay lens is the most important component to look for when performing visual assessments for suitable conditions.

Figure 4: San Diego Thorn-mint (*A. illicifolia*) new growth

Figure 5: San Diego Thorn-mint (*A. illicifolia*) patch in full flower

Figure 6: San Diego Thorn-mint (*A. illicifolia*) close up of flower

Objective

This project investigated potential insect pollinator candidates with the intent of providing information that could be used for land management conservation planning. Lakeside Ceanothus and San Diego Thorn-mint are probably out-crossed species via insects (Wyatt, 1983; Bauder and Sakrison, 1997).

The USFWS in discussing Primary Constituent Elements (PCEs) for San Diego thorn-mint looked at pollination as one of the possible PCEs but did not include it because evidence was not conclusive as to specific pollinators (USFWS, 2007). "PCEs are physical or biological features that are essential to the conservation of the species, and that may require special management considerations or protection. These include but are not limited to, space for individual and population growth and for normal behavior; food, water, air, light, minerals, and other nutritional or physiological requirements; cover or shelter; sites for breeding, reproduction, and rearing of offspring, germination, and seed dispersal; and habitats that are protected from disturbance or are representative of the historic geographical and ecological distributions of a species (USFWS, 2007)." The Multiple Habitat Conservation Plan (MHCP) considered both Lakeside Ceanothus and San Diego thorn-mint as out-crossers that are insect pollinated but concluded that not enough information was available to specifically identify those candidates and therefore considered pollination as unresolved as part of their conservation strategy (Ogden and Conservation Biology Institute, 2000).

The objective of this project is to identify potential pollinator candidates for Lakeside Ceanothus and San Diego Thorn-mint. This objective will 1) describe what is currently known about the biology and phenology of each species; 2) identify insect species observed visiting each plant; 3) determine whether they are potential pollinators, opportunistic feeders or thieves (Buchmann and Nabhan, 1996; Proctor, Yeo and Lack, 1996); and 4) make recommendations to assist land managers and resource agencies in managing these species. The results of the objective and the four sub-objectives will be addressed separately for each of the two plant species.

Lakeside Ceanothus

(1) Describe current known biology and phenology

See Project Background above.

(2) Indentify insect visitors

A. Site selection

To have a better understanding of what insects are visiting the plant, sites were selected that would represent the range of the plant within San Diego County. These sites were selected for their potential impacts from human interactions, stand age (mature stands versus recovering from recent fires), and volume of shrubs within the

stand. Since so little is known about insect visitors and whether pollination is specialized or generalized these sites were selected to observe whether similar or different species of insects are visiting the flower clusters of the Ceanothus under different environmental conditions.

The sites and their descriptions are as follows:

El Capitan Open Space Preserve (ECOSP) is northeast of the Community of Lakeside near Barona along Wildcat Canyon Road. It is under management by the San Diego County Parks and Recreation Department. The study site is recovering chaparral affected by the 2003 wildfires at an elevation of 1,755 feet. GPS: 32° 54.51'N 116° 52.88'W (NAD83)

Crestridge Ecological Reserve (CER) is adjacent to the Community of Crest east of the City of El Cajon. The Reserve is under the management of the California Department of Fish and Game. Most of the Reserve is recovering from the 2003 wildfires. However there is a small patch of chaparral containing mature Lakeside Ceanothus not burned in those fires located in the east central part of the Reserve at an elevation of 1,500 feet. GPS: 32° 50.43'N 116° 51.28'W (NAD83)

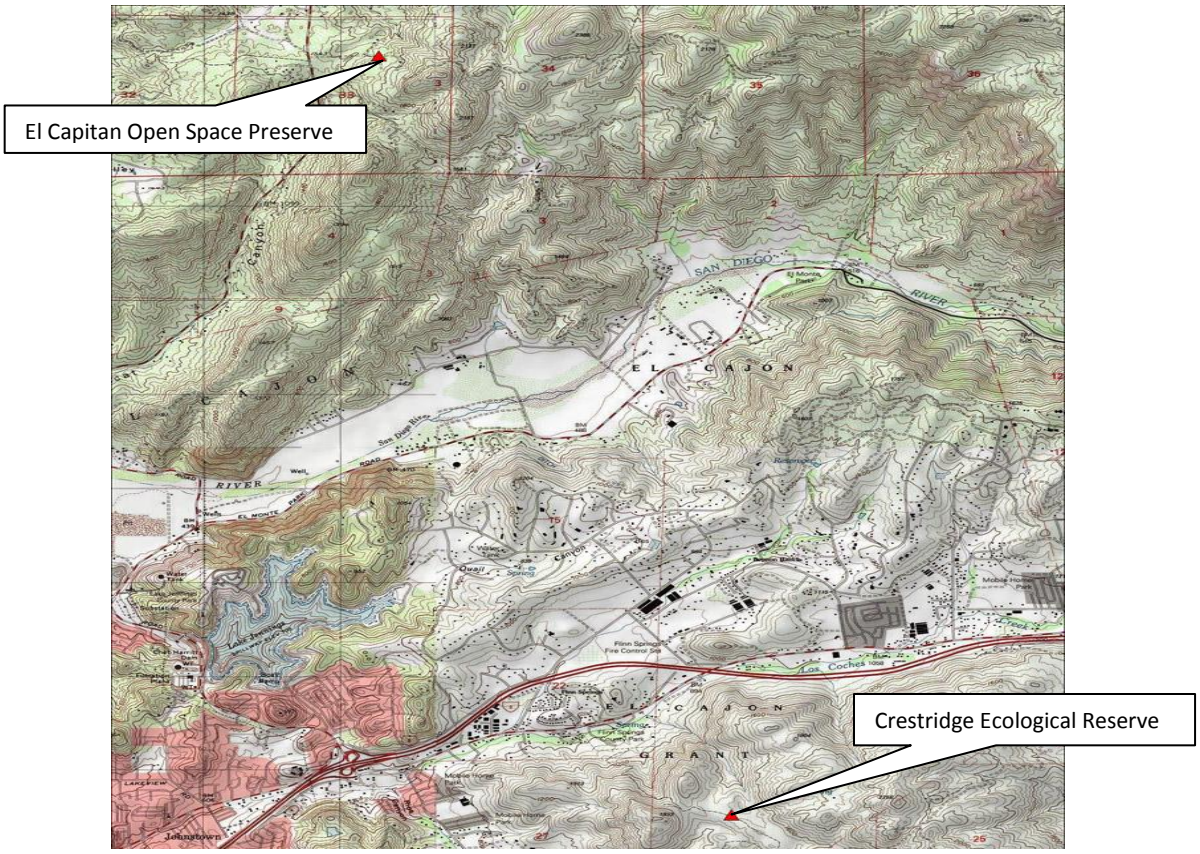


Figure 7: Study Site Locations of *C. cyaneus*

The goal is to visit shrubs at regular intervals during a selected timeframe to optimize insect visitor observation (see Observation Method below). Initially a square hectare study site was chosen at both sites. Once these study areas were chosen it was apparent that a square hectare was too large for studying due to the number of shrubs (ECOSP > 100 shrubs, CER > 200 shrubs) and the difficulty of accessing to all those shrubs because vegetation was too dense. Adherence to regular observation intervals could not be accomplished if sampling was conducted over 100 and 200 shrubs respectively. It was decided to narrow the selection to a manageable number of shrubs as well as allow accessibility to move around the shrubs to note insect visitors. The conclusion was to select 9 shrubs at ECOSP and 6 at CER. Each shrub at each location contained a large number of flower clusters (> 100) which would provide sufficient opportunities for insect visitors. Reducing the number of shrubs observed also allowed for enough observation time between recorded intervals (see Observation Method below).

B. Observation Method

To optimize insect visitor observations, and observe visitor movement through each flower cluster and shrub, a procedure of regularly timed visits to each shrub was developed to capture this. On each regularly timed visit the following data was recorded: the percent sun (clear sky), wind speed and direction, total number of insects observed, total number of species, the number of clusters and/or shrubs each counted insect moved to and any other pertinent observations, i.e. behavior of observed insect such as defending a territory, resting, perching, etc (See Appendix 1 for Protocol used). The timed interval chosen was fifteen minutes. This provided enough time to record all the pertinent data, slowly walk around each shrub noting observations and to collect specimens for analysis (see Objective 3 for more details). Binoculars used were Brunton Epoch 8.5X43 close focusing. These binoculars have the capability to focus as close as three feet. Some insect visitors were photographed using a Konica/Minolta Maxxum 5D SLR digital camera with a 50 mm macro-lens. Insects could be photographed as close as two inches away.

Initial studies were conducted in May and June 2001 under the direction of the Conservation Biology Institute as part of the Draft Management Plan for the Crestridge Ecological Reserve (CDFG, 2002). These initial studies did not result in identification of potential insect pollinators but did provide information useful for this study. These were: insect visitors were active with nearly 100% sun. No visitors were recorded at dawn, dusk or night. High temperatures did not appear to affect insect visitors. Wind speed greater than 12 mph appeared to affect visitors. Temperatures below 55°F resulted in no visitors.

Visits were conducted at the El Capitan Open Space Preserve on May 1, 24 and June 22, 2007; April 16, 28 and June 4, 2008; and May 14 and 21, 2009. Visits were conducted at the Crestridge Ecological Reserve on May 31, 2007; March 22, April 26,

May 4, 13 and June 12, 2008; and May 13 and 19, 2009 (See Appendix 2 for more details).

C. Observation Results

The majority of insect visitors at both study sites were bees in the Order Hymenoptera. The families of bees observed or collected were Andrenidae, Apidae, Colletidae and Halictidae. Ants were the other Hymenoptera family Formicidae. Within the bees, the genera observed or collected were *Agapostemon*, *Andrena*, *Apis*, *Bombus, Halictus*, *Hylaeus, Macrotera, Perdita,* and *Xylocopa*.

The other primary visitors were beetles in the Order Coleoptera. By far the greatest number of beetles was in the family Melyridae. Other beetle families observed or collected were Bupresitidae, Chrysomelidae, Coccinellidae, Dermestidae and Mordellidae.

Heteroptera was the next common insect order visiting the flower. The families observed were Berytidae, Largidae, and Miridae.

Flies in the Order Diptera were not very common but regular visitors. The families of Diptera observed or collected were Bibionidae, Bombyliidae, and Syrphidae. The Syrphidae or hover fly was the most common family visiting the flowers in the Order Diptera.

Lepidoptera was not a common Order visiting the flowers. The families of Lepidoptera that were visiting were Lycaenidae, Noctuidae, Nymphalidae, Pieridae, and Pyralidae.

Within the 'Other" category was one snakefly in the Order Neuroptera in the family Raphidiidae. The remaining observations within the 'Other' category were in the Class Aracnida in the orders Araneae (spiders) and Acari (mites and ticks). The crab spider, family Thomisidae was the only spider observed and spider mite, family Tetranychidae was the mite observed once during the studies.

Order	Family	Genus	ECOSP	CER	Total	Pollinator
Hymenoptera						
	Andrenidae	*Andrena*	95	13	108	X
		Macrotera	23	1	24	X
		Perdita	3	4	7	X
	Apidae	*Apis*	133	161	294	X
		Bombus	1	6	7	X
		Xylocopa	1	1	2	X
	Colletidae	*Hylaeus*	1		1	X
	Halictidae	*Agapostemon*	1	1	2	X
		Halictus		1	1	X
	Formicidae			2	2	

Hymenoptera Total			258 (61.9%)	190 (43.3%)	448 (52.3%)	446 (89.4%)
Coleoptera	Bupresitidae			7	7	
	Chrysomelidae		1		1	
	Coccinellidae		5		5	
	Dermestidae		2	6	8	
	Melyridae		30	201	231	
	Mordellidae			2	2	
Coleoptera Total			38 (9.1%)	216 (49.1%)	254 (29.6%)	
Heteroptera	Berytidae		1		1	
	Largidae		3		3	
	Miridae		60	8	68	
Heteroptera Total			64 (15.4%)	8 (1.8%)	72 (8.4%)	
Diptera	Bibionidae		1		1	
	Bombyliidae			4	4	X
	Syrphidae		40	9	49	X
Diptera Total			41 (9.8%)	13 (2.9%)	54 (6.3%)	53 (10.6%)
Lepidoptera	Lycaenidae		1	2	3	
	Noctuidae			1	1	
	Nymphalidae			7	7	
	Pieridae		1		1	
	Pyralidae			1	1	
Lepidoptera Total			2 (0.5%)	11 (2.5%)	13 (1.5%)	
Other	Neuroptera		1		1	
	Araneae		12	2	14	
	Acari		1		1	
Other Total			14 (3.3%)	2 (0.4%)	16 (1.9%)	

Table 1. Number of floral visitors at the El Capitan Open Space Preserve and Crestridge Ecological Reserve. Numbers in the parenthesis reflect the percentage of the total observed or collected. 'X' indicates they met the definition of a potential pollinator (See 3. (A) below).

Not only were insect visitors active during mostly 100% sunny conditions but also had a preferred visitation timeframe in which they were active as long as the air temperature was at least 55°F (measured at 3 feet above the ground) and winds were below a constant 12 miles per hour. Once cloud cover was 20% or greater no insects were observed visiting the flower clusters. I confirmed the 2001 results related to crepuscular activity with one visit at dawn on May 1, 2007 at ECOSP and one at dusk on June 12, 2008 at CER, as long as no clouds were present. Insects never appeared on the flower

clusters before 0800 and were never observed on flower clusters after 1515. The highest visitor activity was between 1015 and 1445.

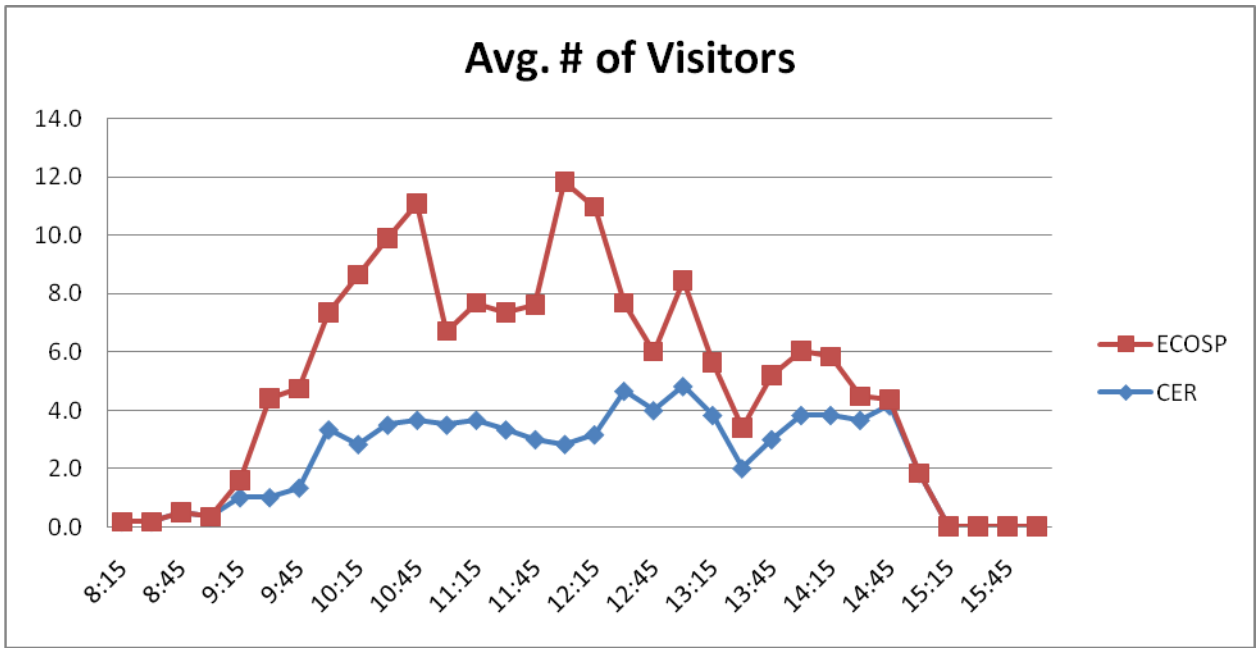


Figure 8: Showing average number of insect visitors at the study sites with peak visitation between 1015 and 1445.

D. Determine whether observed and/or collected insects contained pollen loads of *C. cyaneus*.

Insects observed visiting the flowers displayed different behaviors when on the flower clusters. See Section 3 for a more detailed explanation of their behaviors. Not all visitors were collected. This was to allow potential pollinators to gather pollen and/or nectar for themselves and possibly active nests. Some insects were collected and placed in a cooler to bring their body temperatures down to a point of inactivity. Once they were cooled, they were removed from the cooler with tweezers and their bodies were carefully looked over for the presence of pollen using a 10X hand lens. If they had pollen on their body, some were placed into a killing jar and used as voucher specimens and the others were allowed to warm their bodies back up to a temperature at which they became active again.

Voucher specimens were pinned and either the part(s) of their body that contained pollen was scraped or the body part was removed, i.e. leg, and placed on metal plates with an adhesive to hold the body part or pollen in place (Figure 9). A log was kept as to which specimen, body part or pollen was removed and from which specimen. The adhesive plates were delivered to the Scanning Electronic Microscope (SEM) Laboratory at San Diego State University to determine if the pollen or body part contained pollen from *C. cyaneus*. Photographs of the body part or pollen were taken by the SEM lab in scale from 300X to 1,500X (Figure 10). When the SEM Lab finished

their photographing, the series of photographs were place on an FTP site for me to retrieve. I analyzed the photographs to determine the presence of *C. cyaneus* pollen based on base SEM photographs created by the laboratory after I delivered *C. cyaneus* pollen to them at the beginning of the study.

Figure 9: Plates used to place insect body part or pollen for SEM photographs.

Figure 10: Lakeside Ceanothus pollen grains at 700X.

Bees had *C. cyaneus* pollen on their bodies with the majority collecting the pollen on their hind legs. Some had pollen within the hairs on their thorax and underside. The bees containing the largest concentration of *C. cyaneus* pollen were *Apis melifera*, *Andrena* sp., *Hylaeus* sp., *Macrotera* sp. and *Perdita* sp.

Beetles in the family Melyridae collected some small amounts of *C. cyaneus* pollen on the underside of their bodies but the majority was found around their mouth. The other families Bupresitidae, Chrysomelidae, Coccinellidae, Dermestidae and Mordellidae did not confirm the presence of pollen on them.

Bugs in the family Miridae had small amounts of pollen on their legs. The other families, Berytidae and Largidae, did not have pollen on them.

Flies in the family Bombyliidae showed no *C. cyaneus* pollen on their body but hover flies in the family Syrphidae did have moderate amounts of pollen on the underside of their abdomen.

The only butterflies on which pollen was observed were the painted ladies (*Vanessa cardui*) in the family Nymphalidae. The pollen was observed on their proboscis. None of the other butterflies had any *C. cyaneus* pollen found on them.

(3) Determine whether insect visitors are potential pollinators, opportunistic feeders or thieves

A. Define Pollination

According to the Pollinator Conservation Handbook, (Sheperd, Buchmann, Vaughan and Black, 2003) pollination is "The transfer of pollen grains from an anther to a receptive stigma. Self-pollination is movement within a flower or between flowers on the same plant; cross-pollination is between flowers on separate plants. Pollen may be carried by wind, water, or animals."

According to the non-profit organization, The Pollinator Partnership, in conjunction with The North American Pollinator Protection Campaign "Pollination occurs when pollen is moved within flowers or carried from one flower to another of the same species by birds, bees, bats, butterflies, moths, beetles or other animals, or by the wind. This transfer of pollen leads to fertilization and successful seed and fruit production. Pollination ensures that a plant will produce full-bodied fruit and a complete set of fertile seeds, capable of germinating."

Pollination can occur without fruit or seed production. For this report Pollination occurs as long as the pollen transfer event happens. This study did not look specifically at the desired goal of the transfer event. However, through the study time, results could be observed as to fruit production and conclusions be made that pollination occurred but

that was not the goal of this study. As long as the potential transfer event occurred, pollination occurred.

B. Define Feeders

Once insect visitors had been observed, the next step is to determine whether the visitor produces the transfer event, i.e. potential pollination or visitor remains within the inflorescence. The regularly timed intervals of 15 minutes were a useful measure to determine this. If after a pre-determined time the visitor had not moved to a location different from that at the time of initial observation, it was determined to be a feeder (Buchmann and Nabhan, 1996; Proctor, Yeo and Lack, 1996). A feeder in this case may not necessarily be feeding on pollen or nectar. They feed on petals or even other visitors (Buchmann and Nabhan, 1996; Proctor, Yeo and Lack, 1996). Predators such as spiders, assassin bugs (Order Heteroptera, Family Reduviidae) and snakeflies (Order Neuroptera, Family Raphidiidae) are examples of this type of predacious behavior. Others which are considered feeders, predators or passing visitors are thrips (Order Thysanoptera), springtails (Order Collembola), earwigs (Order Dermaptera, cockroaches (Order Blattodea), booklice (Order Psocoptera), dragonflies / damselflies (Order Odonata), grasshoppers, crickets and katydids (Order Orthoptera), stoneflies (Order Pecoptera), true bugs (Order Heteroptera), lacewings (Order Neuroptera), scorpion-flies (Order Mecoptera) and caddis-flies (Order Trichoptera) (Proctor, Yeo and Lack, 1996).

Categorizing particular types of insects as feeders, predators or passing visitors does not necessarily exclude them as potential pollinators. The regularly timed intervals by direct observation assisted in determining their function. As an example, if a visitor was observed on an anther and during the 15 minute direct observation intervals that visitor had not moved from the initial observed location after three hours, it was determined to be a feeder and not a pollinator. See details below under **D. Pollination Success**.

C. Define Thieves

When studying potential pollinators, visitation is the first step. However, there are cues that can confirm nectar or pollen robbers (Kearns and Inouye, 1993; Inouye, 1980). One of the common cues is holes within the flower or petals removed. Nectar or pollen theft may have a detrimental effect on pollination. The results may also have the benefit of pollinating. Without direct observation this behavior is difficult to determine. One of the other results of nectar or pollen theft is no re-visits by other insect visitors (Buchmann and Nabhan, 1996).

D. Pollination Success

Based on Table 1 I will discuss in more detail these visitors and determine whether they meet the definition of a potential pollinator, feeder or thief.

1) Wind Pollination Success: An additional experiment performed in the first year of this Study at ECOSP was to look at potential wind pollination versus insect pollination. I selected six clusters on three different shrubs (two clusters per shrub) just as the shrub was forming flower buds. The two clusters on each shrub were up to 12 inches apart. Each bud cluster was covered in loose mesh cheesecloth and tied at the cluster stem. The mesh was small enough to deter insect visitors but open enough to allow pollen to come in. During my ECOSP visits in 2007 I monitored each cheesecloth covered cluster to make sure that weather conditions or animals had not removed them or loosened them. At the end of the flowering season when I was observing fruit on the uncovered flower clusters of the 9 shrubs within my study site, I removed the cheesecloth from the six covered clusters. Flower buds were still present and when I touched one of the clusters, the entire cluster dropped dust. I concluded these clusters had not been pollinated and this suggests wind is not a factor in *C. cyaneus* pollination.

Figure 11: Cheesecloth on Lakeside Ceanothus flower clusters to wind pollination test.

2) Hymenoptera Success:

   a) Mining bees (Family Andrenidae) are very small to medium sized bees. Many genera have yellow faces and can be confused with Colletidae. Some genera can have a moderate amount of hair on them while others are similar to Halictidae and have almost no hair on them. They were very

common and regular visitors to the flowers and clusters and would also be observed moving to other shrubs. Vouchers specimens of many were taken because of their identification confusion to Colletidae. Pollen was observed on many of them in the form of pollen balls as well as thick clumps of pollen on the underside of their abdomen. A hand lens was not necessary because the pollen was so dense at times it was easily seen with the naked eye. SEM did confirm the presence of *C. cyaneus* pollen on them as well as pollen from other plants. Based on their visiting intervals, movement through the flowers within multiple clusters and shrubs, I concluded they successfully pollinated.

b) True bees (Family Apidae)

1) Honey bees (*Apis melifera*) were regular visitors to and were normally the first visitors of the daily visits. They are hairy medium sized bees and were observed actively moving through flower clusters on individual shrubs and then moving to clusters on other shrubs. Their movement through the flower clusters was aggressive and caused the individual flowers within the clusters distorted, bent and even broken petals. After flowers had been visited by the honey bee they were never re-visited that day during my regularly timed intervals by not only the honey bee but by any other visitor. Subsequent field visits during the season also indicated these distorted, bent and broken flowers were not re-visited. This is indicative of thievery behavior (Inouye, 1980). A closer look with a hand lens still showed small amounts of pollen on the bent anthers. I did not investigate whether nectar was still available on these broken flowers. At the end of the flowering season, these clusters did produce fruit. I concluded that even though the process was aggressive pollination occurred.

2) Bumble bees (*Bombus* sp.) were not regular visitors. They are hairy large sized bees and were observed actively moving through flower clusters on individual shrubs and then moving to clusters on other shrubs. Their movement through the flower clusters was aggressive and caused the individual flowers within the clusters to be distorted, bent and often resulted in broken petals. After flowers had been visited by the bumble bee they were never re-visited that day during my regularly time intervals by not only the bumble bee but by any other visitor. Subsequent field visits during the season also indicated these distorted, bent and broken flowers were not re-visited. This is indicative of thievery behavior (Inouye, 1980). A closer look with a hand lens still showed small amounts of pollen on the bent anthers. I did not investigate whether nectar was still available on these broken flowers. At the end of the flowering season, these clusters did produce

fruit. I concluded that even though the process was aggressive pollination occurred.

3) Carpenter bees (*Xylocopa* sp.) were not regular visitors. They are hairy large sized bees and were observed actively moving through flower clusters on individual shrubs and then moving to clusters on other shrubs. Their movement through the flower clusters was aggressive and caused the individual flowers within the clusters to be distorted, and bent and even resulted in broken petals. After flowers had been visited by the carpenter bee they were never re-visited that day during my regularly time intervals by not only the carpenter bee but by any other visitor. Subsequent field visits during the season also indicated these distorted, bent and broken flowers were not re-visited. This is indicative of thievery behavior (Inouye, 1980). A closer look with a hand lens still showed small amounts of pollen on the bent anthers. I did not investigate whether nectar was still available on these broken flowers. At the end of the flowering season, these clusters did produce fruit. I concluded that even though the process was aggressive pollination occurred.

c) Yellow-faced bees (Family Colletidae) were not regular visitors. They are small moderately hairy bees. There was one observation from ECOSP on 28 April 2008. A single individual from this family visited flowers within two clusters on one shrub. A voucher specimen noted moderate amounts of pollen of *C. cyaneus* and other pollen from other plants on its head and the underside of its abdomen. It never re-visiting after one timed interval. At the end of the flowering season both clusters it visited produced fruit. Flies and beetles were observed within these same two clusters during the flowering season and may have contributed in successful pollination. Based on their visiting intervals, movement through the flowers within multiple clusters and shrubs it is concluded they successfully pollinated.

d) Sweat bees (Family Halictidae) were not regular visitors. They are small to medium sized bees and were observed moving through flower clusters on individual shrubs. They were never observed moving to other shrubs within the study areas. Using a hand lens, it was observed that small amounts of pollen were deposited on the spurs of their hind legs. They never re-visited flowers or clusters after the initial regularly timed intervals. Unlike the honey bee, bumble bee and carpenter bee, they did not damage the flowers within the clusters they visited, and these clusters were re-visited by other bees, and flies throughout the season. At the end of the flowering season, one cluster produced fruit and the others did not. Since other visitors were observed throughout the season, it is inconclusive as to the ability of the sweat bees to successfully pollinate. However, their behavior was consistent as a potential pollinator.

e) Ants (Family Formicidae) are a diverse family. They are polyphagous feeding on small decomposing insects or other animals as well as plant material. The velvety tree ant (*Liometopum occidentale*) was the only ant species observed during the entire study and that was at the CER only on May 31, 2007. One individual was observed at two different recorded time intervals. It was moving along the base of a single flower cluster removing flower petals. It was never observed in a flower. Since no inter-floral movement (movement between flowers) occurred, no pollen transfer occurred. I concluded they did not pollinate.

3) Coleoptera Success:

a) Soft-winged flower beetles (Family Melyridae) were the second most common visitor only, to be outnumbered by the honey bee. They are a small beetle, < 2 mm. The family is polyphagous feeding on both plant and animal material. The beetle species observed was keyed to the genus *Dasytes*. It is a pollen feeder (Arnett, Thomas, Skelley and Frank, 2002). There were small amounts of pollen on their abdomens with the majority found around their mouth. When observed, they were always found on the same flower and sometimes up to three on the same flower. Since no inter-floral movement occurred, no pollen transfer occurred. I concluded they did not pollinate.

b) Metallic wood-boring beetles (Family Bupresitidae) normally feed on foliage of their larval host plants or visit flowers to feed on pollen or nectar. (Arnett, Thomas, Skelley and Frank, 2002) Some feed on fungus. Many have hirsute bodies and serve as potential pollinators. One individual was observed on one visit at CER on two different recorded time intervals on the same flower. Using a hand lens, pollen was observed in small amounts on the underside of its abdomen. Since no inter-floral movement occurred, no pollen transfer occurred. I concluded they did not pollinate.

c) Leaf beetles (Family Chrysomelidae) feed on living plant material usually consuming leaves or various flower parts including pollen (Arnett, Thomas, Skelley and Frank, 2002). One individual was observed during one recorded time interval on one visit. It was not observed moving to flowers or clusters. Using a hand lens no pollen was found on its body. Since no inter-floral movement occurred, no pollen transfer occurred. I concluded they did not pollinate.

d) Ladybird beetles (Family Coccinellidae) are primarily predacious on small soft-bodied insects and mites but will feed on pollen when prey is not available (Arnett, Thomas, Skelley and Frank, 2002). Two observations of the 7-spotted ladybird (*Coccinella septempunctata*) were recorded during

two recorded time intervals. During the first recorded interval, three individuals were observed and during the next timed interval, one individual was observed on the same flower from the previous recorded interval. They were never observed moving through the flower cluster or between flowers. Using a hand lens I noted small amounts of pollen on the underside of their abdomen. Since no inter-floral movement occurred, no pollen transfer occurred. I concluded they did not pollinate.

e) Carpet beetles (Family Dermestidae) are scavengers and will feed on dried animal or plant material (Arnett, Thomas, Skelley and Frank, 2002). Individuals were observed in dried flowers within the flower cluster mostly during the later part of the flowering season. Pollen was found in small amounts around their mouths. In each recorded observation they were never observed moving to any flowers whether they were dried or not. Since no inter-floral movement occurred, no pollen transfer event occurred. I concluded they did not pollinate.

f) Tumbling flower beetles (Family Mordellidae) are phytophagous but feed primarily on the pollen of many plants (Arnett, Thomas, Skelley and Frank, 2002). One individual was recorded on one timed interval and was not observed moving to other flowers during that recorded interval. Since there was no observed inter-floral movement, no pollen transfer event occurred. I concluded it did not pollinate.

4) Heteroptera Success:

This is a large order which is known mostly as being crop pests and can be vectors for plant and animal diseases. Their functioning mouthpart is hardened and used for sucking fluids either from plants or animals. They are not known to be pollen feeders but can, at times, feed on nectar. Any ability to be potential pollinators would be considered negligible (Arnett 2000). Three families were observed during the study; Stilt Bug (Berytidae), Red Bug (Largidae), and Plant Bug (Miridae). All three families feed on plant fluid by piercing the tissue, damaging the plant. The majority of the observations were at the ECOSP. Individual stilt bug and red bug were observed on separate days at one recorded timed interval each. Neither was observed moving through flowers or flower clusters. Since there was no observed floral movement, no pollen transfer event occurred. I concluded they did not pollinate.

Plant bugs were observed throughout the study at both study sites. Regular observations were noted during some visits and other visits had no recorded observations. Three regular timed intervals recorded up to three individuals on separate flowers on separate shrubs. No individual was observed moving through flowers or flower clusters. Since there was

no observed inter-floral movement, no pollen transfer event occurred. I concluded they did not pollinate.

5) Diptera Success:

This is a diverse Order with species that include parasites, predators, economic pests, vectors for diseases and even pollinators. Only three families were observed during the study. They are described in more detail below.

a) March Flies (Family Bibionidae) are not a very large family within the United States and are mostly found along the west coast. Adults are frequent visitors of flowers and usually in large numbers (Arnett, 2000). Because of their large numbers at flowers they have the ability to be potential pollinators.

One individual was observed on April 16, 2008 at the ECOSP. It was recorded at one timed interval and not observed again. It never moved through flowers or flower clusters. Since there was no observed inter-floral movement, no pollen transfer event occurred. I concluded it did not pollinate.

b) Bee Flies (Family Bombyliidae) are a fairly large family with the majority of species occurring in the southwest United States. They are one of a few Dipteran families which are predatory as larvae and nectar / pollen feeders as adults. This family is documented to include efficient pollinators (Arnett 2000; Proctor, Yeo, and Lack, 1996). Many have hirsute bodies making them ideal pollinating candidates (Arnett, 2000).

They were only observed at the CER on April 26, 2008 and May 4, 2008. Three individuals (*Geron* sp.) were recorded on three successive recorded time intervals in April. During one of the timed intervals one individual was observed moving from one flower cluster to another cluster and during the other two recorded intervals was no observed inter-floral or between-cluster movement. During the May observation one individual (*Bombylius* sp.) was noted moving to three clusters on one timed interval. Using a hand lens, pollen was found on both species. SEM results confirmed the presence of *C. cyaneus* pollen on these individuals. Since there was inter-floral movement, I concluded they are potential pollinators.

c) Flower (Hover) Flies (Family Syrphidae) is a large family and dispersed throughout the United States. This family is also predatory in the larval stage with many being aphid feeders. Most adults feed on nectar and pollen and many are documented pollinators (Arnett, 2000).

Of the three families of Diptera recorded during the study, this one was recorded regularly at both study sites through most of the study seasons. Voucher specimens collected had pollen on their head and the underside of their abdomens. SEM results confirmed the presence of *C. cyaneus* pollen on them. Since there was inter-floral movement, I concluded they are potential pollinators.

6) Lepidoptera Success:

Butterflies, moths and skippers are a large and diverse Order. Their mouthparts are designed to suck fluids and are therefore primarily nectar feeders. Much has been studied about this Order regarding the pollination effectiveness of its members (Proctor, Yeo, and Lack, 1996; Arnett, 2000; Dafni, Kevan and Husband, 2005). In some instances they are specialists like the Yucca moth (Family Prodoxidae, *Tegeticula* sp.) whose relationship with yuccas are symbiotic (Powell and Opler, 2009).

Lepidopterans were not common or regular floral visitors during the study. Families observed were Lycaenidae, Noctuidae, Nymphalidae, Pieridae and Pyralidae. The ECOSP had one date in which Lepidoptera were recorded, April 28, 2009. It was a very warm day with temperatures in the low 90°F range most of the day. Two butterflies were observed, checkered white (*Pontia protodice*, Family Pieridae) and echo blue (*Celastrina echo*, Family Lycaenidae). The checkered white was recorded on one timed interval and not observed moving to flowers or clusters. With a hand lens, no pollen was observed on its body or proboscis. Since there was no observed floral movement, no pollen transfer event occurred. I concluded it did not pollinate. The echo blue was recorded on one timed interval and was observed moving to seven floral clusters. With a hand lens pollen was found on its proboscis. SEM results confirmed no *C. cyaneus* on it. Even though it was observed moving through floral clusters, the lack of pollen found indicates no transfer event occurred and therefore I concluded it did not pollinate.

The CER had three recorded days of Lepidoptera activity: May 31, 2007 and April 26 and May 13, 2008. The May 31, 2007 date recorded two timed intervals. One was a snout moth (Family Pyralidae) and the other was a schinia moth (Family Noctuidae). The moths did not move between flower clusters during the recorded intervals. Since there was no observed inter-floral movement, no pollen transfer event occurred. I concluded it did not pollinate.

Four Lepidoptera visits were recorded on April 26, 2008. Three of them were by painted ladies (*Vanessa cardui*, Family Nymphalidae) and the other was a brown elfin (*Callophrys augustinus*, Family Lycaenidae). The brown elfin did not move to flowers or clusters. Since there was no observed floral movement, no pollen transfer event occurred. I concluded it did not pollinate.

The painted ladies were recorded in two of the three timed intervals where they moved to three flower clusters. With a hand lens pollen was found on their proboscis. SEM results confirmed no *C. cyaneus* on it. Even though it was observed moving through floral clusters, the lack of pollen found indicates no transfer event occurred and therefore I concluded it did not pollinate.

The May 13, 2008 date recorded one Lepidoptera visit. One brown elfin was recorded on one timed interval. It was observed moving to one other flower cluster. With a hand lens no pollen was found on its body or proboscis. Even though it was observed moving through floral clusters, the lack of pollen found indicates no transfer event occurred and therefore I concluded it did not pollinate.

7) Other visitors in the Orders Nerve-wing insect (Neuroptera), true spiders (Araneae) and mites (Acari) were recorded. All three orders are predators (Hogue 1993).

The Neuroptera visitor was a snakefly (Family Raphidiidae) and recorded on one timed interval during the study and was not recorded moving to other flowers or clusters. Since there was no observed inter-floral movement, no pollen transfer event occurred. I concluded it did not pollinate.

The Araneae visitor was an individual crab spider (Family Thomisidae) recorded on numerous timed intervals during the study. It was not recorded moving to other flowers or clusters. Since there was no observed inter-floral movement, no pollen transfer event occurred. I concluded it did not pollinate.

The Acari visitor was a spider mite (Family Tetranychidae) and recorded on one timed interval during the study and was not recorded moving to other flowers or clusters. Since there was no observed inter-floral movement, no pollen transfer event occurred. I concluded it did not pollinate.

E. Conclusions

Wind does not appear to be a pollination method for *C. cyaneus* but further experimental replication may be necessary to demonstrate this conclusively.

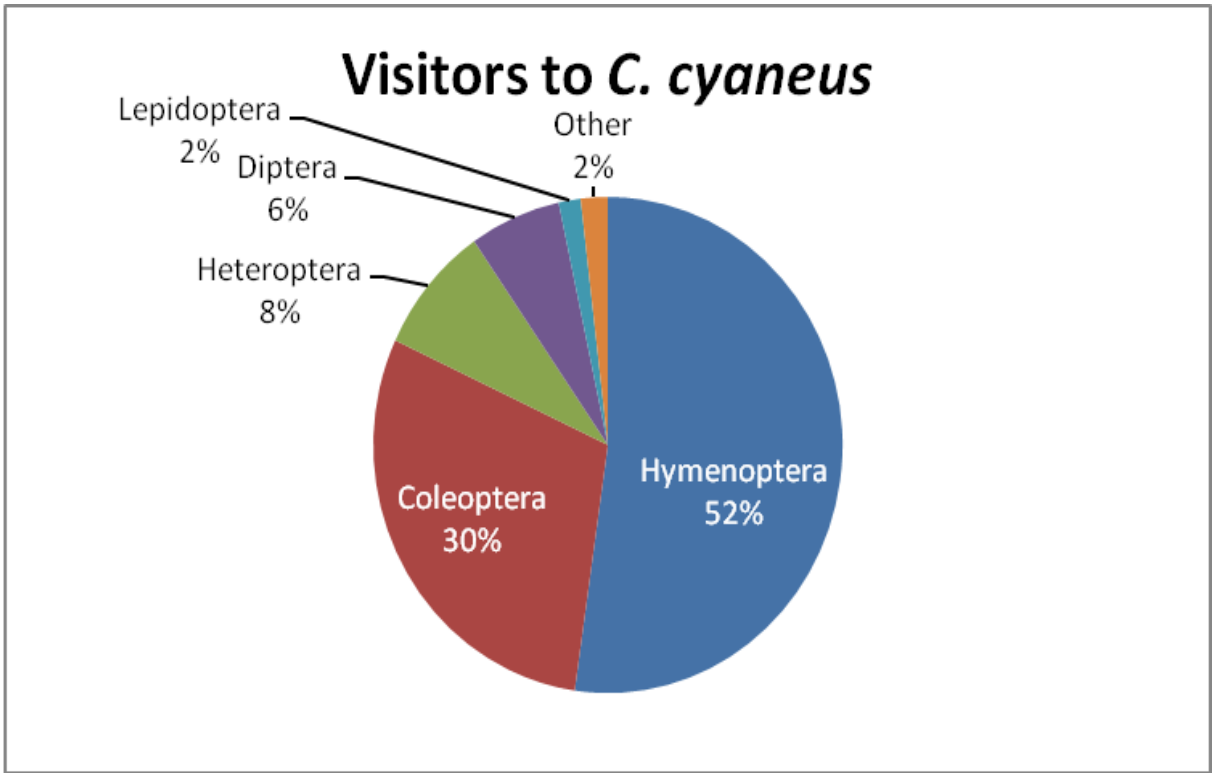
Pollination of *C. cyaneus* does not appear to be performed by specialists. Results indicated many visitors to the shrub also carried pollen from other plants.

Bees appear to be the dominant visitors and perform pollination, especially in the Family Andrenidae. The Andrenid pollinators are no larger than 5 mm. Once flowers and clusters are visited by these bees, re-visits have been observed. Re-visits improves efficiency allowing for successful pollination.

Large bees in the Family Apidae (honey bees, bumble bees, mining bees and carpenter bees) perform pollination at the expense of permanently damaging the flowers. Once the flowers are damaged they are no longer re-visited by any visitor. This appears to be counterproductive for the visitor even though pollination occurs.

Flies in the two observed families Bombyliidae and Syrphidae appear to be effective pollinators of *C. cyaneus*. Even though they are not as regular or frequent visitors as bees they should not be excluded as functioning pollinators. Also, their size is similar to Andrenid bees being no larger than 5 mm. They behave similarly by not damaging the flowers and allowing for re-visits thereby improving pollination success.

None of the other Orders appear to be as efficient pollinators as Hymenoptera and Diptera.



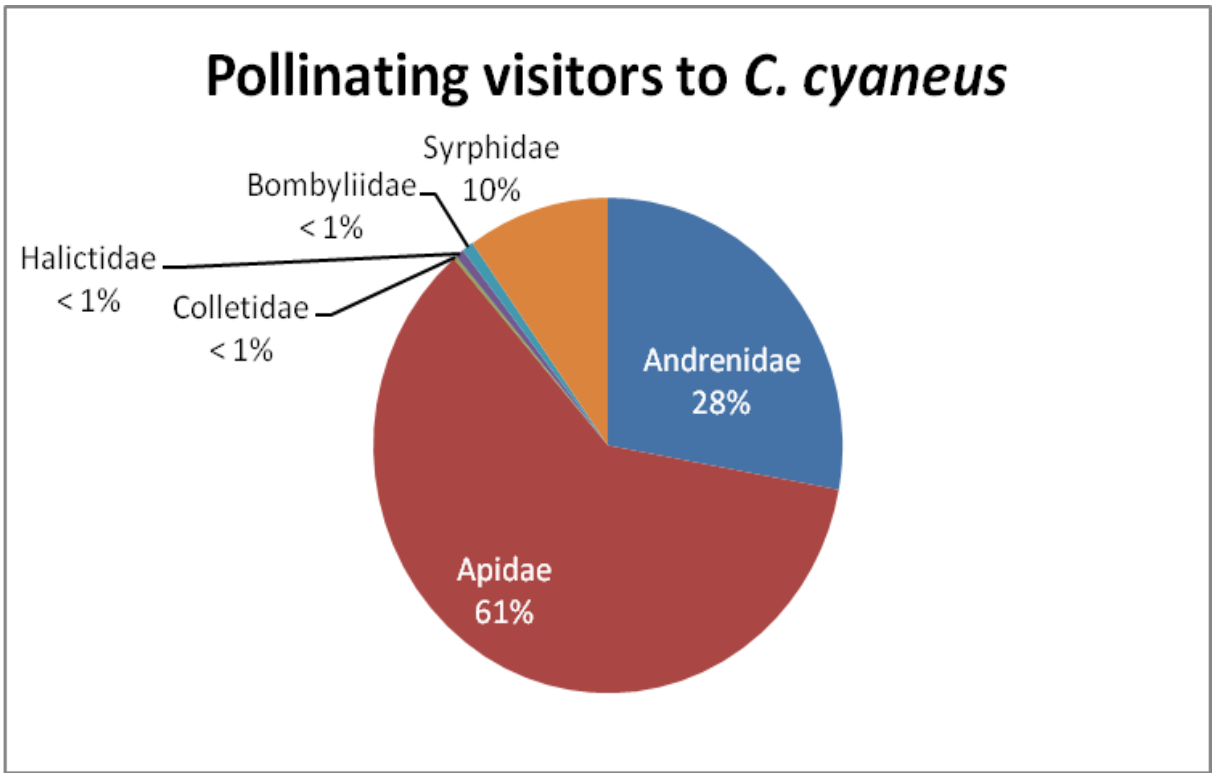


Figure 12: Comparing Total Insect Visitors to the Pollinators of *C. cyaneus*.

(4) Management Recommendations

- If clearing or thinning of the vegetation in the vicinity of *C. cyaneus* is needed to reduce fire risk, it is recommended that this work be conducted outside of the flowering season. If the work cannot be scheduled outside of the flowering season, then it is recommended that the work be done either before 1000 or after 15000. This will allow for optimum flower visitors and pollinators to visit.
- Reduce the density of non-native grasses and forbs, to the extent possible, in the vicinity of *C. cyaneus*. These non-native herbaceous plants out-compete native plants that might attract bees and pollinating flies to the area where they might also then visit the *C. cyaneus*.
- Continue pollination studies to narrow the potential pollinator candidates.
- Future pollination research can be expanded into areas of plant biology and how *C. cyaneus* interacts with pollinating candidates. Plant biology examples: (1) floral pigment or fragrances and how visitors as well as potential pollinators respond to them, (2) When is the flower most receptive for pollen for successful fertilization or when is the pollen at its peak for viability so successful fertilization occurs. As stated earlier, pollination can occur without results of fertilization which in turn may produce fruit and subsequently seeds. However, the goal of pollination is for perpetuation of the species and it is still unknown when the pollen is most viable or when the carpel is most receptive. The potential of understanding these may narrow potential pollinators.

- Future pollination research should include both botanists and entomologists.

San Diego Thorn-mint

(1) Current known biology and phenology

See Project Background above.

(2) Indentify insect visitors

A. Site selection

To have a better understanding of what insects are visiting the plant, sites were selected that would represent the range of the plant within San Diego County. These sites were selected for their distribution throughout San Diego County. Since there is inconclusive evidence as to potential pollinators (Bauder and Sakrison, 1997; Ogden and Conservation Biology Institute, 2000; USFWS, 2007) the site selection strategy throughout the County was to look at different vegetation communities, and elevation to observe whether similar or different species of insects are visiting the flowers under differing environmental conditions.

The sites and description are as follows:

Manchester Habitat Conservation Area (MHCA) is within the City of Encinitas and managed by the Center for Natural Lands Management. The study site is within coastal sage scrub dominated habitat and within approximately five miles of the coast at an elevation of approximately 250 feet. This allows for more temperate weather condition with daytime highs and nighttime lows influenced by the ocean breezes. GPS: 33° 01.84'N 117° 14.98'W (NAD83).

McGinty Mountain (MM) is east of Spring Valley and north of the Community of Jamul near a sub-community of Jamul Highlands and managed by The Nature Conservancy. The study site is on a south-facing slope within mixed chaparral habitat at an elevation of approximately 1,659 feet. Weather conditions in both temperature extremes and rainfall amounts are different than a more coastal influenced environment. GPS: 32° 44.69'N 116° 51.73'W (NAD83).

Wright's Field (WF) is in the Community of Alpine and managed by the Back Country Land Trust. The site is accessed from Tavern Road and South Grade Road along its southern boundary. The study site is within native grassland at an elevation of approximately 1,940 feet. Like McGinty Mountain the site has more diverse weather conditions both in terms of temperature and rainfall amounts as compared to the coastal influence. GPS: 32° 49.02'N 116° 45.94'W (NAD83).

The goal was to visit plants at regular intervals during a selected timeframe to optimize insect visitor observation (see Observation Method below). Initially five plants per study

site were chosen. Since the plants are found in concentrated groups it was determined that five plants was too small of a selection. Each site was looked at and it was concluded that all the flowers within each study site could be observed while performing the study. As the San Diego thorn-mint is an annual, the total number of plants fluctuated each season at each location due to annual rainfall. Study site numbers of plants fluctuated between 10 and 25% during the three year study and I concluded it was not significant enough to skew the insect visitor results. The Manchester Habitat Conservation Area had > 300 plants between 2007, 2008, and 2009. McGinty Mountain had ~180, 235 and 212, respectively between 2007 and 2009. Wright's Field had 32, 36, and 30 respectively between 2007 and 2009. As you can see by these numbers there were plenty of flowering plants at each study site to perform the study.

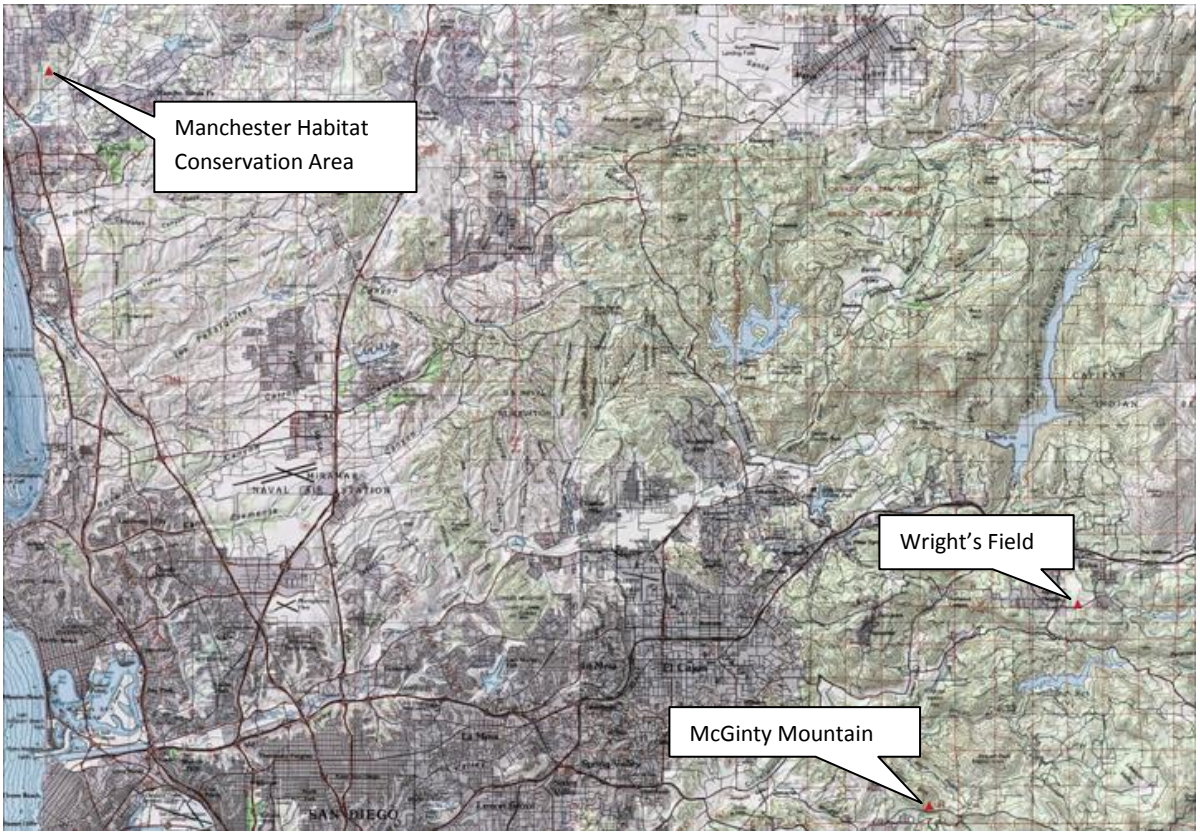


Figure 13: Study Site Locations of *A. illicifolia*

B. Observation Method

To optimize insect visitor observations, and visitor movement through each flower group, a procedure of regularly timed visits to each group was developed to capture this. Observations at each regularly timed visit included: percent sun (clear sky), wind speed and direction, total number of insects observed, total number of species, number of flowers each counted insect moved to and any other pertinent observations, i.e. behavior of observed insect by defending a territory, resting, perching, etc (See Appendix 1 for Protocol used). The timed interval decided on was fifteen minutes. This

provided enough time to record all the pertinent data, slowly walk around each patch noting observations and to collect specimens for analysis (see Objective 3 for more details). Binoculars were used regularly to make the count more accurate. Binoculars used were Brunton Epoch 8.5X43 close focusing. These binoculars have the capability to focus on specimens as close as three feet away. Some insect visitors were photographed using a Konica/Minolta Maxxum 5D SLR digital camera with a 50 mm macro-lens. Insects could be photographed clearly as close as 2-inches away.

Visits were conducted at the Manchester Habitat Conservation Area on April 14, 2007; April 14, and 21, 2008; March 5, April 4, 15, 21, 23 and May 8, 2009. Visits were conducted at McGinty Mountain on April 4 and May 18, 2007; April 15, 23, May 14, 21 and June 2, 10, 2008; April 30, May 12, 18, and 22, 2009. Visits were conducted at Wright's Field on April 4, 15, 16, and 30, 2007; March 23, April 13, 22 and May 7, 11, 2008 and May 10 and 11, 2009 (See Appendix 3 for more details).

C. Observation Results

There were very few visitors overall to the flowers of *A. illicifolia* and they are broken down by the following groups and Orders:

The majority of insect visitors at the study sites were beetles In the Order Coleoptera. The primary families of beetles observed or collected were Cleridae and Melyridae

Bees in the Order Hymenoptera were the next most common visitors. The primary families of bees observed or collected were Apidae and Hallictidae.

Flies in the Order Diptera were observed visiting the flowers were in the families, Bombyliidae and Syrphidae.

Butterflies and moths in the Order Lepidoptera were observed visiting flowers were in the family Hesperidae.

The 'Other' category consisted of a thrip in the Order Thysanoptera

Order	Family	Genus	MHCA	MM	WF	Total	Pollinator
Hymenoptera	Apidae	*Ancyloscelis*		2		2	X
		Apis		4		4	X
		Ceratina	13			13	X
		Diadasia		18		18	X
		Exomalopsis		8		8	X
	Halictidae	*Agapostemon*		2		2	X
		Conanthalictus		1		1	X
		Lasioglossum		1		1	X
Hymenoptera Total			13 (33.3%)	36 (23.0%)	0	49 (24.8%)	49

Coleoptera	Cleridae			1		1	X
	Melyridae		24	88	16	129	(98.0%)
Coleoptera Total			24 (61.5%)	89 (64.8%)	16 (100%)	130 (65.7%)	1 (2.0%)
Diptera	Bombyliidae		2	1		3	
	Syrphidae			1		1	
Diptera Total			2 (5.2%)	2 (1.4%)	0	4 (2.0%)	
Lepidoptera	Hesperidae			14		14	
Lepidoptera Total				14 (10.1%)	0	14 (7.1%)	
Other	Thysanoptera			1		1	
Other Total				1 (0.7%)	0	1 (0.4%)	

Table 2. Number of floral visitors at the Manchester Habitat Conservation Area, McGinty Mountain and Wright's Field. Numbers in the parenthesis reflect the percentage of the total observed or collected. 'X' indicates they met the definition of a potential pollinator (See 3. (A) below).

Not only were insect visitors active during mostly 100% sunny conditions but also had a preferred visitation timeframe in which they were active as long as the air temperature was at least 60°F (measured at 4 inches above the ground) and winds were below a constant 10 miles per hour. Once cloud cover was 30% or greater no insects were observed visiting the flower clusters. I performed a crepuscular activity study with one visit at dawn on May 8, 2009 at MHCA and one at dusk on May 10, 2009 at WF, as long as no clouds were present. Insects did not appear on the flowers before 0930 and were not observed on flowers after 1500. The highest visitor activity was between 1015 and 1430.

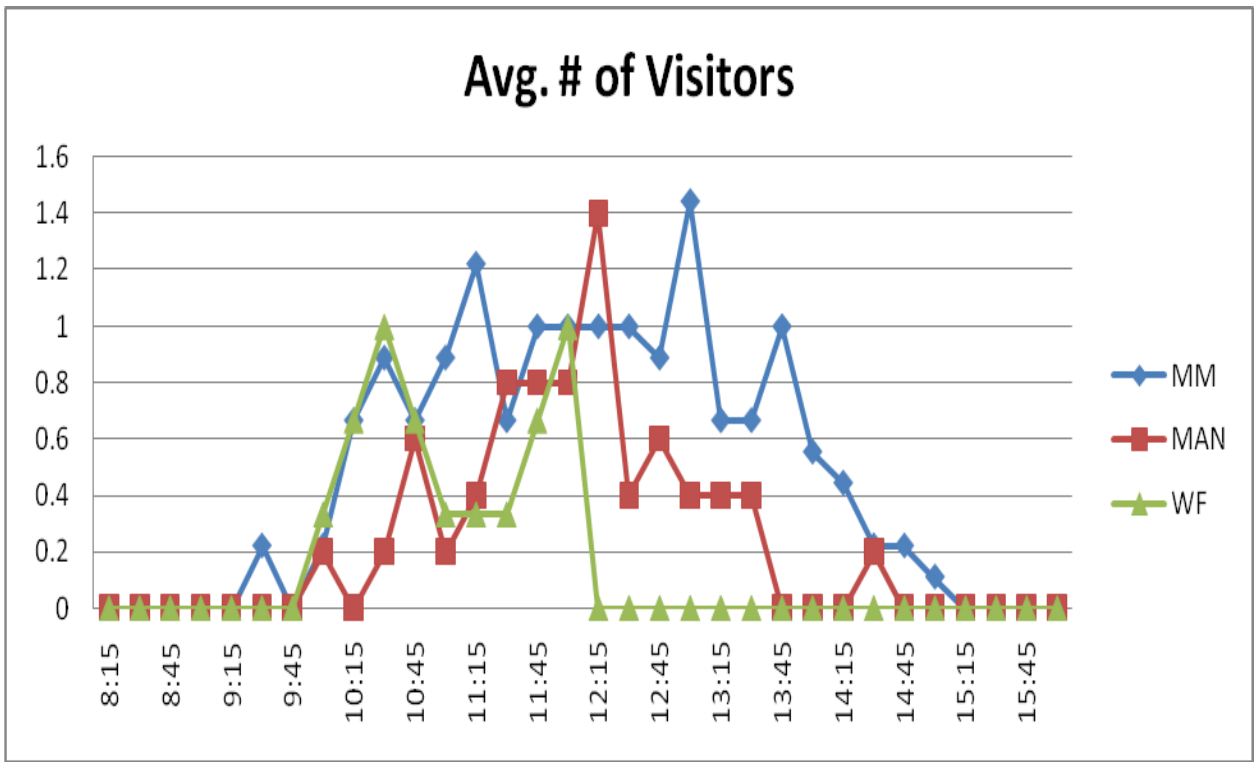


Figure 14: Showing average number of insect visitors at the study sites with peak visitation between 1015 and 1430.

D. Determine whether observed and/or collected insects contained pollen loads of *A. illicifolia*.

Insects observed visiting the flowers displayed different behaviors when on them. See Section 3 for a more detailed explanation of their behaviors. Not all visitors were collected to allow for potential pollinators to gather pollen and/or nectar for themselves and possibly active nests. Some insects were collected and placed in a cooler to bring their body temperatures down to a point of inactivity. Once they were cooled, they were removed from the cooler with tweezers and their bodies were carefully looked over for the presence of pollen using a 10X hand lens. If they had pollen on their body, some were placed into a killing jar and used as voucher specimens and the others were allowed to warm their bodies back up to a temperature at which they became active again.

Voucher specimens were pinned and either the part(s) of their body which contained pollen was scraped or the body part, i.e. leg, was removed and placed on metal plates with an adhesive to hold the body part or pollen in place (Figure 9). A log was kept as to which specimen, body part or pollen was removed and from which specimen. The adhesive plates were delivered to the Scanning Electronic Microscope (SEM) Laboratory at San Diego State University to determine if the pollen or body part contained pollen from *A. illicifolia*. Photographs of the body part or pollen were taken by the SEM in scale from 300X to 1,500X (Figure 15). When the SEM Laboratory was

finished their photographing, the series of photographs were place on an FTP site for me to retrieve. I analyzed the photographs to determine the presence of *A. illicifolia* pollen based on base SEM photographs created by the laboratory after I delivered *A. illicifolia* pollen to them at the beginning of the study.

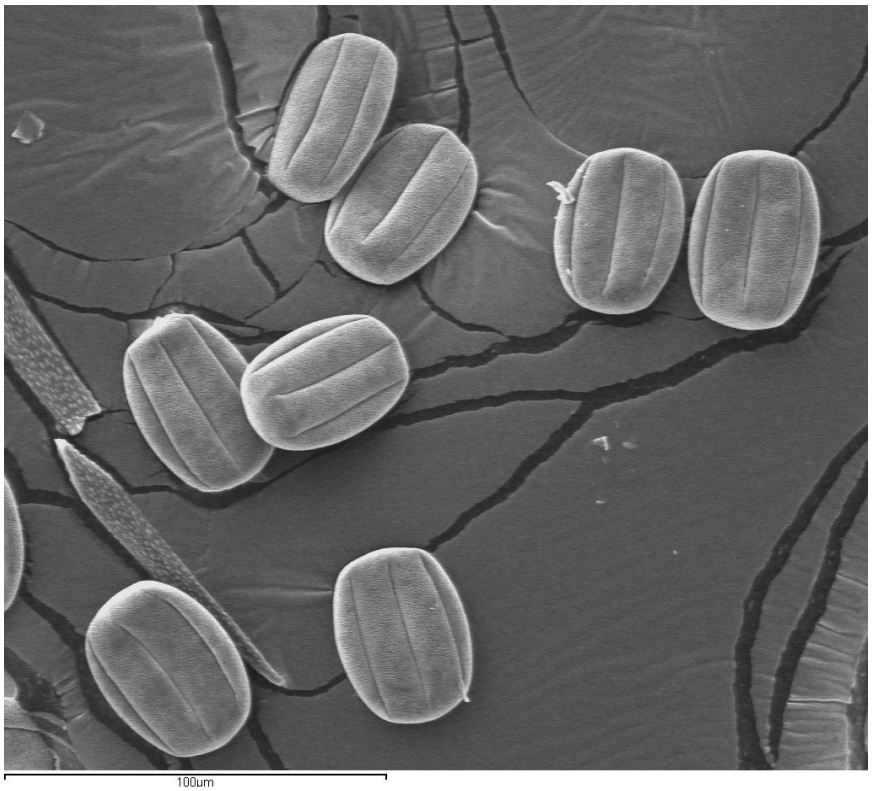


Figure 15: San Diego Thorn-mint pollen grains at 450X.

Bees carried *A. illicifolia* pollen on them with the majority collecting the pollen on their hind legs. Some had pollen within the hairs on their thorax. The bees containing the largest concentration of *A. illicifolia* pollen were the two observed Families; Apidae and Halictidae.

Beetles in the family Melyridae collected some small amounts of *A. illicifolia* pollen on the underside of their bodies but the majority was found around their mouth. Other beetles with *A. illicifolia* pollen were in the family Cleridae.

Flies in the family Bombyliidae and Syrphidae showed *A. illicifolia* pollen on their forehead mostly with small amounts on their mouths.

The only butterfly with pollen on its proboscis was the rural skipper (*Ochlodes agricola*) in the family Hesperidae.

(3) Determine whether insect visitors are functionary pollinators versus opportunistic feeders or thieves

A. Define Pollination

According to the Pollinator Conservation Handbook, (Sheperd, Buchmann, Vaughan and Black 2003) pollination is "The transfer of pollen grains from an anther to a receptive stigma. Self-pollination is movement within a flower or between flowers on the same plant; cross-pollination is between flowers on separate plants. Pollen may be carried by wind, water, or animals."

According to the non-profit organization, The Pollinator Partnership, in conjunction with The North American Pollinator Protection Campaign "Pollination occurs when pollen is moved within flowers or carried from one flower to another of the same species by birds, bees, bats, butterflies, moths, beetles or other animals, or by the wind. This transfer of pollen leads to fertilization and successful seed and fruit production. Pollination ensures that a plant will produce full-bodied fruit and a complete set of fertile seeds, capable of germinating."

Pollination can occur without fruit or seed production. For this report Pollination occurs as long as the pollen transfer event happens. This study did not look specifically at the potential result of the transfer event, i.e. fruit and seed set. However, through the study time, results could not be observed as to fruit production since the flowers wilted and turned brown at the end of the season. As long as the potential transfer event occurred, pollination occurred.

B. Define Feeders

Once insect visitors were observed, the next step was to determine whether the visitor produced the transfer event, i.e. potential pollination or remains within the inflorescence. The regularly timed intervals of 15 minutes are a useful measure to determine this. If after a pre-determined time the visitor has not moved from its initial observed time they are determined to be feeders (Buchmann and Nabhan, 1996; Proctor, Yeo and Lack, 1996). A feeder in this case may not necessarily be feeding on pollen or nectar. They feed on petals or even other visitors (Buchmann and Nabhan, 1996; Proctor, Yeo and Lack, 1996). Predators such as spiders, assassin bugs (Order Heteroptera, Family Reduviidae) and snakeflies (Order Neuroptera, Family Raphidiidae) are examples of this. Others which are considered feeders, predators or passing visitors are thrips (Order Thysanoptera), springtails (Order Collembola), earwigs (Order Dermaptera, cockroaches (Order Blattodea), booklice (Order Psocoptera), dragonflies / damselflies (Order Odonata), grasshoppers, crickets and katydids (Order Orthoptera), stoneflies (Order Pecoptera), true bugs (Order Heteroptera), lacewings (Order Neuroptera), scorpion-flies (Order Mecoptera) and caddis-flies (Order Trichoptera) (Proctor, Yeo and Lack 1996).

Defining certain insects as listed feeders, predators or passing visitors does not necessarily exclude them as potential pollinators. The regularly timed intervals by direct observation assisted in determining their function. As an example, if a visitor was observed on an anther and during the 15 minute direct observation intervals that visitor had not moved from the initial observed location after three hours, it was defined as a feeder and not a pollinator. See details below under **D. Pollination Success**.

C. Define Thieves

When studying potential pollinators, visitation is the first step. However, there are cues that can confirm nectar or pollen robbers (Kearns and Inouye, 1993; Inouye, 1980). One of the common cues is holes within the flower or petals removed. Nectar or pollen theft may have a detrimental effect on pollination. The results may also have the benefit of pollinating. Without direct observation this behavior is difficult to determine. One of the other results of nectar or pollen theft is no re-visits by other insect visitors (Buchmann and Nabhan, 1996).

D. Pollination Success

Based on Table 2, I will discuss in more detail these visitors and determine whether they meet the definition of a potential pollinator, feeder or thief.

1) Hymenoptera Success

a) True Bees (Family Apidae)
Of the five genera observed and / or collected all were in the subfamily Apinae with the exception of the *Ceratina* which is in the subfamily Xylocopinae. In the subfamily Apinae, *Apis* is in the Tribe Apini, *Ancyloscelis* and *Diadasia* are in the Tribe Emphorini and *Exomalopsis* is in the Tribe Exomalopsini. This is important because not only does Tribe breakdown assist in identification due to physical characteristics but also in their environmental function (Michener, 2000). Each genus / species will be discussed in more detail below.

1) Honey bee (*Apis melifera*) is a hairy medium sized bee. During the study individuals were observed attempting to land on the lower bi-lobed petal without success. During the recorded timed intervals they could be observed attempting to land on the lower petal as many a seven times. Eventually they would give up and fly off. This observed was consistent at all study sites until May 12, 2009 at the McGinty Mountain site. Four recorded intervals on that date observed an individual visiting over thirty flowers on one interval, ten on the second interval, ten on the third interval and nine on the fourth interval. With a hand lens pollen was observed throughout its body. Pollen was collected from its different locations on its body and SEM confirmed the

presence of *A. illicifolia* pollen on its forehead between its eyes. Since there was floral movement, I concluded pollination occurred.

2) *Ceratina* is a slender and small form of carpenter bee. They vary from black to brilliant metallic green which can result in confusing them with many species of sweat bees (Family Halictidae). Facial features as well as body hair are some of the keys in distinguishing them apart Michener, 2000).

   Although they lack dense hairs like many other Apidae, they are still effective flower visitors. They were only observed at the Manchester Habitat Conservation Area. They were the second most recorded visitor of bee to all of the study sites. They were regular observed crawling into the flowers of *A. illicifolia* and spending as much as five seconds in the flower. They moved to other flowers on the same plant as well as other plants during recorded timed intervals. With a hand lens, pollen was found on their body with the majority found on their head. SEM confirmed the presence of *A. illicifolia* pollen on its forehead between its eyes. Since there was floral movement, I concluded pollination occurred.

3) *Ancyloscelis* is a small bee ranging in size from 6 – 10mm. They are a long-tongued (L-T) bee which makes them suitable for getting nectar from tubular flowers. One of the physical characteristics of this species is the pronounced hind tibial scopa (pollen collecting hairs) (Michener, 2000).

   When observed at the McGinty Mountain study site they would make repeat visits to *A. illicifolia* flowers. Pollen was observed in balls on their scopa as well as their hairy head and thorax. SEM results confirmed the presence of pollen of *A. illicifolia* as well as pollen from other plants. Since it was observed making many re-visits as well as floral movement, I concluded pollination occurred.

4) *Diadasia* is a small bee ranging in size from 5 – 20mm. It is in the same Tribe as *Ancyloscelis*, so their physical characteristics are similar in that they are an L-T bee with pronounced hind tibial scopa. From there they can vary with some species containing dense hairs throughout their bodies to minimal hairs with the exception of the scopa (Michener, 2000).

   This was the most numerous group of bees observed visiting *A. illicifolia* and they were observed making re-visits. Pollen balls were observed on them as well as on their body from head to the underside of their abdomen. SEM results confirmed the presence of pollen of *A.*

illicifolia as well as pollen from other plants. Since it was observed making many re-visits as well as floral movement, I concluded pollination occurred.

5) *Exomalopsis* is in the Tribe Exomalopsini which has similar physical characteristics of the digger bee Tribe, Anthophorini. They can be minute to moderate sized bees with this genus ranging in size from 4 – 12mm. They lack the hind tibial scopa like the previous genera but they do have a well defined scopa on their metastoma (underside of their abdomen). They do have hairs on other parts of their body but they are more diminished (Michener, 2000).

   They were not a regular visitor to *A. illicifolia*. However, when observed they moved through the McGinty Mountain study site regularly and to numerous flowers on plants and numerous plants. They were observed on occasions moving through the upper crimped lobe and when collected, pollen was found on their metastoma scopa. SEM results confirmed the presence of pollen of *A. illicifolia* as well as pollen from other plants. Since it was observed making many re-visits as well as floral movement, I concluded pollination occurred.

b) Sweat Bees (Family Halictidae) are small bees with little hair on its body. They were not regular visitors but when observed they moved to between two and four flowers. With a hand lens, pollen was found on their body. SEM results confirmed *A. illicifolia* on its head and thorax. Other pollen from other plants was found on their hind legs. Since there was inter-floral movement, I concluded pollination occurred.

2) Coleoptera Success

a) Checkered beetles (Family Cleridae) are brightly colored with dense hairs on their bodies. It is not a large family within the United States. Some adults feed on stored products and carrion and some feed on pollen. The hairy body and pollen feeding behavior makes this family a potential pollinator. An individual was observed on one timed interval. It was observed moving to one flower on each of three different plants. With a hand lens pollen was found on its head with most of it around its mouth. SEM confirmed the presence of *A. illicifolia* around its mouth along with other pollen. Since there was floral movement, I concluded pollination occurred.

b) Soft-winged flower beetles (Family Melyridae) were the most common visitor. They are a small beetle, < 2 mm. The family is polyphagous feeding on both plant and animal material. The beetle was keyed to the genus *Dasytes*. It is a pollen feeder (Arnett, Thomas, Skelley and Frank,

2002). There were small amounts of pollen on their abdomen with the majority found around their mouth. When observed, individuals were always found on the same flower within the crimped upper lobe where the anther is located. Since no inter-floral movement occurred, no pollen transfer occurred. I concluded they did not pollinate.

3) Diptera Success

This is a diverse Order that includes parasites, predators, economic pests, vectors for diseases as well as pollinators. Two families were observed during the study. They are described in more detail below.

a) Bee Flies (Family Bombyliidae) are a fairly large family with the majority of species occurring in the southwest United States. They are one of a few Dipteran families which are predatory as larvae and nectar / pollen feeders as adults. This family documented to have efficient pollinators (Arnett 2000; Proctor, Yeo, and Lack, 1996). Many have hirsute bodies making them ideal pollinating candidates (Arnett, 2000).

Only two genera of bee fly were observed during the study; *Bombylius* and *Conophorus*. They were not regular visitors but when observed they visited multiple flowers. With a hand lens pollen was found on the proboscis of the *Bombylius* and on the head of the *Conophorus*. SEM confirmed no pollen from *A. illicifolia* on these species. Even though there was inter-floral movement, since no pollen was found on them, I concluded they did not pollinate.

b) Flower (Hover) Flies (Family Syrphidae) is a large family and dispersed throughout the United States. This family is also predatory in the larval stage with many being aphid feeders. Most adults feed on nectar and pollen and many are documented pollinators (Arnett, 2000).

One observation in the genus *Eupeodes* was at McGinty Mountain on May 12, 2009. It was noted on one timed interval visiting two flowers within the study area. When it visited the second flower it crawled into the tube for approximately two seconds. It never made contact with the crimped lobe petal above which is where the pollen is located. A hand lens conformed pollen on the underside of its abdomen. SEM confirmed no *A. illicifolia* pollen but pollen from other plants. Even though there was inter-floral movement, since no pollen was found on it, I concluded they did not pollinate.

4) Lepidoptera Success

Butterflies, moths and skippers are a large and diverse Order. Their mouthparts are designed to suck fluids and are therefore primarily nectar feeders. Much has been studied about this Order regarding the pollinating abilities of its members (Proctor, Yeo, and Lack, 1996; Arnett, 2000; Dafni, Kevan and Husband, 2005). In some instances they are specialists like the Yucca moth (Family Prodoxidae, *Tegeticula* sp.) whose relationship with yuccas are symbiotic (Powell and Opler, 2009).

The only recorded Lepidoptera was rural skipper (*Ochlodes Agricola*, Family Hesperidae). It was a repeat visitor only at McGinty Mountain visiting up to as many as five flowers during the recorded time intervals. It has a long proboscis and was able to dip it into the flower tube without making contact with the crimped upper lobe where the pollen is located. With a hand lens no pollen was found anywhere on its body. After taking a voucher specimen, the proboscis was unfurled and with a hand lens confirmed no pollen present. Even though there was inter-floral movement, since no pollen was found on it, I concluded they did not pollinate.

5) 'Other' included one observed individual. It was a thrip in the Order Thysanoptera. They are very small insects usually one millimeter or less. They can be common on flowers, under leaves or under bark. Some are predacious on mites and small insects but most feed on many flower parts causing damage (Arnett, 2000). One observation was recorded on May 12, 2009 at McGinty Mountain. No inter-floral movement was recorded during its one recorded time interval. Since no floral movement occurred, no pollen transfer occurred. I concluded it did not pollinate.

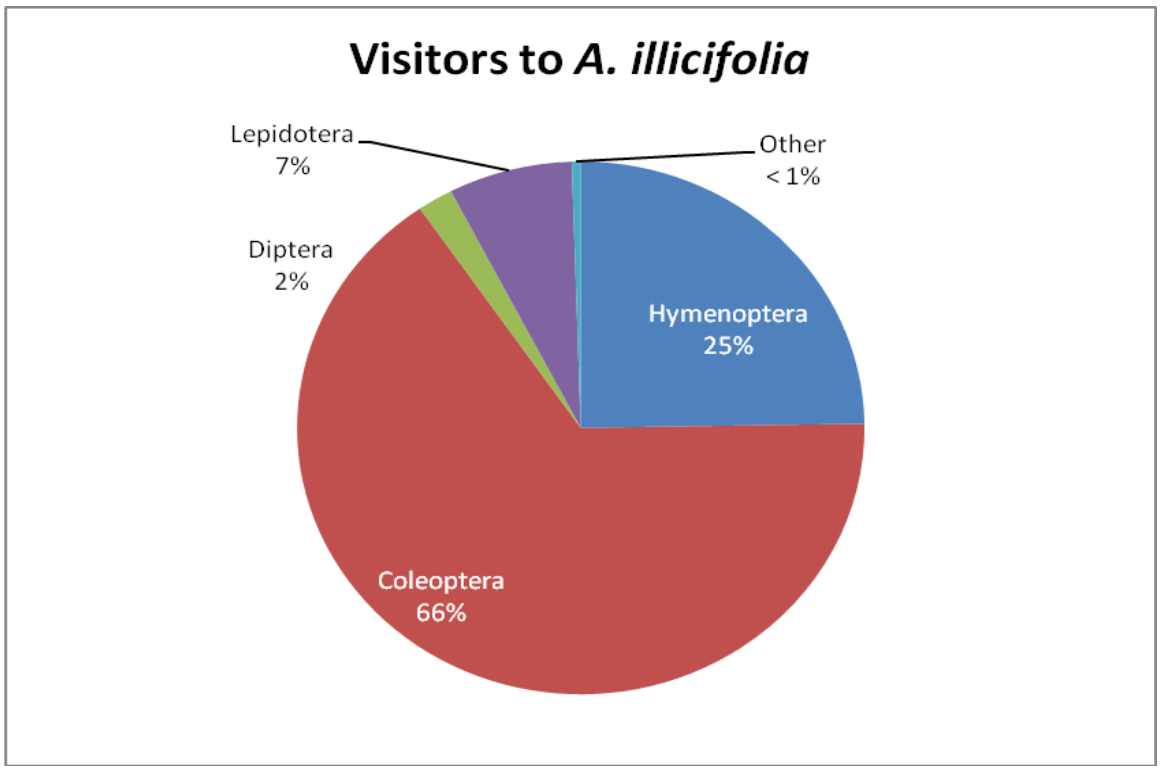
E. Conclusions

Overall there were very few visitors to *A. illicifolia*. This would appear to narrow potential pollinators. Based on the limited visitors, it does not appear *A. illicifolia* is pollinated by a specialist or specialists. Those visitors concluded to be pollinators also had pollen from other plants on them.

The dominant visitors were bees in the two Families Apidae and Halictidae. These appear to be the more effective pollinators.

Pollen of *A. illicifolia* was found on some visitors in the other Orders but data was inconclusive as to whether they are as effective as the Apidae and Halictidae bees.

The more effective pollinators appear to be 6 mm or smaller.



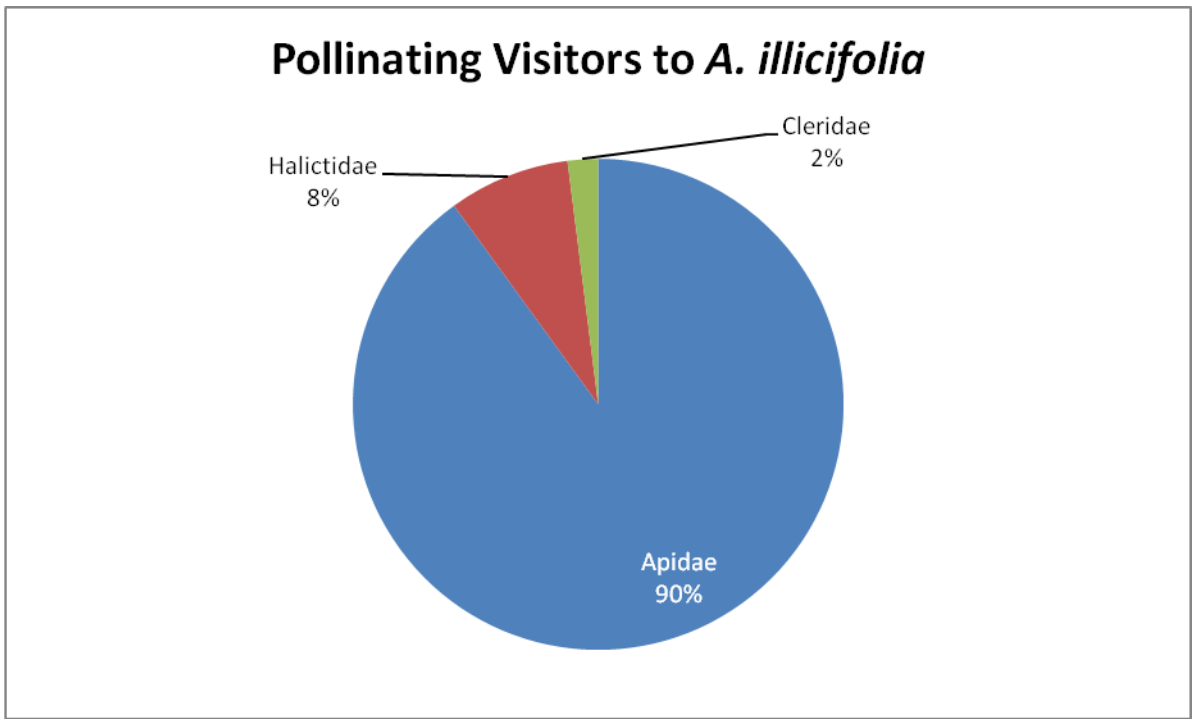


Figure 16: Comparing Total Insect Visitors to the Pollinators of *A. illicifolia.*

(4) Management Recommendations

- Continue studies of *A. illicifolia* to confirm smaller visitors are the more effective pollinators.
- Remove annuals from directly around and within *A. illicifolia* patches to allow for visitors to make repeat visits. Study sites at Wright's Field and Manchester Habitat Conservation Area were overrun with both native and non-native annuals. This hindered visitors from not only visiting once but repeatedly. Also of the pollinating candidates listed in Table 2, the majority of them are ground nesters and excess annuals affect their nests (Michener, 2000). Since *A. illicifolia* does not grow very tall, clearing of annuals taller than thorn-mint should occur. There are no studies showing how large of an area around a patch is minimal to allow for insect visitors. This could be a future study to assist land managers.
- Continue pollination studies to narrow the potential pollinator candidates.
- Future pollination research can be expanded into areas of plant biology and how they interact with pollinating candidates. Plant biology examples: (1) floral pigment or fragrances and how visitors as well as potential pollinators respond to them, (2) When is the flower most receptive for pollen for successful fertilization or when is the pollen at its peak for viability so successful fertilization occurs. As stated earlier, pollination can occur without results of fertilization which in turn may produce fruit and subsequently seeds. However, the goal of pollination is for perpetuation of the species and it is still unknown when the pollen is most viable or when the carpel is most receptive. The potential of understanding these may narrow potential pollinators.
- Future pollination research should include both botanists and entomologists.

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Appendix 1. Protocols used for *C. cyaneus* and *A. illicifolia* for recording data of regularly time intervals.

1) Monitoring will begin approximately 15 minutes prior to the emergence of the sun if the marine layer is present. It is possible that daily overcast conditions will influence insect activity and therefore it is important to be at the monitored site while conditions are still overcast and then be able to begin the monitoring once the clouds have burned away.
2) Monitoring will be completed when the coastal clouds have blocked the sunshine, rain/drizzle, or no insect visitation has occurred for one hour.
3) Temperature is not currently a factor because it is unclear whether there are potential pollinators which utilize cooler than normal or warmer than normal conditions in which to perform pollination.
4) Monitored plants are based on each study site (See Section (2) A. Site Selection for *C. cyaneus* and *A. illicifolia*.
5) At fifteen minute intervals the following will be recorded on a Data Input Sheet:
    a) Data #
    b) Time
    c) % sun
    d) wind speed and direction
    e) temperature, prefer Fahrenheit
    f) total # of insects on all the plants
    g) total # of species on all the plants
    h) plant #'s moving to, i.e. how many of the 10 plants observed it moving to
    i) any other observations or comments
6) Each plant will be inspected for a total census of insects and species.

Photographing of activity is recommended to document potential pollination activity.

Appendix 2: Lakeside Ceanothus Pollination Field Visit Information

Date	Survey Hours	Weather Conditions	Purpose of Visit,
5/01/07	0530-0700	Partly cloudy to clear; no breeze; 58-62°F	El Capitan Ceanothus crepuscular study
5/22/07	1300-1430	Overcast; gentle W breeze; 60°F	Crestridge Ceanothus Study – Overcast and cool.
5/24/07	0915-1430	Sunny; no breeze to SW @ 7 mph; 72-81°F	El Capitan Ceanothus Study
5/31/07	0915-1515	Marine Layer to Sunny; No breeze to NW @ 4-5 mph; 63-83°F	Crestridge Ceanothus Study
6/22/07	0915-1015	Sunny; no breeze; 72-75°F	El Capitan Ceanothus Wind pollination results
3/22/08	1000-1300	Sunny; no breeze; 68-84°F	Crestridge Ceanothus – Assessment to determine flowering season
4/16/08	0930-1430	Sunny; No breeze to N @ 7 mph; 58-69°F	El Capitan Ceanothus Study
4/26/08	0745-1345	Sunny, no breeze to N @ 3 mph; 78-96°F	Crestridge Ceanothus Study
4/28/08	0930-1430	Sunny; no breeze to W @ 6-10 mph; 85-91°F	El Capitan Ceanothus Study
5/04/08	0945-1445	Sunny to partly cloudy; SW-NW @ 2-5 mph; 70-74°F	Crestridge Ceanothus Study
5/13/08	0915-1115	Sunny; no breeze to N @ 2 mph; 62-71°F	Crestridge Ceanothus Study
6/04/08	1020-1035	Sunny; no breeze; 73°F	El Capitan Ceanothus Study – season complete no shrubs in flower
6/12/08	1800-2030	Crestridge; no breeze; 68°F	Crestridge Ceanothus Study – crepuscular study
5/13/09	0920-1515	Sunny; no breeze to W @ 3-6 mph; 71-73°F	Crestridge Ceanothus Study
5/14/09	1015-1415	Sunny; NW-W @ 1-6 mph; 73-75°F	El Capitan Ceanothus Study
5/19/09	0850-1400	Filtered sun to sunny; NE-N @ 1-3 mph; 68-87°F	Crestridge Ceanothus Study
5/21/09	0945-1445	Sunny; W-NW @ 2 mph; 65-80°F	El Capitan Ceanothus Study

Appendix 3: San Diego Thorn-mint Pollination Field Visit Information

Date	Survey Hours	Weather Conditions	Purpose of Visit
4/04/07	0900-1600	Partly cloudy to Sunny; W @ 4-8 mph; 58-74°F	Scouting trip with DPLU, CDFG and USFWS to check out Wright's Field, and McGinty Mountain
4/14/07	0900-1030		Scouting trip with USFWS and CNLM to MHCA site to determine suitability for thorn-mint study
4/15/07	1000-1030	Partly cloudy; W @ 3-6 mph; 58°F	WF: checking status of plants
4/16/07	1000-1015	Overcast; W @ 4-5 mph; 59°F	WF: Plants not yet in flower
4/30/07	1145-1245	Sunny; W @ 4-5 mph, 72°F.	WF: Some plants in flower and already senescing
5/18/07	1030-1430	Hazy to Sunny; W @ 2-6 mph; 71-69°F.	MM: Some plants in flower but most have senesced.
3/23/08	1330-1400	Sunny; E @ 10 mph; 82-84°F	WF: checking status of plants
4/13/08	1345-1415	Sunny; N @ 3 mph; 91°F	WF: checking status of plants
4/14/08	1115-1515	Sunny; W – SW @ 2-6 mph; 78-74°F	MHCA: stopped after over one hour of no visitors
4/15/08	1015-1045	Sunny, W @ 2-5 mph: 62-63°F	MM: only one plant with one flower present
4/21/08	0830-1330	Hazy to sunny; E – W @ 2-6 mph; 62-74°F	MHCA:
4/22/08	1215-1415	Sunny; NW @ 3-8 mph; 71-75°F	WF: Stopped due to no insect visitors
4/23/08	0945-1500	Sunny to Hazy; W @ 2-6 mph; 59-71°F	MM: only flower beetle activity
5/07/08	1015-1130	Overcast; gentle breeze to N @ 3 mph; 56°F	WF: Stopped due to overcast and cool conditions
5/11/08	0945-1200	Sunny; no breeze to W @ 4 mph; 59-75°F	WF: Stopped due to one insect (Flower Beetle) visiting. No other visitors.
5/14/08	0915-1430	Sunny; SW @ 2-3.5 mph; 71-82°F	MM: decent visitor activity

Date	Survey Hours	Weather Conditions	Purpose of Visit
5/21/08	1130-1300	Mostly Cloudy; SW @ 6 mph; 72-70°F	MM: Stopped due to no insect activity because of cloud cover.
6/2/08	1045-1430	Sunny; W @ 6-3 mph; 73-80°F	MM: stopped, no visitors since 1300
6/10/08	1030-1400	Marine layer-Hazy sun; SW @ 7-8 mph; 71-74°F	MM: Stopped due to no visitors to the flowers
3/05/09	1100-1230	Sunny; W @ 3 mph; 61°F	MHCA: Monitor potential timing for surveys
4/04/09	1330-1430	Sunny, W @ 5 mph; 64°F	MHCA: Plants beginning to flower need about 1 week for potential surveys
4/15/09	0945-1230	Sunny; NNW @ 12 – 25 mph, 51 - 60°F	MHCA: Started survey stopped due to winds. WF: Monitored status. Plants at least 2-3 weeks away from flowering.
4/21/09	0930-1245	Sunny; no breeze to SW @ 3 mph; 80 - 85°F	MHCA: Many plants already beginning to senesce.
4/23/09	1100-1215	Overcast; SW 6-9 mph; 58 - 55°F	MHCA: stopped survey due to overcast conditions and no insect activity.
4/30/09	0830-1300	Sunny	MM, WF, MHCA assessing plant status. MM had over 70 plants in flower
5/08/09	0600-0730	Partly cloudy to clear; no breeze; 59-61°F	MHCA crepuscular study
5/10/09	1830-2030	Clear; W @ 4-6 mph; 68-63°F	WF crepuscular study
5/11/09	0945-1145	Sunny; W @ 3-4 mph; 70-82°F	WF: stopped due to no visitors
5/12/09	0920-1510	Sunny; WSW-W @ 3-5 mph; 67-77°F	MM: decent visitor activity
5/18/09	0850-1500	Sunny; no breeze to W @ 2 mph; 82-88°F	MM: decent visitor activity
5/22/09	0915-1515	Sunny: W @ 3 mph; 73-70°F	MM: visitor activity but declining. Flowers are beginning to senesce