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Endangered plant conservation : do pollinators matter to the robust spineflower?

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ENDANGERED PLANT CONSERVATION:
DO POLLINATORS MATTER TO THE ROBUST SPINEFLOWER?

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by
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ABSTRACT

ENDANGERED PLANT CONSERVATION: DO POLLINATORS MATTER TO THE ROBUST SPINEFLOWER?

by Kathryn Ann Murphy

Pollinators can be crucial to reproductive success and long-term survival of endangered plant species. As is the case for many endangered plants, no pollination studies have been conducted for the robust spineflower (*Chorizanthe robusta* var. *robusta*), a federally endangered annual plant endemic to the central coast of California. This study of two robust spineflower populations evaluates pollinator importance, frequency, and habitat needs through extensive observation and experimental bagging of inflorescences. It also correlates pollinator visitation frequency with environmental conditions.

Research results indicate that although *C. robusta* var. *robusta* is self-compatible, pollinator access to flowers increased seed set significantly, indicating that pollinators increase plant fitness. Pollinator visitation rates varied within sites and between sites, and correlated with environmental conditions. The observed high diversity of pollinators may allow for visitation within different microhabitats. Results suggest that protecting pollinator habitat and diversity may be important to the survival of this taxon.

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Background

The relationship between plants and their pollinators is essential to the long-term survival of most plant species, ensuring the exchange of genetic information and reproductive success. Changes in plant-pollinator relationships can particularly affect endangered plant species, as they are often comprised of small populations vulnerable to ecological change (Schemske et al.1994). Knowledge of plant-pollinator relationships can often be important in managing for the survival and recovery of endangered species. Currently, little is known about the pollination of the robust spineflower (*Chorizanthe robusta* Parry var. *robusta*), a federally endangered annual plant found in Santa Cruz County in California. The purpose of this project is to investigate aspects of the pollination ecology of the robust spineflower. The information gained from field research and review of existing research is then analyzed to make management recommendations on how to best protect the relationship between *C. r.* var. *robusta* plants and their pollinators.

Since the passage of the Endangered Species Act (ESA) in 1973, the United States has made a commitment to protect species in danger of extinction, and to develop plans for their recovery. The ESA states that the habitat of a species must also be protected if the species is to survive and reproduce (Silander and Primack 1978). The Act specifies that research on habitat components necessary to the species is required for recovery plans (Schemske et al. 1994). Though pollinators can be essential to the habitat of many plant species, often little is known about the pollination ecology of endangered species. The needs of pollinators are rarely specified in recovery plans (Neal 1998).

By relying on pollinators, plants can increase the chances that some of the pollen deposited on a flower is from a different plant. Most plants rely on this type of pollination, referred to as outcrossing, for at least some of their reproduction (Proctor et al. 1996). Outcrossing ensures greater genetic variability within plant populations, enabling populations in general to adapt more successfully to environmental change and survive over time. Outcrossing can occur among different plants within a plant colony, among plants in different colonies, or among plants in separate populations. For plants that rely on insects for pollination, outcrossing patterns are determined by the foraging behavior of the pollinating insects and the distance between plant populations, colonies, and individuals (Handel 1983).

Many types of human activity, such as agriculture and urbanization, have resulted in habitat loss and fragmentation for many plant and insect species. While habitat changes can directly affect plant species by reducing the number and size of populations, habitat modifications can also harm plant populations indirectly by affecting the pollinator community.

Habitat fragmentation, in which a contiguous area is broken up into smaller islands surrounded by new human-modified environments, can also harm pollinators (Allen-Wardell et al. 1998, Jennersten 1988). Reductions in the size of insect populations can lead to a reduced frequency of pollinator visits, and a lower diversity of insects that pollinate. Populations with lower frequency and diversity of pollination can have reduced seed and fruit set resulting from an insufficient amount of compatible pollen being delivered to the flowers (Jennersten 1988).

Fitness levels in plant populations can also be reduced through inbreeding depression. Inbreeding depression results from high levels of selfing or from interbreeding among closely related individuals. Populations that have recently suffered from habitat modification or reduction are likely to experience changes in gene flow (Barrett and Kohn 1991). If the changes in gene flow result in increasing levels of genetic exchange between related individuals or increased levels of selfing, then these populations could be more susceptible to inbreeding depression (Lesica 1993). One way in which inbreeding depression can occur is through habitat changes that limit the ability of pollinators to forage among separate plant populations or colonies (Allen-Wardell et al. 1998).

Like many other endangered species, the robust spineflower has been affected by habitat loss and habitat fragmentation. Historically, the range of the robust spineflower extended from Alameda County south to Monterey County (US FWS 2002). It is now limited to only eight known populations, all occurring in Santa Cruz County. In 2002, the US FWS designated critical habitat for this variety. The low number of individuals and populations puts the plant at great risk of extinction. The robust spineflower has already suffered from habitat loss due to development. The population at Sunset Beach is now surrounded on three sides by agricultural fields. The populations occurring on private property are further threatened with habitat loss and habitat fragmentation due to potential development (US FWS 2000). These factors, which are at least in part the cause of endangerment, also have the potential to threaten pollinators. If the plant receives inadequate pollination, this could affect the ability of the plant to reproduce and reduce the amount of genetic exchange occurring within the populations. Lower reproduction, changes in gene flow, and potential inbreeding depression could ultimately threaten the ability of the variety to survive.

Although knowledge of pollination ecology can be essential in managing for the recovery and survival of endangered plants, currently we know very little about the pollination ecology of the robust spineflower. The information on pollination of other *Chorizanthe* taxa is scattered or conflicting. No one has conducted pollinator observations or studies of the robust spineflower (US FWS 2000). *Chorizanthe pungens* var. *pungens*, a threatened spineflower variety growing in Monterey County, had poor seed set and germination when pollinators had limited access to plants (Harding Lawson Associates 2000). This result could indicate that pollination is necessary for this species to reproduce. On the other hand, Moldenke (1976) determined through bagging and greenhouse experiments that some species of *Chorizanthe* were capable of self-fertilization. He predicted that the genus *Chorizanthe* habitually self-fertilized. The US Fish and Wildlife Service has recognized the need for more information on the pollination biology of the robust spineflower in its Draft Recovery Plan (2000).

The goal of this project is to bridge the gap that currently exists in our knowledge of the pollination ecology of this endangered taxon. This research encompasses a study of two populations of robust spineflower: the first is a large population at Sunset State Beach in Santa Cruz County, and the second is a smaller population at Pogonip Park in

Santa Cruz County. Both field and library research was conducted to address the following questions: To what degree can the plant self-pollinate? Does the progeny of self-pollinated flowers show reduced levels of fitness? What are the most frequent pollinators, and what are their habitat needs and foraging ranges? How diverse is the pollinator community? What environmental factors might affect the frequency of pollination? The results of this research can then be used in managing the taxon and protecting the relationship between the plant and its pollinators.

Objectives

The goal of this project was to investigate aspects of the pollination ecology of *C. robusta* var. *robusta*, and apply this information to assist in the survival and recovery of this variety of spineflower. Four objectives encompassed the project:

1. Determine the importance of pollinators to plant reproduction and fitness in *C. robusta* var. *robusta*:

   H1: Pollinator access improves plant fitness in *C. robusta* var. *robusta*.

2. Identify insect pollinators of *C. robusta* var. *robusta* and record their frequency, abundance and diversity.

3. Evaluate what environmental factors might affect visitation frequency:

   H1: There is a positive relationship between frequency of insect visitation and plant height, the density of robust spineflower, the density of other plant species, and percent bare ground.

4. Identify the habitat requirements and foraging ranges of pollinators.

RELATED RESEARCH

This literature review examines pollination studies of plants in altered habitats, and reviews literature on pollination studies of rare and endangered species. This review then discusses existing research on *C. robusta* var. *robusta* and discusses how pollination may be important to the taxon. The review concludes by discussing how the present research on pollination ecology will assist in the survival and recovery of the robust spineflower and add to our knowledge of the pollination of endangered species.

Theoretical framework for research

There is increasing evidence for a worldwide decline of honeybee pollinators, and indications of a decline in the populations of other invertebrate pollinators as well (Allen-Wardell et al. 1998). In a study commissioned by the Society for Conservation Biology, Allen-Wardell et al. (1998) reviewed the status of pollinators globally, and discussed how declines in pollinator populations threatened the stability of food crop yields and animal and plant biodiversity. The paper emphasized the importance of pollination research on rare and endangered plants, and made specific research recommendations. These recommendations included monitoring of invertebrate pollinators as part of critical habitat management, including the habitat needs of critically important pollinators in the management and recovery plans, and monitoring seed and fruit set and pollinator visitation rates in the management of endangered species.

Several factors can make rare and endangered plants more susceptible to disruptions in the pollination system. Species that have recently experienced changes in distribution may experience changes in gene flow due to changes in pollinator foraging habits (Huenneke 1991). If pollinators are limited to foraging among a small population of closely related individuals, or if the rate of self-fertilization increases, then inbreeding depression can occur. This can result in reduced fitness in the population. Inbreeding depression is also likely to occur in species that have limited geographic distributions (Karron 1991). Habitat fragmentation is also common with endangered plant species. Habitat fragmentation can lead to reduced frequency or diversity of pollinators and result in reduced seed set in the population (Jennersten 1988, Steffan-Dewenter 1999).

Despite the fact that the reproductive success and long-term survival of many endangered species could be affected by disruptions to plant-pollinator interactions, very few recovery plans discuss the needs of pollinators. Kathryn Kennedy, previously with the US Fish and Wildlife Service, presented a review of the recovery plans of endangered plant species to the Ninth Annual International Conference of the Society for Ecological Restoration. In her review, she found that only a few plans had information on pollination ecology, and none of the plans reviewed in the study included plans for managing pollinator habitat (Neal 1998).

Many studies on the pollination ecology of rare and endangered plants discussed how understanding the pollination biology and protecting pollinator habitat may be crucial to the survival and recovery of the species. Disruptions to the pollination system of rare plants can reduce plant reproduction and recruitment, and potentially contribute to extinction. Studies by Boyd (1994), Pavlik (1992), Lesica (1993), and Sipes et al. (1995) focused on the pollination ecology of rare and endangered plants. These studies determined the importance of pollination to plant reproduction through pollinator exclusion experiments, identified the most important pollinators, estimated the overall abundance of key pollinators, and analyzed whether pollination limited reproduction. These particular studies found pollination to be crucial to plant reproduction, and found key pollinator populations to be disrupted or threatened. The authors of these studies included general or specific recommendations on how to protect pollinator populations.

Lesica (1993) conducted a pollination study of *Silene spaldingii*, a partially self-compatible grassland perennial listed by the state of Montana as endangered. The anthers of this species mature and dehisce before the stigma matures, a breeding system that discourages but still permits self-fertilization. To assess the most frequent pollinators, Lesica observed insect visitors over three consecutive days in 1988 and in 1990. In each year he observed only three visits, all by bumblebees. To assess the effects of pollinator exclusion on plant fitness, Lesica selected 35 pairs of plants, and covered the inflorescence of each treatment plant to exclude pollinators. The reduction in fecundity of the seeds produced by flowers that were covered to prevent pollinator access was estimated to be 97%. The differences in fecundity were attributed to inbreeding depression in the plants that self-fertilized due to pollinator exclusion.

Lesica concluded that pollination is vitally important to plant fitness and recruitment, and to the survival of the species. He cautioned that burning, spraying, and grazing grasslands could negatively affect bumblebee populations, which nest at or just below the surface of the ground. He urged that consideration be given to the pollinator habitat requirements in managing areas near *Silene spaldingii*.

Sipes et al. (1995) analyzed the breeding system and pollination of a rare orchid, *Spiranthes diluvialis.* The study determined that a pollinating agent was required for pollination to occur. Insect visitation was rarely observed, but a few bumblebees were seen visiting the flowers and carrying pollinia. The authors concluded that protecting bumblebee populations could be crucial to the survival of the species. Recommendations to protect bumblebee populations included maintaining the insecticide-free buffer zone around populations of the plant as recommended by the FWS, and extending the buffer zone to cover the entire growing season. The authors also recommend providing nesting boxes, protecting above ground structures where the bees are likely to nest, and protecting floral diversity to provide sources of pollen for the bees throughout their foraging season.

Other studies have focused more specifically on the habitat needs and foraging behavior of pollinators. Pollinator foraging patterns can determine how pollen is transferred among flowers, and thus may affect gene flow and outcrossing rates both within and between plant populations (Handel 1983). Pollinator foraging studies can help determine how pollinator movements might affect specific plant species. Leong et al. (1995) documented the foraging movements of the most common pollinator of a vernal pool plant species, *Blennosperma nanum* var. *nanum*. By using mark and recapture techniques, the authors found that the native solitary bee pollinator, *Andrena blennospermatis*, foraged primarily within one plant patch. The authors occasionally observed the bees foraging between patches 25 meters apart, but never between patches

80-100 meters apart. The authors discussed how this is likely to affect genetic exchange within *Blennosperma*, since the bee is the most common pollinator.

Studies on habitat requirements of insects can provide information for their conservation, as well as information on the availability of suitable habitat for pollinators of particular plants. Wettstein and Schmid (1999) analyzed how different types of habitat modification and fragmentation affect both butterfly and grasshopper abundance at wetland sites. The authors found that as habitats became increasingly fragmented, species richness of butterflies decreased. The authors discussed how maintaining habitats with diverse management techniques and vegetation structure could help conserve a diversity of insect species. They also discussed how maintaining connectivity between habitats could be especially helpful to butterfly species.

Taxonomy, life history, and pollination

Chorizanthe robusta Parry var. *robusta* is a small slightly hairy annual in the *Pungentes* section of the buckwheat family (Polygonaceae) (US FWS 2000). The taxon was listed as endangered in 1994. This variety of spineflower was first collected and named by Charles Parry in 1889. The plants are branched from the base, erect or spreading, and growing up to 8" tall. The basal rosette of leaves is greenish or reddish in color. The involucre of bracts subtending each flower has hooked teeth, from which the genus derives it name. The margins of the bracts are pink or white and membranous. The flower heads are aggregate and range in size from 1.5 to 2.0 cm, which is relatively large for a spineflower, hence the name robust.

The morphology of the *C. r.* var. *robusta* flower indicates that it could be pollinated by a wide array of pollinators. The white to rose-colored flower is radially symmetric. The cylindrical flowers range in length from 2.5 to 4mm (Reveal 1989). Many species that have flowers with short corolla tubes are visited by bees, beetles, butterflies and flies (Kearns and Inouye 1994). A shorter corolla tube generally indicates that nectar is accessible to insects with short as well as long proboscises.

Each flower contains one ovule that if successfully fertilized produces one seed. Flowers that produce only one seed per ovule theoretically need only one pollen grain for fertilization (Gomez and Zamora 1999). Hence, it is possible for pollinators that carry only a small amount of pollen to transmit enough pollen to fertilize the flower in a single visit. In addition, since the anthers and stigma are well extended above the flower and are in close proximity to each other, it is likely that most insects would make contact with the reproductive parts of the flower while foraging.

There are several other related *Chorizanthe* taxa in Santa Cruz County that are also listed under the California Endangered Species Act or the Federal Endangered Species Act. The Ben Lomond spineflower (*Chorizanthe pungens* var. *hartwegiana*) is a federally endangered variety growing in the Ben Lomond Sand Hills. McGraw and Levin (1998) identified that survivorship, growth and reproduction of the Ben Lomond spineflower were low under high shade, indicating that shade intolerance may restrict the distribution of this variety of spineflower to open sandy areas. Pollinators of this spineflower variety include wasps, bees, flies and butterflies (US FWS 2001). *Chorizanthe robusta* var. *hartwegii*, the Scotts Valley spineflower, is also federally endangered, and Critical Habitat has been designated for this variety. The Monterey spineflower, *Chorizanthe pungens* var. *pungens*, is threatened. The US FWS has designated critical habitat for this taxon as well.

Ertter (1996) has attempted to clarify the taxonomy of the robust spineflower and other related spineflowers, but further taxonomic work needs to be done to clearly distinguish the different species and varieties from one another. Many of the characters used to identify these plants are plastic, and there are some disagreements as to the identification of some specimens. *C. r.* var. *robusta* is characterized as erect, with white inflorescences forming distinct heads. This is in contrast with the closely related *C. pungens* var. *pungens*, which is more prostrate and has smaller heads that may be distinct or indistinct (Ertter 1996).

Although the basic life history of the robust spineflower is known, little detailed research has been conducted on habitat needs and other requirements for the long-term survival of the taxon. The robust spineflower is an annual with indeterminate growth. It germinates with winter rains, and blooms from April through June or July or even later depending on growing conditions. Seeds disperse by involucre teeth that attach to the fur of passing animals (US FWS 2000). The plant grows in sandy soils. At Sunset State Beach, plants grow in openings in coastal scrub habitat. At the more inland populations, the robust spineflower grows in openings in chaparral or woodland. At one population of *C. robusta* var. *robusta*, Baron (1998) found a mean survivorship rate of 42% for seedlings. Baron identified desiccation as a major cause of mortality, and identified a correlation between size of plant and overall reproductive output.

Research on pollination in California, as well as pollination syndromes and trends within the different plant associations among which the robust spineflower grows, can give some indication of pollinator availability for the robust spineflower. In California in general, bees are probably the most important pollinators, especially bumblebees, Anthophoridae and semi-social Halictid bees (Moldenke 1976). Generalist bees are probably the most significant pollinators for plants that are locally common, but have inconspicuous flowers or low rewards per flower. Bee flies pollinate about 10-20% of the flora in low to mid elevations in California. Wasps visit at least 40 genera of plants, including *Eriogonum*. Butterflies are also important pollinators in California.

The robust spineflower population at Sunset State Beach grows in a coastal scrub habitat. A survey of bee abundance and diversity at a cool windy and foggy coastal scrub site in Marin County found one-half the number of bee species as were found at a more inland chaparral site (Dobson 1993). The cool, foggy, and windy climate was found to limit flight time for small bees and metallic bees, which need higher temperatures for longer periods to warm their body temperatures. Bumblebees, which can regulate their body temperatures, are more common in coastal foggy climates. Coastal scrub in northern California also has about 50% less overall pollinator diversity than inland chaparral sites (Moldenke 1976). Muscoid flies are common in immediate coastal areas, and occasionally pollinate. Bee flies are also fairly common pollinators in coastal areas. Hover flies are common but tend to be inefficient pollinators due to lack of facial pubescence.

Due to climatic conditions unfavorable to most pollinators, self-compatibility is more common in coastal foggy areas. Moldenke (1976) estimated that 30% of the flora in the coastal scrub are self-compatible or habitual self-fertilizing species, as compared to 18% of the genera in California overall. Most of the habitual selfers or self-compatible plants are annuals, which often stand a better chance of reproducing in one season if they do not have to rely on pollinators for reproduction (Moldenke 1976). Wind pollination is also more common in coastal areas.

Other robust spineflower populations grow in openings in chaparral or woodland. In chaparral areas, bee diversity and overall pollinator diversity is higher (Moldenke 1976). Halictid bees, which are generalist pollinators, as well as smaller specialist bees, are more common in chaparral vegetation. Ground nesting bees are more common, since they require dry areas less favorable to fungus and an abundance of dry twigs that are present after fires. Most chaparral shrubs are genetically self-incompatible. Nocturnal moths are also present in chaparral, pollinating masses of small white flowers.

Effective ant pollination is somewhat uncommon, since their method of entry into the flower frequently allows them to take nectar without contacting any pollen. Ants can actually be harmful to plants by secreting chemicals that can interfere with pollen viability (Proctor et al. 1996). However, pollination by native ant species has been documented for another plant in the Polygonaceae, *Polygonum cascadense* (Hickman 1974). The plant is prostrate, and has inconspicuous flowers close to the stem. Separate plants often grow intertwined. Since the plant has a similar growth habit to the robust spineflower and is in the same family, it is possible that native ants could also pollinate the robust spineflower.

Although no pollination studies have been conducted for the robust spineflower, limited pollinator observations have been conducted on other species in the genus *Chorizanthe*. Randy Morgan observed wasps, bees, flies and butterflies on *Chorizanthe pungens* var. *hartwegiana,* the Ben Lomond spineflower. Moldenke (1976) observed several different types of insects visiting *Chorizanthe* flowers, including wasps, beeflies, unspecified tiny species of insects, and generalist bees.

In the annual species of *Chorizanthe* studied by Reveal (2001), the anthers mature the first day the flower is open, and the stigma receptivity is delayed until the following day. When the stigmas become receptive, the three style branches uncoil and the stigmas are exerted from the center of the flower. Self-pollination may occur at the end of the first day if the flower has not been cross-pollinated. Some other annual *Chorizanthe* species have the anthers positioned in such a way that the degree of selfing is reduced. Greenhouse work on some annual species of *Chorizanthe* indicated that selfing is important to those species (Reveal 2001). However, *Chorizanthe pungens* var. *pungens*, a threatened spineflower variety growing in Monterey County, had poor seed set and germination when pollinators had limited access to plants (Harding Lawson Associates 2000). This result could indicate that pollination is necessary for this species to reproduce.

Status as an endangered species

The robust spineflower was listed as endangered in February 1994 due to the small number and sizes of the remaining populations. Currently there are only eight known populations, all occurring in Santa Cruz County (US FWS 2002). The historical range of the taxon included sandy areas near the coast from Alameda County south to Monterey County, a range of 100 miles. The primary cause of endangerment for the robust spineflower is loss of habitat to agricultural and urban development (US FWS 2000).

The largest population occurs along a half-mile stretch of backdunes at Sunset State Beach (FWS 2002). The population at Sunset Beach in 1995 was estimated to consist of 100,000 individuals. The Pogonip Park site, owned by the City of Santa Cruz, contains two small colonies confined to a small area of habitat. Another population occurs on a grassy site in the city of Santa Cruz near where Highway 1 crosses Branciforte Creek. In the City of Aptos, a robust spineflower population grows between openings in maritime chaparral. At the Buena Vista site, a population occurs on a private parcel west of the City of Watsonville, growing in openings in oak forest and maritime chaparral. Another population is found in Freedom, growing in a grassy opening also amongst oak woodland and maritime chaparral. In 2002, an additional population was discovered at a site south of Empire Grade and north of Wilder Ranch. Another potential population was found in 2002 at two locations in Manressa State Beach, although it has not been determined with certainty whether the plants were *C. r.* var. *robusta* or *C. pungens* var. *pungens* (US FWS 2002). Threats to robust spineflower populations include loss of habitat, particularly those populations occurring on private property. Recreational use and competition from non-native plants also pose threats. Extirpation due to stochastic events also threatens smaller populations.

The US Fish and Wildlife Service published a draft recovery plan for the robust spineflower in 2000 (US FWS 2000). In 2002, US FWS designated critical habitat for *C. robusta* var. *robusta*. As indicated in the draft recovery plan, the immediate recovery goal is to avoid the loss of any existing populations by protecting population sites from further development (US FWS 2000). The next goal would be to develop and implement management plans so that the existing populations can remain self-sustaining, and remain stable or increase in size, for at least 10 years. If this goal can be achieved, the variety would be considered for downlisting from endangered to threatened. The long-term goal would be to locate additional existing populations, or to establish new populations, so that there are at least 10 populations of 1,000 or more individuals that persist for at least 10 years. At that point, the robust spineflower would be considered for delisting.

To achieve these goals, the FWS lists six priorities (FWS 2000):

1. Protect habitat. To further protect *C. r.* var. *robusta* habitat from destruction or modification, the US FWS (2002) has designated critical habitat consisting of 469 acres. The critical habitat designation also identifies special management actions that *C. r.* var. *robusta* populations may require. The critical habitat designation includes areas outside the geographic area occupied by *C. r.* var. *robusta* that include "primary constituent elements" of the species (FWS 2002). The primary constituent elements are identified as sandy soils, plant communities that support species associated with *C. robusta* var.

robusta, plant communities that contain little or no cover by invasive non-native species, and physical processes that support the natural dynamics of coastal dunes. The critical habitat designation only protects habitat on federal lands, or on non-federal lands if a proposed action involves federal funding or permitting.

2. Manage habitat. In the critical habitat designation, the US FWS recommends developing management plans to help sustain ecosystem processes that are essential to each population of robust spineflower. Recommendations include maintaining plant communities to provide for the habitat needs of pollinators, as well as limiting the use of pesticides. The report also recommends maintaining connectivity among sites in close proximity to support pollinator movement between sites. In addition, the report recommends limiting activities such as building roads or fences that could fragment habitat. To maintain openings for *C. r.* var. *robusta*, the report recommends using prescribed fire, thinning or other vegetation management. The report also recommends controlling invasive species.
3. Conduct Management -Oriented Research. This research should help identify what is required for long-term species viability. Research should include taxonomic and life history studies, habitat requirements including pollination, and population monitoring.
4. Establish new populations in appropriate habitats within the taxon's historical range.
5. Revise recovery criteria, as new information becomes available. This could include information on habitat requirements and the number of individuals required for a viable self-sustaining population. Management recommendations should also be revised as results of ongoing management efforts become available.

6. Develop an education and outreach program to educate the public and enlist their support in protection and recovery efforts.

This research project was designed to assist the FWS and other agencies with the conservation of *C. robusta* var. *robusta* by contributing information about pollination useful to the development and revision of recovery and management plans, and by identifying habitat that may be important to pollinators. In addition to assisting the US FWS with recovery efforts, this research also helps to clarify and add to our knowledge of the pollination ecology of the robust spineflower. The research also adds to existing information on pollination systems of endangered species.

METHODS

The methods used to study the pollination biology of *C. robusta* var. *robusta* included field research, as well as laboratory and library research. Observational field research included systematic observations of the frequency and foraging behavior of insect visitors. Field observations were also conducted to determine whether frequency of visitation correlated with habitat features and population characteristics of the robust spineflower, such as density of robust spineflower and density of other plant species. Examination of insects for pollen was performed in the lab. Experimental research was conducted to determine whether plants could self-pollinate, and whether there was any difference in fitness between self and cross-pollinated plants. Library research included reviewing existing studies for information on foraging behavior and habitat requirements of probable pollinators. Both qualitative and quantitative methods were used to analyze data. Recommendations on how to protect the plant-pollinator relationship were based on results of field research in addition to information from existing research.

Study sites

This study examined two populations of robust spineflower: a small population at Pogonip Park in the City of Santa Cruz, and a larger population at Sunset State Beach in southern Santa Cruz County (figure 1). The two populations differ in their size and the types of habitats in which they occur. Six other populations exist in southern Santa Cruz County (US FWS 2002).

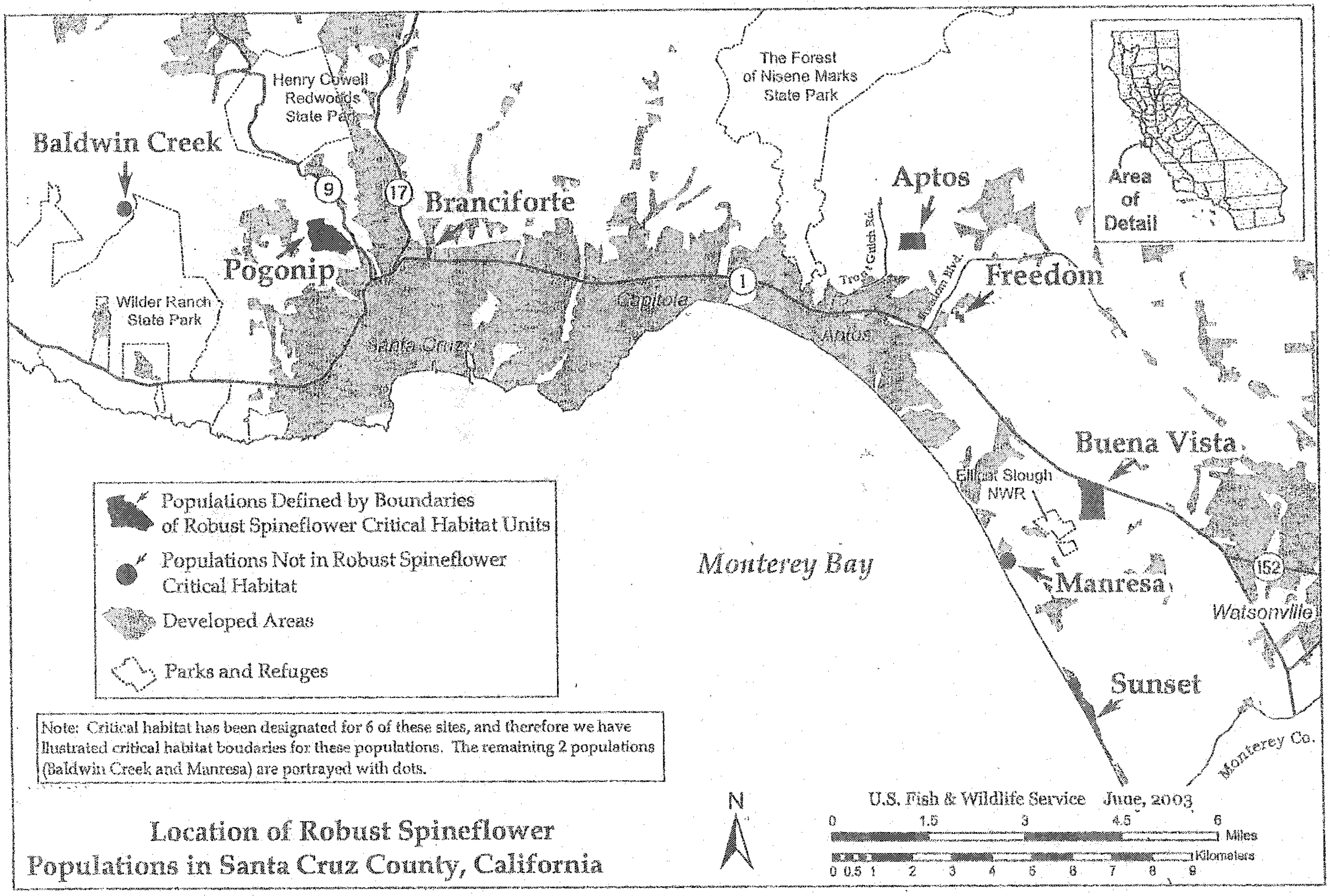


Figure 1: Distribution of the robust spineflower in Santa Cruz County.
(US Fish and Wildlife Service: June 2003.)

At the Sunset State Beach population, robust spineflower plants grow in sandy soil in openings within coastal dune scrub habitat. In 1995, the population was estimated to consist of more than 100,000 individuals (US FWS 2000). The population forms a long narrow strip on the back dunes. Large agricultural fields surround the site on three sides.

Other plant species found at the site include mock heather (*Ericameria ericoides*), seaside woolly sunflower (*Eryiophyllum staechadifolium*), coastal sagewort (*Artemisia pycnocephala*), and coyote bush (*Baccharis pilularis*) (US FWS 2000). *Bromus diandrus*, a non-native grass, was also observed growing at the site in 2001. The Monterey spineflower (*Chorizanthe pungens* var. *pungens*), a federally listed species, is also found at Sunset State Beach growing on the foredunes closer to the water (US FWS 2000). Sand gilia (*Gilia tenuiflora* ssp. *arenaria*), a federally endangered annual plant, also occurs at Sunset.

The population at Pogonip consists of two colonies located within one kilometer of each other. Both of these colonies occur south of the Brayshaw Trail (US FWS 2000). In 1990, the total number of robust spineflower plants at Pogonip was estimated to consist of 340 individuals. In the year 2000, the Pogonip population was estimated at 800 individuals (US FWS 2002). The population appears to be stable (US FWS 2000). In 2002 as part of this study, I estimated the larger of the two colonies to consist of approximately 200 individual plants.

The two colonies occur in coastal terrace prairie habitat (Kirk Lennington, personal communication 2002). Other rare species found at Pogonip in central coast prairie habitat include Santa Cruz clover (*Trifolium buckwestorium*), San Francisco popcorn flower (*Plagiobothrys diffusus*), and the federally endangered Ohlone tiger beetle (*Cicindela ohlone*) (City of Santa Cruz 2002).

Study design, data collection, and data analysis

At Sunset, field research and data collection began on May 11 and ended on August 22, 2002. Most of the data were collected between May 28 and July 21, when most of the sample plots had open flowers and insects actively visited flowers. At Pogonip, field research began May 26 and ended on July 6, with most data collected between May 26 and June 22.

To prepare for the 2002 study, I conducted a pilot study in 2001 from May 11 through July 21 at Sunset. During this study, I experimented with different approaches to determine the best methods for the 2002 study. I spent over 6 hours observing insect visitors of *C. r.* var. *robusta* flowers. I also collected insects I observed foraging on *C. r.* var. *robusta*. Using the information gathered in 2001, I was able to make some comparisons between the pollinator assemblage at Sunset in 2001 and 2002.

Assessing pollinator importance

Bagging experiments: To determine whether self-fertilization occurs, I compared seed set of flowers to which pollinators did not have access with the seed set of flowers freely accessible to pollinators. I conducted this experiment only at Sunset State Beach. The experiment was conducted within 20 randomly selected sample plots.

To select 20 sample plots, I first approximated the extent of the area that robust spineflower plants occupied by walking the trail that bisected the population and counting the number of paces. Based on a trail length of 410 m, 20 random numbers were selected using a random number table. Any number from 1 through 410 was accepted as a valid number. To ensure interspersion of plots, if two numbers were less than 3 m apart, another number was selected. For each random number drawn, another random number was drawn from a set of numbers equal to the length in paces the population extends to the right or left. The location of each sample plot was determined by walking the number of paces specified by the first random number, turning right or left as specified by a coin toss, and walking the number of paces specified by the second random number. Once I walked the specified number of paces, I continued walking in the same direction until I found a *C. r.* var. *robusta* patch with at least five plants. The center of each patch was marked with a flag.

At each of the twenty randomly selected plots, I selected three plants. On May 30th when the plants were selected, many of the flowers had already opened. Three plants at each plot were selected in close proximity to each other that had few or no open flowers. I randomly assigned one plant as the treatment plant, one as the control, and one to measure whether the treatment had any effect on seed set. I selected an inflorescence on each plant that had few or no open flowers, and removed any open flowers.

For the treatment plant, the selected inflorescence was enclosed in a white nylon drawstring bag. In other experiments evaluating materials for pollination bags, nylon had the least effect on temperature and humidity inside the bag (Kearns and Inouye 1993). The material had a very fine mesh (36X36 threads/cm) to exclude small pollinators. Individual inflorescences were covered, rather than entire plants, to prevent ground-dwelling insects from pollinating treatment inflorescences.

To check whether the bags had any effect on the seed set, I covered selected inflorescence on the second plant with a nylon bag that had an opening cut in the center of the bag to allow for pollinator access. For the closed bag treatment and open bag control, a layer of Vaseline was applied around the stem to discourage small insects from getting into the bags. For the plant used to measure the effects of treatment and unrestricted pollinator access, I marked the selected inflorescence with a red string. No Vaseline was applied to the stem. To prevent mature flowers breaking off the inflorescences, I tied open nylon drawstring bags around the untreated inflorescence after all the flowers on the inflorescence had closed but before they had matured.

Monitoring of closed bags revealed the presence of small insects inside some of the bags a few days after the bags had been applied. To prevent any further insect access, the closed bags were removed. A thicker layer of Vaseline was applied to the stem around the area where the bag had been tied. The bag was then retied more tightly onto the stem beneath the inflorescence. The bags were carefully monitored every 2-5 days throughout the flowering season, and no additional insects were found inside the closed bags.

Of the 20 plots, either treatment or control inflorescences were damaged at 12 of the plots, leaving 8 undamaged plots. Damage at some plots appears to have been a result of human tampering, and at other plots a result of possible rabbit herbivory. When the selected inflorescence had completely dried and all flowers had turned brown at each of the 8 plots, I removed the inflorescence from the plant. I recorded the following information for each inflorescence: 1) the plot number 2) whether the seeds were from a closed bag, an open bag, or a "no bag" treatment, 3) the collection date, 4) if the stem was broken or intact when collected. In the lab, I counted and recorded the number of flowers that had set seeds and the number of flowers that had not set seed on each inflorescence.

Of the 8 intact plots, in two plots the stems of the inflorescences in either the open or closed bags broke prior to collection. The data from these plots was therefore not used in the analysis. In a third sample plot, the open bag had been picked 30 days prior to the closed bag. Since the inflorescence was picked so much earlier than the other inflorescences, it is probable that the stem may have broken early and prevented maturation of the seeds. This data set was therefore also not used for analysis. Seed set data from the remaining five sample plots was used for data analysis.

In the initial study design, the effect of pollinators on seed set was to be evaluated by comparing the rate of seed set of the flowers on the untreated inflorescence with the seed set of flowers in the closed bags. However, a one-way analysis of variance ($?_{ij} ? ?_i ? ?_i ? E_{ij}$, Sokal and Rohlf 1987) showed there was a significant difference between the rate of seed set of inflorescences receiving the open bag treatment and the inflorescences that had no treatment during flowering (calculated p-value = <0.0001). These results indicated that treatment had an effect on seed set. Therefore, the rate of seed set of the closed bags was compared with the rate of seed set of open bags, using the open bags as the control treatment.

To measure whether pollinator access had an effect on seed set, the seed set of open bags was compared to the seed set of closed bags using a one-way analysis of variance ($p = 0.05$). The rate of seed set was defined as the proportion resulting from dividing the total number of seeds per cluster of flowers by the total number of flowers per cluster. The null hypothesis was that the mean seed set of the open bags was the same as the mean seed set of the closed bags. Relative fecundity was calculated as the seed set from the closed bag inflorescences divided by the seed set from the open bag inflorescences (Lesica 1993).

Germination experiment: To determine whether seeds produced from self-pollinated flowers had different levels of fitness than seeds produced from open-pollinated flowers, I tested the germination of the seeds from the different treatments. Before germination tests, seeds were stored in dry indoor conditions for a period of 7 to 9 months. For treated inflorescence that contained more than 15 seeds, I randomly selected a sample of 15 seeds from that inflorescence. All the seeds resulting from one treatment at each plot (maximum of 15) were placed in a separate petri dish on moist filter paper. There were 3 petri dishes for each of the 5 plots, for a total of 15 petri dishes. The plot number and type of treatment was recorded for each dish. To simulate the colder temperatures the seeds would have experienced in the field, the petri dishes with the seeds were placed in cold treatment (4-6 degrees C) for 48 hours. The seeds were monitored twice daily for moisture levels while in cold storage. After 48 hours, the petri dishes were placed in random order on a tray and placed in a well-lit room with temperatures ranging from 14 to 21 degrees Celsius. The tray was rotated twice daily to counteract any temperature or light gradients that could affect germination. Seeds were monitored twice daily for moisture levels.

After six days, I recorded the number and percentage of seeds in each dish that had germinated. I considered a seed to have germinated if the radical had emerged from the seed. In one petri dish containing 15 seeds from an open bag treatment, none of the seeds had germinated and the seeds appeared to have developed a fungal infection. Therefore, the seeds from this dish were omitted from the statistical analysis. An analysis of variance was used to test the null hypothesis that the germination rate of open pollinated seeds was equal to the germination rate of seeds that had no insect visitation ($p=0.05$).

Breeding system: To determine how long flowers were able to distribute and receive pollen, and to determine how the flower structure and maturation sequence might affect the ability of the flower to self-pollinate, I observed flower development both in the field and in the lab.

I followed 24 flowers for 2 days after opening (table 1). Observations were conducted within 5 plots randomly selected from the twenty sample plots used for the bagging experiments. At each plot, I selected 4 to 5 flower buds that appeared ready to open and could be easily marked. I marked the bracts of each selected flower with acrylic paint using a toothpick. I assigned each flower a number, and placed a small numbered flag next to each marked flower. I observed each flower daily until the flower opened. I recorded the date each flower opened, and the date the anthers were exerted and began to shed pollen. I recorded the position of the stigma inside the corolla tube, noting when it began to uncoil. I also recorded which day the stigma matured, as indicated by the uncoiling of the 3 style branches (Reveal 2001). I noted the position of the anthers relative to the stigma. I used a dissecting microscope in the field and the lab to assist with observations.

Table 1: Breeding system observations: 24 flowers observed on days 1 and 2 of opening, and 18 flowers observed on day 3 of opening.

Number of days flower open	1	2	3
Time and date			
Flowers with anthers shedding pollen			
Flowers with anthers gone			
Flowers with mature stigmas			
Open flowers			
Closed flowers			

Since flower development continued beyond the second day, I observed another group of 18 flowers on the third day after they had been open (table 1). I randomly selected 4 sample plots from the five sample plots used for the first observations. At each plot, I selected 5 flower buds that appeared ready to open. I marked the flowers as above. I observed each flower daily until the flower opened, and recorded the date. I observed the flowers again two days after they had opened, and recorded the position and maturity of the stigmas and anthers, and whether the flower was still open.

Data on the breeding system was analyzed qualitatively. Morphological traits and the maturation sequence were analyzed to determine how they might affect the ability of the plant to self- pollinate effectively without the aid of a pollinator. The length of time that flowers remained open was evaluated relative to how long the flower was available for pollination. Protandry, where the anthers mature before the stigma, could potentially discourage self-fertilization. Positions of the stigma and anthers that make contact unlikely could also discourage self-fertilization.

Pollinator frequency, abundance, and foraging

To determine the frequency with which different insect taxa visited flowers of *C. r.* var. *robusta*, I spent a total of 11.5 hours at Sunset and 5.5 hours at Pogonip systematically observing insects. At each site I identified visitors to the most specific taxonomic level possible: species, type specimens, family, or size class and order. For size classes, minute insects were less than 2 mm (the approximate diameter of a flower). Small insects were between 2 mm and 4 mm, and large insects were larger than 4 mm.

At Sunset, observations were conducted within the 20 sample plots used for the bagging experiment. Five additional plots were selected using the same methodology for a total of 25 observation plots. I conducted frequency observations from May 27 through July 21, for a total of 8 weeks.

At Pogonip, observations were conducted within 12 randomly selected sample plots. To select plots, I chose 12 random numbers from the numbers 1 through 20, which equaled the length of the trail in paces. If robust spineflower plants grew away from the trail at the randomly selected location, then I selected a random number from a set of numbers equal to the number of paces the plants extended from the trail. If no plants were found at the randomly selected location, I continued walking down the trail until a group of at least five plants was found. I marked the center of each plot with a flag. Some of the sample plots occurred within the same patch due to the small population size. I conducted observations for six weeks only from May 26 through July 6 due to the shorter flowering season.

In order to observe frequency of visitation throughout the entire season, I observed insect visitation at sample plots throughout each site during early, mid and late season. The order for visiting the sample plots was randomly selected using a random number table, and was repeated 3 times. Most sample plots were observed at least 3 times: once during early, mid and late season. Some plots were observed only twice due to early plant senescence. A total of thirty 10-minute observations were conducted at Pogonip, and 64 at Sunset. Fewer total observations were conducted at Pogonip due to the smaller population size. After first determining when insects were most active, I conducted frequency observations from 10 a.m. to 5 p.m. at Sunset, and from 10 a.m. to 4 p.m. at Pogonip.

For frequency observations, I observed insects within a 0.4 m^2 area in the center of each sample plot. From the 2001 pilot study, I determined that an area of 0.4 m^2 was large enough to include at least some insect activity within a 10-minute observation period but small enough to observe each insect visit. To mark the observation area, four 0.6 m bamboo stakes were placed on the ground forming a square, with the stake in the center of the sample plot forming the center of the observation plot.

Once the observation area was marked, I sat quietly for 5 minutes to allow the insects to become accustomed to my presence. I then observed insects for 10 minutes from a distance of 1 meter so as not to disturb the insects (Gomez and Zamora 1999). To observe insects, I used a pair of compact binoculars (8X23) to which I attached a close-up adaptor lens made for a 35 mm camera (Lorquin Entomological Society 1999). Using this device I was able to sit one meter away from the plot so as to minimally disturb the insects, while still being able to observe minute insects and details of foraging behavior. During observations, I continued to observe an insect unless another insect appeared, at which point I observed the new insect. On a few occasions where there were more than 2 insects foraging at one time, I approximated the number of visits per insect. For each insect, I recorded the following information on data sheets:

1) The identity of the insect. The insect was identified as a type specimen when possible. If there were no visible defining characters to identify the insect as a type specimen, I identified the insect as a member of a larger group of insects such as bee flies or muscoid flies (Conner and Neumeier 1995). Very small insects were grouped by order and size class. I noted on the data sheet if the insect was captured after observation so that observations for that insect could be later matched to the specific taxon.

2) The number of flowers the insect visited, and if the flower was on the same or a different inflorescence. (It was not possible to determine if the insect visited a different plant because the most plants grew intertwined.) This information was used to compare relative foraging speeds of insects within the sample plots, and to assess the likelihood that the insect transmitted outcrossed pollen.

3) Whether the insect contacted both the anthers and stigma when foraging. This behavior indicated the insect was likely to be an effective pollinator (Pavlik et al. 1993, Gomez and Zamora 1999).

In order to identify insects that visited flowers of *C. r.* var. *robusta*, I spent approximately ten hours at Sunset and six hours at Pogonip collecting insect visitors of *C. r.* var. *robusta* flowers for identification throughout the flowering season. I collected most insects after I finished frequency observations, so that I could match the data on frequency and behavior of insects with a specific insect. As I walked through each site, I collected any additional insects I observed foraging on flowers of *C. r.* var. *robusta*. I captured large insects with a sweep net, and small insects with an aspirator. After capture, I placed each insect in a kill jar containing ethyl acetate and blotter paper (Dafni 1992).

In the lab, all insects captured were pinned or pointed and labeled as type specimens. For each type specimen, I identified any morphological features such as size, color patterns or shape of the abdomen that could be used to identify insects on the wing. Type specimens were then used to identify insect visitors seen during other observations. All captured insects were examined in the lab for pollen under a dissecting microscope (Lesica 1993). Pollen grains were sufficiently large for individual grains to be visible under a dissecting scope. For each insect, I also noted where the pollen was located on the insect. Presence and location of pollen on the insect were analyzed to determine if the insect was a probable pollinator (Thompson 2001). I identified each insect to the most specific taxonomic level possible, generally to family, using a dissecting microscope. For the most frequent visitors, I consulted with entomologists at San Jose State University and California Academy of Sciences for identification to genus or species level where possible.

To estimate the total number of open flowers in the sample plot, I counted the number of open flowers in five randomly selected inflorescences. I then multiplied this by the total number of inflorescences in the sample plot (Conner and Neumeier 1995). To count the inflorescences, I subdivided the 0.4 m^2 plots into sixteen 15 X 15 cm squares using 6 additional 0.6 m bamboo stakes. I then counted the number of inflorescences within each 15 X 15 cm square. Visitation frequency per plot was calculated as the proportion of flowers visited in a 0.4 m^2 plot within a 10-minute observation period. I also calculated the expected mean number of visits per flower throughout its receptive period using the formula:

$$E_v = (M_{pa}/\text{day}) * (P_{fv}/\text{minute}) * D_{sr}$$

where E_v = Expected mean number of visits per flower, M_{pa}/day = minutes pollinators are active per day, P_{fv}/minute = proportion of flowers visited per minute, and D_{sr}= number of days stigmas are receptive (Jennersten 1988).

I calculated the expected probability of a flower receiving at least one visit while the stigma was receptive using the Poisson Distribution:

$$P=1-1/e^x$$

where x is the mean number of visits a flower received while the stigma was receptive (Jennersten 1988). I calculated the expected probability of a flower receiving at least one visit while the stigma was receptive for each plot. I estimated the expected probability of a flower receiving at least one visit at each site using the mean of the visitation probabilities of all plots at each site.

I used ANOVA to determine if there were significant differences in the expected number of visits per flower between the two sites ($p = 0.05$). I also determined whether there were significant differences in the probability of visitation by season at each of the 2 sites using ANOVA ($p = 0.05$).

I also compared the rate of visitation at each sample plot at both sites. A non-random spatial pattern of insect visitation indicated that insects visited some plots more often, and other plots less often, then could be expected due to chance alone. This could indicate that the distribution of resources affected the visitation patterns of pollinators. To determine whether there was a pattern to insect visitation, I used the non-parametric Kolmogorov-Smirnov test to compare the rate of insect visitation by plot to the Poisson distribution ($p = 0.05$). If the null hypothesis was rejected, then I concluded that there was a spatial pattern to insect visitation. To help determine whether resource distribution related to insect visitation patterns, I analyzed insect visitation in relation to selected environmental variables using Principle Components Analysis.

To gain additional information on frequency of visitation throughout the entire site, as well as to observe insects that may have been disturbed by my presence during frequency observations, I conducted abundance surveys along transects at locations dispersed throughout each population. I spent 3.5 hours conducting 14 abundance surveys at Sunset, and 2.5 hours conducting 10 surveys at Pogonip. To ensure interspersion, I divided the population into 5 separate areas and conducted surveys sequentially in the five areas. For each area, I randomly selected a starting point along the trail using a random number table, and a direction of travel using a random number table and a compass. I measured frequency of insects visiting *C. r.* var. *robusta* flowers along 36 meter transects. Every 9 meters, I recorded all visitors of *C. r.* var. *robusta* flowers on either side of a 2-meter transect located one meter in front of where I was standing for a period of 1 minute. I used the compact binoculars with the close-up lens attached to observe insects, and recorded insect visitors using the categories outlined for the frequency observations.

To gain more information on how foraging behavior might affect pollination effectiveness and floral constancy, I conducted additional observations on the foraging behavior of frequent visitors to *C. r.* var. *robusta* flowers. For each insect observed, I recorded what parts of the insect's body made contact with the anther and stigma of the flower, and whether the insect appeared to be foraging for nectar or pollen. I then observed the insect foraging for one minute, noting how many flowers it visited on each inflorescence. I continued watching until the insect flew out of view and recorded the total amount of time the insect visited flowers of *C. r.* var. *robusta*. If I observed the insect visiting another plant species, I recorded the species the insect visited.

To gain additional information on whether specific insect taxa might be specializing on *Chorizanthe*, I observed flowers of other plants blooming at the same time as robust spineflower. I recorded whether any of the same taxa that visited *C. r.* var. *robusta* flowers were also visiting flowers of other plant species. I also recorded when other plant species came into bloom, to note any possible sources of competition for pollinators.

After analyzing the data from frequency, abundance and foraging observations, I qualitatively assessed the ability of each insect visitor to act as a pollinator. An effective pollinator was defined as any insect that repeatedly visited flowers of *C. r.* var. *robusta* for pollen or nectar, and made contact with any body parts of any of the flower's reproductive organs (Petanidou and Vokou 1993). I used additional information on frequency of visitation, adherence of pollen to the insect, foraging speed, and assessment of pollination effectiveness from other research to determine which insects were likely to be important pollinators in 2002. Insect frequency was measured as the number of times the insect was seen visiting flowers during frequency observations, and calculated as the percent of total insect visits (Spears 1983). If the insect was also seen visiting flowers of *C. r.* var. *robusta* in 2001, then this indicated that the importance of the insect as a pollinator might extend to other years as well.

If the insect visited more than 1 inflorescence, then it was considered probable that the insect was transmitting pollen from one plant to another thereby facilitating outcrossing. A qualitative evaluation of the potential floral constancy of pollinators was based both on observations of foraging behavior and information from existing research. Assessment of potential foraging ranges of pollinators was based on an evaluation of existing research. Information on habitat requirements of important pollinators was obtained through a review of existing research.

Visitation frequency and environment

To examine relationships between environmental conditions and population characteristics of the robust spineflower with the frequency of insect visits, I estimated percent cover of bare ground, robust spineflower and other plant species within the 0.4 m^2 plots after each frequency observation. To measure potential relationships between frequency of visitation and characteristics within a larger area, I measured these same variables within a 6 m^2 area surrounding each observation plot. These measurements were then correlated with the frequency of insect visitation.

To collect data, I conducted observations within 24 of the 25 sample plots at Sunset, and within the 12 sample plots at Pogonip. I conducted observations at each plot a total of two to four times throughout the season (from May 27 through July 21 2002), depending on how long flowers remained in bloom at each plot. Measurements for the 6 m^2 plots were taken only once late in the season at both study sites. These measurements were made using 16 0.4 m^2 subplots within the 6 m^2 area. At the Pogonip site, the height of the plant closest to each of the four corners of the 0.4 m^2 plot was also measured late in the flowering season. These were averaged to obtain the mean plant height within each 0.4 m^2 plot. At both sites, insect visitation was measured as the proportion of flowers visited within each 0.4 m^2 plot within each 10-minute observation period. In some plots receiving many insect visits, the number of visits the plot received was estimated.

Because of seasonal changes in the visitation rate, early, mid and late season observations were placed into separate groups and analyzed separately for both the Sunset and Pogonip sites. Since the data for the 6 m^2 plots was collected late in the season at both sites, these data were analyzed only for the late season observations.

For early and mid season observations, I compared the proportion of flowers visited to the percent cover of robust spineflower, other plant species and bare ground using Pearson's Product Moment Correlation. The number of observations for each season ranged from 18 to 20 for the Sunset site and 8 to 9 for the Pogonip site. Correlation coefficients were compared to the critical value of r using a t-test (Sokal and Rohlf 1987). A correlation was considered to be significant when the calculated correlation coefficient was greater than the r-statistic (p=. 05).

For late season observations, relationships among the variables at both the 0.4 m^2 plots and the 6 m^2 plots were examined using Principle Components Analysis. The rate of visitation was then compared to the factor scores generated from the Principal Components Analysis using Pearson's Product Moment Correlation. Correlations with a calculated correlation coefficient > r were considered significant (p=. 05). A significant finding indicated that there was a correlation between the rate of insect visitation and the variables under consideration.

After reviewing the results of this study, a set of recommendations was developed for the US FWS and local agencies. Recommendations included any special management actions that could help to protect the plant-pollinator relationship. Results from existing research were incorporated into these recommendations.

RESULTS

Assessing pollinator importance

Effects of the treatment bags on seed set

The treatment bags had an unintended but significant effect on seed set, independent of the effect of pollinator access. A one-way analysis of variance showed there was a significant difference between the rate of seed set of inflorescences receiving the open bag treatment and the inflorescences that had no treatment during flowering (calculated p-value = <0.0001).

Effects of pollinator exclusion on seed set

Inflorescences that were enclosed in bags set seed, indicating that *C. robusta* var. *robusta* is self-compatible and is capable of self-fertilization (figure 2). The percent seed set for the five samples ranged from a low of 1.5% to a high of 9.1%, with a mean seed set of 5.7%.

Seed set of inflorescences that were accessible to pollinators was significantly greater than the seed set of inflorescences that self-pollinated without the assistance of a pollinator (figure 2). Average seed set of inflorescences that were in open bags was 29.9%, compared with an average seed set of 5.7% for flowers in closed bags. Flowers in closed bags set only 18.8% as many seeds as did flowers in open bags. A one-way ANOVA indicated that the average seed set of inflorescences in open bags was significantly greater than the average seed set of inflorescences in closed bags (calculated p-value = .031).

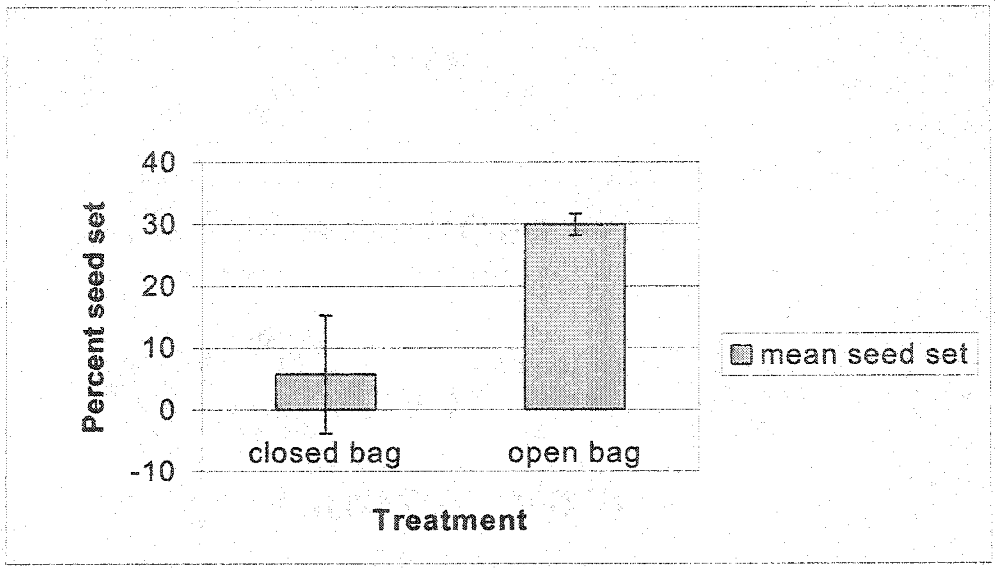


Figure 2. Mean percent seed set of inflorescences in open and closed bags. Closed bags were inaccessible to pollinators (treatment), and open bags were accessible to pollinators (control). Error bars represent one standard error.

The results of the ANOVA revealed the presence of two outliers of similar magnitude and opposite in value in the open bag treatments (studentized residual = 2.358 and -2.897). The presence of outliers can be attributed in part to the small sample size (n=5). Since ANOVA results do not tend to be affected by minor violations of normality (Sokal and Rohlf 1987), it is probable that the results of the ANOVA were not affected by the presence of outliers. Skewness of less than two for open bag (skewness = 0.308) and closed bag (skewness=0.568) treatments also indicate that the data are normally distributed.

Pollinator exclusion and seed germination rates: The germination rate of seeds from open-pollinated flowers did not differ significantly from the germination rate of seeds from flowers that were self-pollinated (table 2). The mean germination rate of seeds from self-pollinated flowers (51%) appears to be higher than seeds from open pollinated flowers in open bags (24%) and flowers with no bags (36%). However the results from the ANOVA do not indicate a significant difference among the means (calculated p-value = 0.31). Although the sample size was small, the large calculated p-value suggests that a larger sample size would not yield significant differences among the means.

Table 2. Seed set and germination rates: Comparison of germination rates of seeds from *Chorizanthe robusta* var. *robusta* plants with and without pollinator visitation.

Bagged x ? SE (n)	Open bag x ? SE (n)	No bag x ? SE (n)	Calculated P-value	Significant Difference?
50.7 ? 30.3 (5)	24.3 ? 23.2 (5)	36.0 ? 8.05 (5)	0.31 > .05	No

Breeding system

Out of the 24 flowers that were observed for the first and second day the flowers had been open, 100% of flowers had pollen present on anthers mid-day on the first and second days (table 3). None of the flowers had exserted stigmas on either day. Dissecting 5 flowers in the evening of the 2nd day revealed the stigma still tightly coiled in three of the corolla tubes, and beginning to uncoil but still not exerted in two of the corolla tubes. One hundred percent of flowers were still open midday of the second day.

Table 3: Breeding system: Days 1 and 2 of flower opening (n=24)

Number of days flower open	1	2
Time	12:30pm - 1:30pm 6/25/02	12:30pm - 1:30pm 6/26/02
Percent of flowers with anthers shedding pollen	100	100
Percent of flowers with exserted stigmas	0	0

Of the 18 flowers that were observed on the third day the flowers had been open, 94% of flowers were open and 6% percent of flowers had closed by midday (table 4). By early evening, 89% of flowers had closed and 11% were still open. 100% of flowers had stigmas exerted by midday. Stigma exertion and pollen dehiscence overlapped midday for 61% of flowers. Stigma exertion and pollen dehiscence continued to overlap into the evening for 17% of flowers. For 39% of flowers, all anthers appeared to have completed dehiscence by midday and there was no observed period of overlap for anther and stigma maturation. Dissecting several of the flowers on the third day of opening revealed pollen on the surface of the stigma.

Table 4: Breeding system: Day 3 of flower opening (n=18)

Time and date	12:30pm - 1:30pm 7/02/02	4:30pm - 5:30pm 7/02/02
Flowers with ruptured anthers containing pollen	61%	17%
Flowers with anthers gone	39%	83%
Flowers with exserted stigmas	100%	-
Flowers closed	6%	89%
Flowers open	94%	11%

Observations of the flower morphology revealed some spatial separation between stigmas and anthers in addition to the temporal separation. Upon maturity, the stigma surfaces of the three style branches were close together above the center of the flower, while the anthers were curved outward above the edges of the petals. The mature anthers and exserted stigma were close in height, both extending slightly above the petals.

Pollinator identification, frequency, and diversity

Pollinator identification and frequency

Results of frequency observations indicate that Diptera, Hymenoptera, Lepidoptera and Coleoptera orders were frequent visitors to *C. robusta* var. *robusta* flowers at both Sunset (figure 3) and Pogonip (figure 4). At the Sunset population, Hemiptera (true bugs) were also frequent visitors to *C. robusta* var. *robusta*.

During frequency observations of sample plots at Sunset, Hymenoptera accounted for 40% of all flower visits, Diptera for 27%, Coleoptera for 22%, Lepidoptera for 7%, and Hemiptera for 3% (figure 3). At Pogonip, Hymenoptera accounted for 61% of all flower visits, Lepidoptera for 23%, Diptera for 13%, and Coleoptera for 3% (figure 4). Examination of insects revealed that insects in these orders carried pollen.

Within the insect orders listed above, 14 insect families were frequent visitors at Sunset (figure 3) and 13 frequent families at Pogonip (figure 4). At Sunset, frequent visitors included flies (Bombyliidae, Calliphoridae, Milichiidae, Sarcophagidae and Tachinidae), solitary bees (Halicitidae and Anthophoridae), and social bees (Apidae) and wasps (Sphecidae Formicidae and Brachonidae). Beetles in the family Dasytidae, moths in the family Gelechiidae, and true bugs in the family Lygaeidae were also frequent visitors. At Pogonip, frequent visitors included solitary bees (Anthophoridae and Halicitidae), social bees (Apidae), wasps (Sphecidae and Bembicinae) and flies (Bombyliidae, Calliphoridae, Tachinidae). Butterflies were also frequent visitors at Pogonip (Lycaenidae, Nymphalidae and Satyridae), as well as beetles (Bruchidae and Chrysomelidae).

Figure 3: **Frequency of important pollinators at Sunset: insect orders (center graph) and taxa within orders (surrounding graphs).**

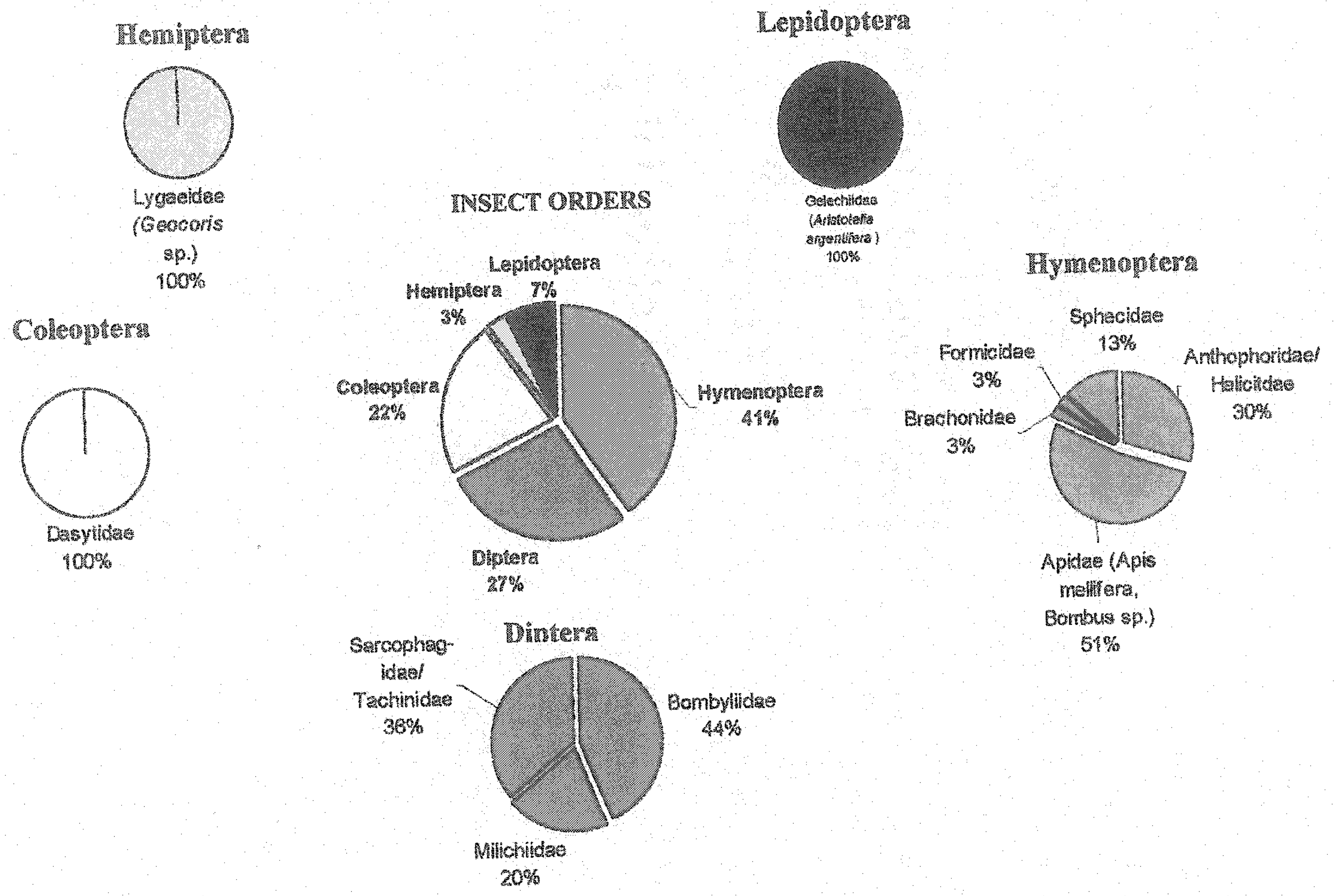
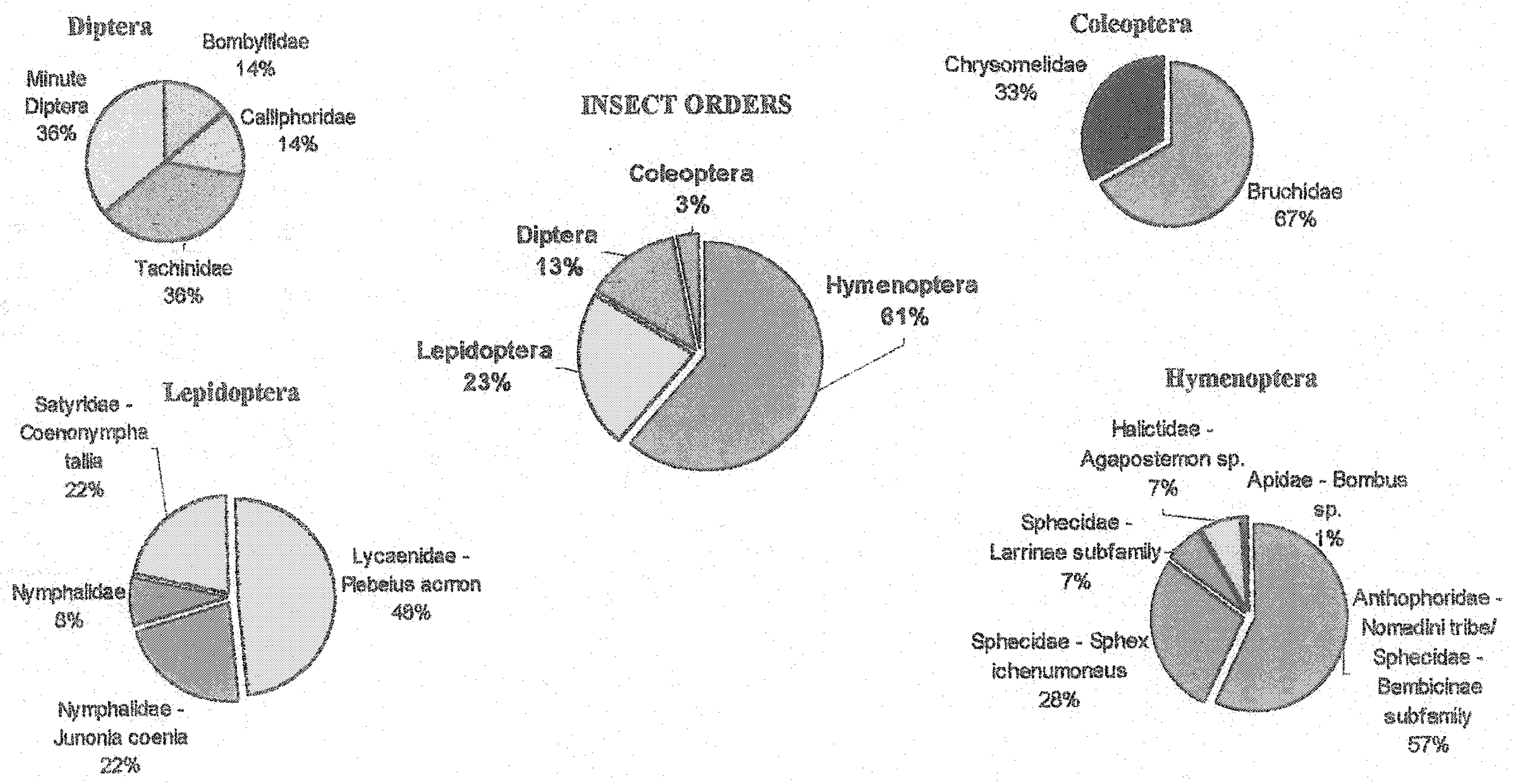


Figure 4: Frequency of important pollinators at Pogonip: frequencies of insect orders (center graph) and of taxa within orders (surrounding graphs).



Frequent visitors that could be recognized as type specimens in the field were identified to the most specific level possible. Within the frequent pollinator families at Sunset, one subfamily, 4 genera, and 2 species were identified (table 5). At the Pogonip site, three subfamilies, one tribe, 3 genera, and 3 species were identified as frequent visitors (table 6). A total of 18 insect taxa were identified as frequent visitors of *C. robusta* var. *robusta* at Sunset and 19 insect taxa at Pogonip.

Table 5: **FREQUENT POLLINATOR TAXA AT SUNSET IN 2002**

Frequent Pollinators (important taxa in bold)	**Avg. # flowers/ Insect/ 10 min.**	**# visitors**	**Pollen on Insect?**	**Abundance along transects**	**Other taxa**
ORDER: HYMENOPTERA (40% of total flower visits)					
Anthophoridae/ ------------------- **Halicitidae**	3.4	17	yes	6	*Ceratina* sp. --------------- 1) Tribe *Halictini* 2) *Agapostemon* sp.
Apidae ***Apis mellifera* L.**	12	2	yes	1	
Apidae ***Bombus* sp.**	41	2	yes	4	
Brachonidae	1.8	4	yes	4	*Chelonus* sp.
Formicidae	3	1	no	1	
Sphecidae Subfamily **Larrinae**	2.6	10	yes	3	
Sphecidae ***Ammophila* sp.**	----	none	yes	4	*Ammophila nasalis Provancher*

ORDER: **DIPTERA** (27% of total flower visits)

Bombyliidae ***Lepidanthrax sp.***	2.9	19	yes	8	
Bombyliidae			yes	1	
Calliphoridae			yes	2	
Milichiidae	4.5	6	yes	1	
Sarcophagidae/ ----------------- **Tachinidae**	7.8	6	yes yes	5	------------------ *Ptilodexia* sp.

ORDER: **COLEOPTERA** (22% of total flower visits)

Important Pollinators (important taxa in **bold)**	**Avg. # flowers/ Insect/ 10 min.**	**# visitors**	**Pollen on Insect?**	**Abundance along transects**	**Other taxa**
Dasytidae (Melyridae)	2.6	43	yes	32	

ORDER: **HEMIPTERA** (3% of total flower visits)

Lygaeidae ***Geocoris* sp.**	2.1	7	yes	7	

ORDER: **LEPIDOPTERA** (7% of total flower visits)

Gelechiidae *Aristotelia argentifera*	3	12	?	7	

Table 6: **FREQUENT POLLINATOR TAXA AT POGONIP IN 2002**

Frequent visitors	**Avg. # flowers/ Insect/ 10 minutes**	**# visitors**	**Pollen on insect?**	**Abundance along transects**	**Representative taxa**

ORDER: HYMENOPTERA (61% of total flower visits)

Anthophoridae **Nomadini tribe/** ------------------------ Sphecidae **Bembicinae Subfamily**	5.6	18	yes	13	---------------- *Steniolia elegans*
Sphecidae ***Sphex ichenumoneus***	5.7	9	yes	3	
Sphecidae **Larrinae** Subfamily	3.7	3	yes	6	*Tachysphex* sp.
Halictidae ***Agapostemon* sp.**	4	3	yes	1	
Apidae ***Bombus* sp.**	4	1	yes	0	
Sphecidae ***Ammophila* sp.**	----	none	yes	4	

ORDER: **LEPIDOPTERA** (23% of total flower visits)

Lycaenidae ***Plebeius acmon***	11	3	yes	19	
Nymphalidae ***Junonia coenia***	7	2	yes	9	
Nymphalidae	7	1	Yes	0	
Satyridae ***Coenonympha tallia***	7	2	yes	5	

ORDER: **DIPTERA** (13% of total flower visits)

Frequent visitor taxa	**Avg. # flowers/ Insect/ 10 minutes**	**# visitors**	**Pollen on Insect?**	**Abundance along transects**	**Representative taxa**
Bombyliidae	2	3	yes	1	
Calliphoridae	5	1	yes	2	
Tachinidae	4.7	3	Yes	2	
Minute Diptera	2.2	6	?	1	***Glabellula* sp.** (Bombyliidae)

ORDER: **COLEOPTERA** (3% of total flower visits)

Frequent visitor taxa	**Avg. # flowers/ Insect/ 10 minutes**	**# visitors**	**Pollen on Insect?**	**Abundance along transects**	**Representative taxa**
Bruchidae	3.5	2	yes		
Chrysomelidae	3	1	yes		

Pollinator foraging behavior

Intra-plant foraging: During frequency observations, most insects were observed foraging at more than one inflorescence. Although it could not be determined whether the inflorescences on which they were foraging belonged to different plants, pollinator foraging among separate inflorescences is likely to result in foraging among separate plants. Observations of foraging behavior confirmed that most pollinators foraged at separate plants at least some of the time while foraging. Both frequency and foraging observations indicate that most pollinators facilitate at least some outcrossing while foraging.

Hymenoptera at Sunset: The three Halicitidae from Sunset examined carried pollen, most of it clinging to dense branched setae. One had most of the pollen on its head and near its mouthparts; another had pollen primarily on its ventral thorax. A third had pollen distributed over its entire body, including thick clusters of pollen on the setae of the tibia. Since Halictidae were observed contacting both anthers and stigma while foraging, it is likely that some of the pollen carried on their body would be transmitted to the stigma. The one Anthophoridae examined for pollen had pollen primarily on its dorsal thorax. Since the three branches of the stigma arch over the top of the flower when receptive, it is likely that pollen would be transmitted to the stigma while foraging.

Bumblebees carried liberal amounts of pollen and made solid contact with the reproductive parts of the flower, indicating that they were likely to transmit pollen efficiently. Of the 3 bumblebees checked for pollen, pollen was found on the femur, tibia, mouthparts, ventral thorax, and scattered over the body. While foraging, the head and legs of the bumblebees made contact with the anthers and stigmas of the flower on which they were feeding, while the tip of the abdomen made contact with flowers as they crawled across the inflorescence.

While foraging, one bumblebee visited *Chorizanthe* flowers for 5 minutes before moving on to forage on *Lotus scoparius*. Another visited 23 flowers in one minute before also moving on to a *L. scoparius* plant. Bumblebees were also seen visiting flowers of *Dudleya* sp., *Lupinus arboreus*, and *Eriophyllum staechadifolium*.

The 2 honeybees examined carried copious amounts of pollen. One honeybee that was foraging for pollen had pollen sacs partially full, where pollen is generally inaccessible to flowers. However, the bee also carried pollen in the setae on her head, and the ventral side of her thorax and abdomen. The other bee examined that was apparently foraging for nectar carried pollen on her head and mouthparts. During behavioral observations, the honeybee's legs, head and tip of the bee's abdomen contacted the flower, indicating probable pollen transmittal.

Observations of honeybees in the field show repeated visits to *C. r.* var. *robusta* flowers. A honeybee observed foraging for nectar visited a total of 22 flowers of *C. r.* var. *robusta* on 8 different inflorescences before flying out of view. Another honeybee was observed foraging at one patch for longer than 4 minutes before flying out of view.

Observations of wasps in the Larrinae subfamily (Sphecidae) indicate that the head and dorsal thorax of these insects contacted the reproductive parts of the flower during foraging. Only a few pollen grains were found on the insect's head and body, indicating that the amount of pollen delivered may be relatively low. Wasps in the genus *Chelonus* (Brachonidae) were found to carry small amounts of pollen. Observations of foraging behavior indicated that they made contact with the reproductive parts of the flower while foraging. None of the argentine ants (Formicidae) examined were found carrying pollen, indicating that their pollen transmittal could be minimal.

Diptera at Sunset: Lepidanthrax specimens examined had minimal amounts of pollen on their bodies, although they had dense setae. This could be due in part to their foraging behavior, with bee flies frequently grooming themselves and flying to the ground between flower visits. One specimen had large amounts of pollen inside its mouthparts. Since it appeared during behavioral observations that *Lepidanthrax* foraged on both nectar and pollen, it is possible that pollen on the mouthparts reached the stigma. *Lepidanthrax* demonstrated a high degree of floral constancy. One insect observed while foraging visited a total of 16 flowers on three different plants.

The muscoid flies examined carried variable amounts of pollen ranging from a few grains to copious amounts. The pollen was carried primarily on the proboscis, and on bristles on the ventral and dorsal thorax. While foraging, the flies made contact with the reproductive parts of the flower with their proboscis and their thorax, indicating they were likely to transmit pollen effectively. Their foraging speed was quite variable. While one fly was observed visiting 42 flowers during a 10-minute period, the other 5 flies visited only 1 or 2 flowers during an observation period.

The muscoid flies exhibited some degree of floral constancy. During behavioral observations, one Tachinidae visited 25 flowers in succession on 8 different inflorescences before flying out of view. Another muscoid fly visited 30 flowers of *C. r.* var. *robusta* in succession before flying out of view.

The Milichiidae flies examined carried small amounts of pollen on their heads, thorax and mouthparts. While foraging, Milichiidae flies visited an average of 4.5 flowers within the plot in a 10-minute period, indicating a relatively slow foraging speed. One Milichiidae fly visited a total of 5 flowers on two different plants within a 5-minute period. While foraging, the fly tipped forward into the flower head first, and contacted the reproductive portions of the flower with its head, and dorsal and ventral thorax.

Phoridae flies were not found to carry pollen, although they appeared to contact the reproductive parts of the flower while foraging.

Coleoptera at Sunset: Melyridae (Dasytidae) beetles examined had small amounts of pollen. The beetles crawled across the inflorescence while foraging for pollen, with their legs, head and ventral side of their bodies making contact with the reproductive portions of the flower. Foraging behavior indicates that Melyridae were likely to transmit pollen. At Sunset, Melyridae beetles were frequently observed flying from plant to plant. Examination of a few flowers on which the beetles had been foraging did not reveal any noticeable damage to the stigma or anthers of the flower. Melyridae beetles were also observed foraging on *Eschscholzia californica* at the site.

Lepidoptera and Hemiptera at Sunset: No pollen was found on any of the *Aristotelia argentifera* moths examined. However, while foraging for nectar the proboscis, head and legs of the moth made contact with the anthers and stigmas of the flowers. One moth visited 4 flowers on 2 different plants within a 4-minute period during foraging.

The two *Geocoris* (Lygaeidae) that were examined carried small amounts of pollen, and were observed contacting anthers and stigma while foraging.

Hymenoptera at Pogonip: Bees in the Nomadini tribe (Anthophoridae) and in the Bembicinae subfamily (Sphecidae) family were observed making contact with the reproductive parts of the flower while foraging. All insects examined had liberal amounts of pollen on their legs, dorsal and ventral thorax, ventral abdomen, and head.

In the Sphecidae family, *Sphex ichneumoneus* insects examined were found to carry copious amounts of pollen on their heads. During foraging, the head of *S. ichenumoneus* contacted the reproductive parts of the flower. While foraging, *S. ichenumoneus* frequently flew among separate inflorescences and flower. Larrinae wasps and wasps in the genus *Ammophila* that were examined all carried pollen, and while foraging made contact with the reproductive parts of the flower.

Halicitidae bees were observed contacting the anthers and stigmas of the flower while foraging. Bees examined had pollen on their legs and mouthparts.

Lepidoptera and Diptera at Pogonip: A few pollen grains were seen on the heads and proboscis of all the butterflies examined. Mouthparts and legs of butterflies were observed contacting the flowers while foraging. Calliphoridae, Tachinidae and Bombyliidae flies that were examined had pollen scattered throughout their bodies and heads.

Coleoptera at Pogonip: Beetles examined in the Bruchidae and Chrysomelidae families were found to carry small amounts of pollen. While foraging, these beetles were observed making solid contact with the flower. The Dermestid seen during frequency observations stayed in one flower, indicating that its rate of pollen transfer to other flowers may be low. In addition, the flower on which it was feeding had anthers missing after the beetle had foraged.

Other floral resources at Sunset and Pogonip

At Sunset, early flowering species included *Amsinkia spectabilis*, *Geranium dissectum*, *Anagalis arvensis*, and *Cryptantha leiocarpa*. All of these species appeared to have mostly finished blooming by May 27, which was still early in the flowering of *C. robusta*. Plants with extended blooming periods included *Lotus scoparius*, which began blooming before May 1. By June 17, there were only a few flowers remaining on *L. scoparius* plants seen in the field. *Lupinus arboreus* was in bloom by May 1, and continued flowering throughout May and June. California poppies appeared to have a similar blooming period. *Eriophyllum staechadifolium* began blooming by May 15 and continued through June. Plants that began flowering late relative to the flowering season of *C. r.* var. *robusta* (mid to late June) include *Dudleya casitas*. *Ericameria ericoides* began flowering in July, after peak flowering in *C. r.* var. *robusta*.

Some pollinators of robust spineflower also visited flowers of other species while *C. r.* var. *robusta* was in bloom. In addition to visiting *C. r.* var. *robusta*, Bumblebees were observed on *Lotus scoparius*, *Eriophyllum staechadifolium,* and *Dudleya caespitosa*. Dasytidae beetles were found foraging on flowers of California poppy plants growing adjacent to *C. r.* var. *robusta*. Sphecid wasps were observed visiting flowers of yellow bush lupine (*Lupinus arboreus*). These species would appear not to specialize exclusively on robust spineflower.

At Pogonip, there were no other flowers blooming within the patch of *C. r.* var. *robusta* throughout most of the flowering season. In early July, which was the end of the flowering season for robust spineflower at Pogonip, both Anthophoridae and Bembicinae were observed foraging both at *C. r.* var. *robusta* and at plants in the Asteraceae family flowering nearby.

Pollinator diversity

There were 18 insect families visiting *C. r.* var. *robusta* at Sunset, and 14 at Pogonip. Combined, there were a total of 21 different families. Since only the most frequent visitors were identified to the level of genus or species, diversity at the species level is unknown.

While overall diversity in pollinator families was similar at the two sites, there were important differences in the pollinator assemblage. At Sunset, beetles were the most frequent flower visitors, representing 32% of visits by the most common pollinators. Hymenoptera, with 28% of visits, and Diptera, with 24% of visits, were next common. Lepidoptera, primarily the moth *Aristotelia argentifera,* represented 9% of all frequency visits. Hemiptera, primarily *Geocoris* sp., represented 7% of frequency visitation. At Pogonip, Hymenoptera were clearly the most frequent visitors, representing 62% of all frequency visits. Diptera represented 20% of all frequency visits. Lepidoptera, exclusively butterflies, represented 12% of all visits. Coleoptera represented 6% of all frequency visits. Hemiptera were not frequent visitors at Pogonip.

Visitation frequency at Sunset and Pogonip

Calculation of the overall frequency of insect visitation reveals that there were significant differences in the frequency of visitation at the two sites. The mean proportion of flowers visited in 10 minutes in a $0.4m^2$ plot at Sunset was 0.04, whereas the mean proportion of flowers visited in 10 minutes in $0.4m^2$ plot at Pogonip was 0.12. ANOVA results indicate that the mean visitation rate at Pogonip was significantly higher than the mean visitation rate at Sunset ($p<0.001$).

At Pogonip, the rate of visitation during early season differed significantly from the rate of visitation during late season. From May 26 - June 6 the mean proportion of flowers visited in 10 minutes in a $0.4m^2$ plot was 0.03. This visitation rate was significantly lower than the mean visitation rate of 0.22 per 10-minute observation from June 22 through July 6 ($p<0.001$). There was no significant different in rates of visitation according to the season at Sunset ($p=0.311$).

While the visitation rate at Sunset did not fluctuate significantly throughout the season, the visitation rate by plot varied considerably. The total number of expected visits a flower would receive at each of the 24 different plots ranged from a low of 0.12 to a high of 3.91. The mean number of expected visits per flower within the entire site was 1.81. Since pollinators were active for approximately 7 hours per day (10am to 5pm) and the stigma was receptive for 1 day, there were 420 minutes during which a flower could be pollinated. Using the Poisson Distribution, the probability of a flower at Sunset receiving at least one pollinator visit while the stigma was receptive ranged from a low of 11% at one plot to a high of 98% at another plot. In 7 of the 24 plots, each flower had less than a 50% probability of being visited by at least one pollinator.

At Pogonip, the visitation rate by plot also varied considerably. The total number of expected visits a flower would receive at each of the 12 different plots ranged from a low of 0.69 to a high of 7.83. The mean number of expected visits per flower for the entire site was 3.54. At Pogonip, the site became shaded by oak trees after 4pm, reducing the number of hours pollinators were active. Since pollinators were active for approximately 6 hours per day (10am to 4pm) and the stigma was receptive for 1 day, there were 360 minutes during which a flower could be pollinated. Using the Poisson Distribution, the probability of a flower receiving at least one pollinator visit while the stigma was receptive ranged from a low of 49% at one plot to a high of 100% at another plot. In only one of the 24 plots did a flower have less than a 50% probability of being visited by at least one pollinator.

Analysis of visitation frequency by plot indicated that there was greater spatial variability in pollinator visitation rates than would be expected due to chance alone at both Sunset and Pogonip. Using the Kolmorgov-Smirnov test to analyze spatial distribution of pollinator visitation and comparing the results to the Poisson Distribution, the results indicated a non-random pattern to the spatial distribution of pollinator visitation for both Sunset and Pogonip ($p < 0.001$). Spatial variability in pollinator visitation frequency could indicate that the distribution of resources might affect the frequency with which pollinators visit different plots.

Environment and insect visitation

Sunset State Beach

The correlation analysis confirmed that the non-random spatial pattern of pollinator visitation rates related to environmental variables at both Sunset and Pogonip. At the Sunset Beach site, there were unexpected correlations between variables at the 0.4 m^2 plots and visitation frequency during early and mid season. For the 18 observations conducted between May 27 through June 9 (early season), insect visitation was higher in plots with a high density of plants of species other than *C. r.* var. *robusta.*

A high density of other plant species in the 0.4 m^2 plots was significantly correlated with a high visitation rate of *C. r.* var. *robusta* flowers (Pearson's Product Moment Correlation = 0.481 > r 0.478). During the middle of the flowering season (June 11 - June 24), visitation was highest in plots with a low density of spineflower within the 0.4 m^2 plots. There was a significant negative correlation between the rate of visitation to *C. r.* var. *robusta* and density of spineflower within the 0.4 m^2 plots (Pearson's Product Moment Correlation = -0.576 > r 0.482).

Late in the flowering season (June 25 - July 21) a high rate of visitation to *C. r.* var. *robusta* flowers was again positively correlated with a high density of other plant species within the 0.4 m^2 plots (figure 5). Plots with a high density of plant species other than robust spineflower had high visitation rates. There was a significant positive correlation (Pearson's Correlation Coefficient = 0.501 > r .456) between the visitation rate to *C. r.* var. *robusta* flowers and factor 2 of the Principal Components Analysis. In factor 2, density of other plant species within the 0.4 m^2 plots was the only important component (component loading = .997).

An analysis of plot variability shows that plots with a high spineflower density within the 0.4 m^2 plots also tended to have a high spineflower density within the 6.0 m^2 area. Density of other species within the 0.4 m^2 plots was not associated with density of spineflower (figure 6). Spineflower density within the 0.4 m^2 plots varied according to plot exposure and location. All 4 plots that were on west-facing slopes had positive factor 1 scores, indicating high density of spineflower both within the 0.4 m^2 plots and the 6 m^2 area (figure 6). Plots along the trail and to the right of the trail had both low and high densities of spineflower. All 3 plots that were on the east side of a large water tank had negative factor 1 scores indicating low density of spineflower. Density of other plant species also appeared to vary according to plot exposure. All of the west-facing plots with the greatest wind exposure had negative factor 2 scores, indicating a low density of plants of other species.

Figure 5: CORRELATION OF INSECT VISITATION RATE AND PLOT VARIABILITY AT SUNSET: JUNE 25 - JULY 21 2002

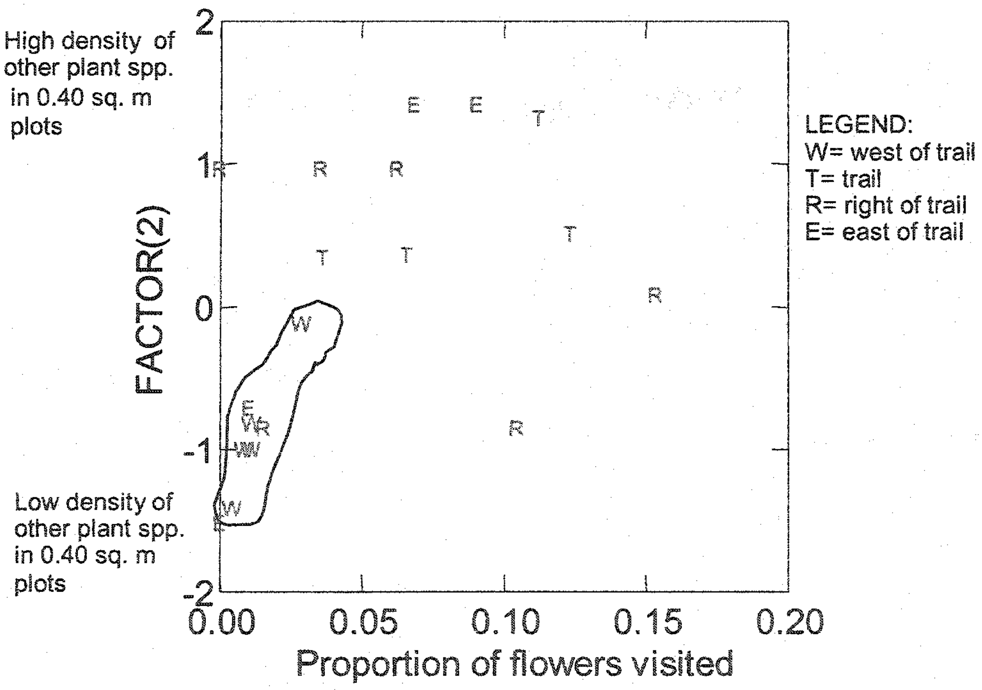
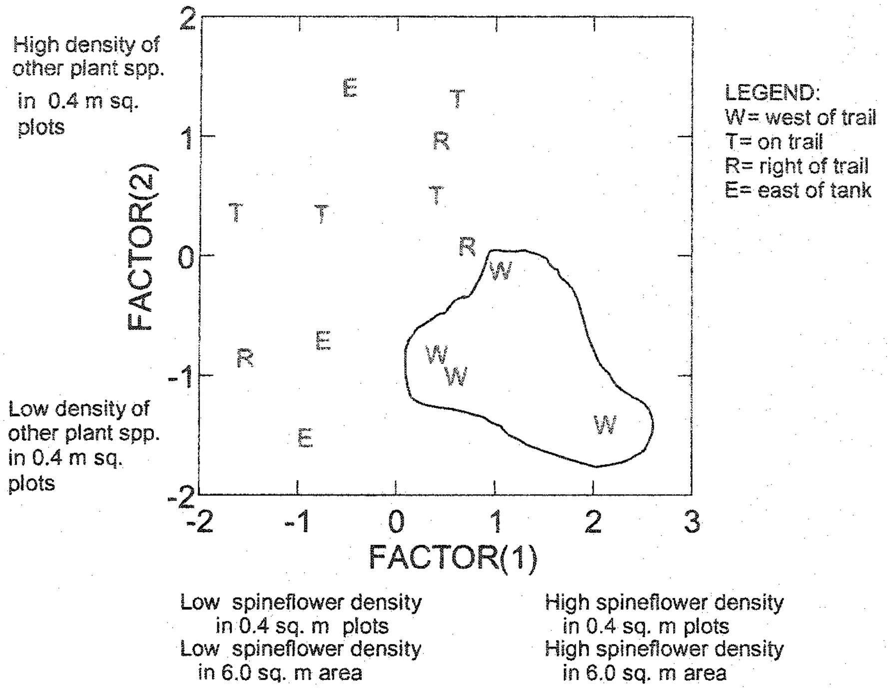


FIGURE 6: PLOT VARIABILITY AT SUNSET:
JUNE 25 - JULY 21 2002



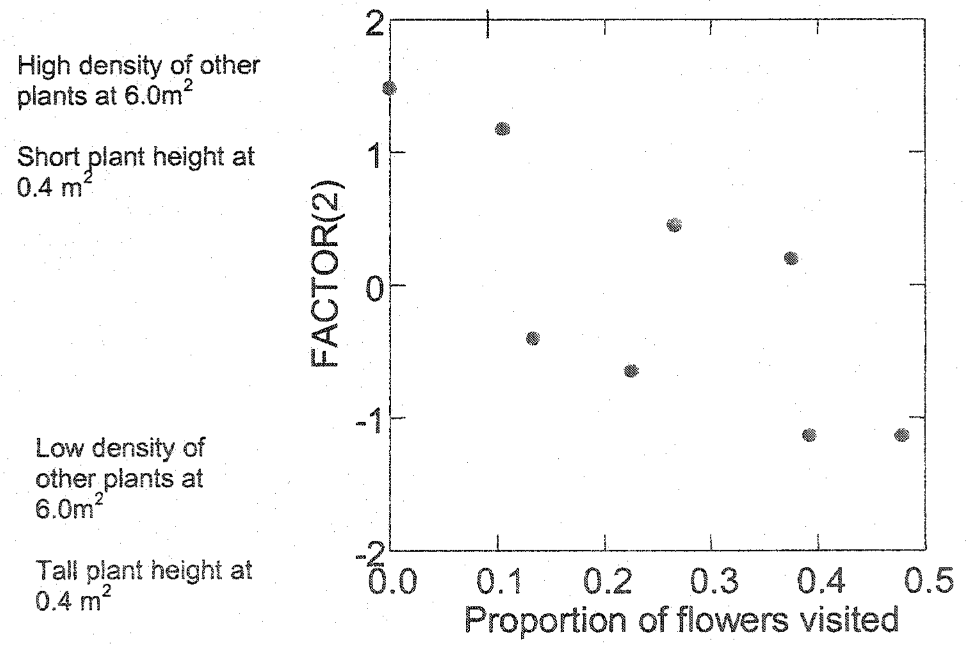
Plot exposure may also have some relationship with insect visitation. The plots on west-facing slopes all had very low visitation rates; in all plots on west-facing slopes, insects visited less than 5% of flowers in each plot during each 10-minute observation period (figure 6). Visitation to plots along and to the right of the trail was variable (0% to 15%). Visitation rates to plots on the east side of the tank were low to moderate (0% to nearly 10%).

Pogonip Park

For the early season observations (May 26 - June 6) and mid-season observations (June 14 - June 20) at Pogonip, there were no relationships found between insect visitation and the variables analyzed. Within the 0.4 m^2 plots during the early and midseason, correlations between rates of insect visitation with density of spineflower, density of other plant species, and percent cover of bare ground with were all less than ? 0.247? .

However, I did find significant relationships among the variables for the late season observations (June 22 - July 6). There was a significant negative correlation (calculated r = -0.761 > r ? . 707?) between rates of insect visitation and factor 2 of the Principle Components Analysis. Plots with low factor 2 scores had a low density of other plant species within the 5.95 sq. m area and a tall average vegetation height within the 0.37 sq. m plots (figure 7). These plots had high visitation rates.

FIGURE 7: CORRELATION OF INSECT VISITATION RATE AND PLOT CHARACTERISTICS AT POGONIP: JUNE 22 - JULY 6 2002



DISCUSSION

Three main conclusions emerge from this research:

1. *C. robusta* var. *robusta* is self-compatible. However, pollination by insects increases seed set, and is therefore important to plant fitness.

2. *C. r.* var. *robusta* is pollinated by a wide array of insects comparable in diversity to that of some other generalist pollination systems.

3. The probability of a flower being visited by a pollinator differs between the two populations. Probability of visitation also varies according to location within a site, time of season, the density of *C. r.* var. *robusta* and the density of other plant species. Pollinator response to these variables differs in the two populations studied.

Pollinator importance to Chorizanthe robusta var. robusta

Self-compatibility: This study demonstrated that *C. robusta* var. *robusta* is self-compatible and can self-pollinate autogamously. While the data was taken from just one population, this trait is likely to be consistent within the variety. Reveal (2001) cites greenhouse studies that demonstrate that selfing plays an important role in the reproduction of several other annual *Chorizanthe* species as well. In the annual *Chorizanthe* species discussed by Reveal, passive self-fertilization occurs in flowers that have not been cross-pollinated when the flower closes. Self-compatibility is also a trait found in other coastal dune scrub annuals. Moldenke (1976) estimated that 30% of coastal dune scrub species were self-compatible or habitually self-fertilizing, and that this percentage was even higher among coastal dune scrub annuals.

There is no evidence for inbreeding depression among self-fertilized progeny as measured by their germination rate. However, reduced fitness of self-fertilized progeny can manifest during other stages of plant development as well, including seedling survival and development of seedlings to maturity. Although it is possible that inbreeding depression may affect fitness of self-fertilized progeny, it is likely that self-fertilization still plays a role in reproduction and recruitment in *C. r.* var. *robusta*: Flowers with no pollinator visitation set viable seed, as did flowers from plots with low visitation rates.

From a survival standpoint, self-fertilization is consistent with a windy and frequently cold environment where pollinators may be unreliable. This can be particularly true for annuals, which often stand a better chance of reproducing in one season if they do not have to rely on pollinators for reproduction (Moldenke 1976). A survey of bee abundance and diversity at a cool windy and foggy coastal scrub site in Marin County found one-half the number of bee species as were found at a more inland chaparral site (Dobson 1993). The cool, foggy and windy climate was thought to limit flight time for small bees and metallic bees, which need higher temperatures for longer periods to warm their body temperatures. In another study, coastal scrub in northern California was found to have about 50% less overall pollinator diversity than inland chaparral sites (Moldenke 1976).

Effects of the treatment bags on seed set: I evaluated plausible causes for the differences in seed set between open bag and no bag treatments. While each of these events could have caused changes to the "open bag" and "no bag" treatments that affected seed set, their effects to the "open bag" and "closed bag" treatments were likely to be of similar magnitude. Therefore the difference in seed set between the open and closed bags should reflect the difference between the seed set of flowers that were cross-pollinated and the seed set of flowers that were self-pollinated. Since the treatment bags may have inhibited seed set, it is possible that the ability of *C. r.* var. *robusta* to self-fertilize without a pollinator could be higher than that found with this experiment.

One possible reason for the differences observed between the open bag treatment and the no-bag treatment could have been changes to temperature, humidity levels and solar radiation inside the bags. One study indicated temperature differences of 1 to 2 degrees C inside nylon bags with 25 openings/cm (Kearns and Inouye 1993). Since the mesh used in this experiment was an even tighter weave (36 openings/cm), it is possible the bag treatments altered temperature and humidity levels. The resulting changes could have been significant enough to reduce seed set within the bag treatments, and could have accounted for the differences in seed set between the "open bag" and "no bag" treatments (Dafni 1992). However, it is likely that any effects on temperature and humidity of the open and closed bags was similar, since only the top of the "open bag" was cut to allow for pollinator access. Any differences in the temperature and humidity levels within "open bags" and "closed bags" were probably minor and were not likely to result in different levels of seed set in the two treatments.

Another possible cause of the different levels of seed set in the "no bag" and "open bag" treatments could be the possibility that the string used to tie the bag on the stem interfered with the cambium layer in the stem. This could interfere with flower maturation and seed set. Although this could account for different levels of seed set in the open and no bag treatments, any effects of the string to the cambium layer should be the same to both the open and closed bag treatments.

A third possible explanation for the differences in the seed set of open bags and no bag treatment was changes in pollinator behavior caused by the bags. Since the seed set was lower in the open bag treatments than the no bag treatments, it is possible that the presence of the bags discouraged potential pollinators from visiting the flowers. It is also possible that the presence of the bag interfered with the ability of pollinators to access the flowers. This could be especially true for the larger pollinators that may have had difficulty getting inside the open bags. If the presence of the bag reduced the frequency or effectiveness of pollinator visits, then the lower seed set in the open bag treatments than the no bag treatments could be a result of reduced pollinator access. If this is the case, then it is possible that pollinator access has a greater effect on seed set than was reported in this study. This could strengthen the conclusions of this experiment that pollinator access has a significant effect on seed set.

Effects of pollinators on plant fitness: Although *C. r.* var. *robusta* is self-compatible, pollinator access to flowers increased seed set at the Sunset Beach population. This indicates that pollinator access is important to fitness in *C. robusta* var. *robusta*. Flowers that were prevented from receiving pollinator visits set only 19% as many seeds as did flowers that had open pollination. The significantly greater seed set in flowers that were open-pollinated suggests that pollinator visitation may be very important to recruitment levels in the *C. robusta* var. *robusta* population at Sunset. This study is consistent with the results of a study for a related variety of spineflower, *Chorizanthe pungens* var. *pungens*. This species had poor seed set and germination when pollinators had limited access to plants (Harding Lawson Associates 2000).

This research did not determine the cause of lower seed set in flowers without pollinator access. Although the high germination rate of self-fertilized progeny suggests a lack of inbreeding depression, it is possible that inbreeding depression could cause lower rates of seed set through abortion of self-fertilized ovules. The plant may have self-incompatibility mechanisms to prevent pollen from the same flower developing a pollen tube. Other reasons for lower seed set could be inadequate levels of pollen deposition to stigmas of self-fertilized flowers. Limited spatial and temporal separation of the male and female phases of the flower could also hinder pollen transfer to the stigma.

Because visitation by pollinators increases seed set, pollinators are clearly important to fitness in *C. r.* var. *robusta*. Whether the potentially increased levels of seed set resulting from pollinator visitation would have an impact on the long-term viability of populations depends both on life-history characteristics of *C. robusta* var. *robusta* and environmental conditions. Research on other annual species of *Chorizanth*e indicates that population levels fluctuate considerably from year to year based on local climatic conditions (Reveal 2001). In some annual *Eriogonum* species, populations show an increase in a year with favorable environmental conditions after several years of decreasing numbers of individuals in the population. This indicates that the seed bank might persist for a number of years. However, annual *Chorizanthe* species appear to have less persistent seed banks than annual *Eriogonum* species. Many *Chorizanthe* species also demonstrate a poor ability to recover from severe disturbances to the population (Reveal

2001). If seeds remain viable only for a few years, it is possible that several years of low seed set could have a negative impact upon the longevity of a particular *C. robusta* var. *robusta* population. Baron (2003) has also found high levels of seed predation in populations of *C. r.* var. *robusta*. Baron has also documented high levels of seedling mortality. At one population of *C. r.* var. *robusta*, Baron (1998) found a mean survivorship rate of 42% for seedlings. Under these circumstances, the increased seed set resulting from high levels of pollinator visitation could be essential to the long-term survival of the population.

Even small reductions in seed set that could result from decreased pollinator activity could have a major impact upon small populations of *C. robusta* var. *robusta*. For smaller population such as those occurring at Pogonip Park in Santa Cruz and in the City of Aptos, a reduction in the number of seeds produced over several years could reduce recruitment levels to the point at which the populations were no longer viable.

Low germination rates and low seed viability could also increase the impact of annual fluctuations in seed set. Although 33% of *C. r.* var. *robusta* seeds from untreated flowers germinated under laboratory conditions, the germination rate is under field conditions is unknown. The relatively high germination rate of seeds in laboratory conditions could also indicate high seed viability in 1-year old seeds. However, it is possible that field conditions could also affect seed viability.

Breeding system: Flower morphology and timing of stigma and anther maturation are consistent with flowers that permit self-fertilization and encourage outcrossing. In the *Chorizanthe robusta* var. *robusta* flowers that were observed, the flowers observed remained opened for three days. The anthers shed pollen for 2-3 days, and the stigma was receptive on the third. While the male-only phase of the flower could encourage cross-fertilization (Dafni 1992), the overlapping of the stigma receptivity with the pollen shedding could potentially allow for self-fertilization.

Other annual species of *Chorizanthe* are also protandrous with a day of overlap between stigma receptivity and pollen dehiscence. However in the annual *Chorizanthe* species discussed by Reveal (2001) the stigma becomes receptive typically on the second day the flower is open. The delay in stigma receptivity in *C. robusta* var. *robusta* until the third day could indicate a greater propensity for outcrossing. Horizontal separation of the anthers and stigma, with the anthers facing outward above the petals, could further discourage passive self-pollination in the robust spineflower.

Frequency, diversity, and variability of pollinators

Flower morphology and pollination: The pollinators at each site differ in their overall frequency of visitation, foraging speed, and amount of pollen carried and probably transmitted. However, given the generalist flower morphology, and the fact that only one pollen grain could potentially fertilize the entire flower, a wide array of insects are likely to be effective pollinators. Other studies have demonstrated that a wide array of pollinators deposited measurable amounts of pollen to stigmas of generalized flowers (Kearns and Inouye 1994, Gomez and Zamora 1998).

Evaluating pollinators at Sunset

Hymenoptera: Bees in the Anthophoridae and Halictidae families are likely to be among the most important pollinators at Sunset. Combined, the families accounted for

12% of all visits to *C. r.* var. *robusta* flowers in 2002. They were also quite frequent along transects, accounting for 26% of all Hymenoptera seen during transect surveys. Anthophoridae and Halictidae bees visited an average of 3.4 flowers within the sample plots during the 10-minute observation period. This is less than the average of 10.6 flowers for hymenoptera overall. However, the frequency of these bees compensates for their possibly slower foraging speed. Anthophoridae and Halicitidae were also observed visiting flowers of *C. r.* var. *robusta* at Sunset in 2001, indicating that they were likely to have been important pollinators in that year as well.

Review of relevant research indicates that Both Halicitidae and Anthophoridae are likely to be effective pollinators of *C. r.* var. *robusta*. These bee families are two of the most important pollinator groups in California (Moldenke 1976). Along the coast, Halictids are the dominant pollinators for about 25% of bee-pollinated flora (Moldenke 1979). Halictids are particularly effective pollinators in plant communities where the peak flowering time of different plant species follows in succession rather than overlapping, as is the case at Sunset.

Like most bees, Halictids and Anthophoridae generally exhibit a high degree of floral constancy, which increases their effectiveness as pollinators (Moldenke 1976). Essentially all Halictids are generalist feeders, but are facultative specialists on the plant producing the most floral resources at that time (Moldenke 1979). Therefore, Halictids are generally highly efficient pollinators of the flowers they visit. Research analyzing the pollen loads carried by several species of Anthophoridae indicates a high degree of floral constancy for this family as well (Proctor et al. 1996). Some Anthophoridae are specialist feeders, feeding on a particular genus, family or closely related plant taxa. However, a survey of a coastal dune site at Point Reyes found that only about 5% of plant species had specialist bee pollinators (Moldenke 1979). At a coastal site in Northern California, both Halictidae and Anthophoridae species behaved as generalist feeders (Dobson 1993). Although it may be unlikely that *C. r.* var. *robusta* has specialist pollinators at Sunset, it is still a possibility.

Bumblebees (*Bombus* sp., family Apidae) were likely to be an important pollinator at Sunset in 2002. During abundance observations, four bumblebees were observed, accounting for 16% of all flowers visited during frequency observations. Though only two were observed foraging during frequency observations (accounting for 6% of all Hymenoptera), the combined total number of flowers they visited in the sample plots was 82. The high foraging speed of bumblebees makes up for their relatively low abundance, and indicates that they are probably important to the pollination of *C. r.* var. *robusta*. Several bumblebees were seen foraging on *C. r.* var. *robusta* in 2001, indicating that they may have been important for pollination in 2001 as well.

Bumblebees observed in the field exhibited a high degree of floral constancy. However, their presence on other species suggests they are generalist pollinators, assuming that the same species of bumblebees were visiting different species of plants. A review of the literature also indicates that bumblebees are generalist feeders, but repeatedly visit the same plant species (Moldenke 1976). Although generalists, the long amount of time bumblebees spent visiting robust spineflower and the large number of flowers they visited before moving on to other species indicates a high degree of floral constancy. This results in more effective pollination, as a greater proportion species-specific pollen delivered is delivered to the flower stigma (Kunin 1993).

Apis mellifera (honeybees) were also a potentially important pollinator of *C. r.* var. *robusta* at Sunset. Honeybees were not very abundant at the site, with only 2 seen during frequency observations and 1 during abundance observations. However, like bumblebees they visited a relatively high number of flowers during frequency observations, averaging 12 flowers per plot during a 10-minute observation. The low abundance of *Apis mellifera* relative to other bee species bodes well for the native bees, which can be out-competed by the non-native honeybee (Moldenke 1976).

Observations of honeybees in the field show repeated visits to *C. r.* var. *robusta* flowers, indicating some degree of floral constancy. Other research indicates that most honeybees show strong floral constancy (Proctor et al. 1996). This is especially true when honeybees are collecting pollen, since it is easier for bees to pack pollen from only one species into one pollen load. A high degree of floral constancy is likely to increase their effectiveness as pollinators.

Wasps in the Larrinae subfamily (family Sphecidae) are also likely to be important pollinators at Sunset. They were quite frequent visitors to *C. r.* var. *robusta*, with the 10 observed during frequency observations accounting for 5% of all flowers visited. During abundance observations there were 3 observed, accounting for 13% of all hymenoptera. Their high frequency and abundance suggests they are a significant pollinator, although the foraging speed and the amount of pollen delivered per visit could be somewhat low.

Sphecidae are frequent visitors of *Eriogonum,* also in the Polygonaceae family (Moldenke 1976). Sphecids are also frequent visitors to 40 other genera of plants in California. A species of *Tachytes* in the Larrinae subfamily was considered a highly efficient pollinator of Apiaceae plants (Proctor et al. 1996).

Species in the genus *Chelonus* (family Brachonidae) were likely to be of some importance in the pollination of *C. r.* var. *robusta* at Sunset. They accounted for approximately 1% of all flowers visited during frequency observations, and 17% of hymenoptera observed during abundance observations.

Argentine ants (*Linepithema humile*) visited more flowers than any single other hymenoptera. However, ants are frequently ineffective pollinators. Ants can rob nectar from plants without effectively pollinating and secrete chemicals that can interfere with pollen viability (Proctor et al. 1996). None of the argentine ants examined were found carrying pollen, indicating that their pollen transmittal could be minimal. Because of their questionable effects on pollination, argentine ants are not considered probable pollinators of *C. r.* var. *robusta*.

Diptera: Along with Hymenoptera, Diptera pollinators were also likely to be very important to the pollination of *C. r.* var. *robusta* at Sunset. In the Bombyliidae family, the only frequent visitors were flies in the genus *Lepidanthrax*. These bee flies were very frequent, accounting for 11% of all flowers visited. Eight *Lepidanthrax* were observed during transect surveys, accounting for 47% of all Diptera. Lepidanthrax visited an average of 2.9 flowers per 10-minute observation. Given their high frequency and abundance, they are likely to have a significant impact on pollination of *C. r.* var. *robusta* at Sunset despite their slow foraging speed and the small amount of pollen carried. Several *Lepidanthrax* were also observed during 2001, indicating that they may have been important pollinators in that year as well.

Lepidanthrax demonstrated a high degree of floral constancy. However, their tendency to fly to the ground between visits to different plants could limit the amount of outcrossed pollen that was transferred.

Bombyliidae are frequent pollinators in plant communities in central California occurring at low to middle elevation (Moldenke 1976). For some species of Bombyliidae the proboscis is the only part of the insect that contacts the flower, so most pollen transfer occurs from the proboscis to the stigma. Most species are suspected of being consumers of nectar as well as pollen.

Three families of short tongued (muscoid) flies are also likely to be important pollinators of robust spineflower at Sunset: Tachinidae, Sarcophagidae and Calliphoridae. Six muscoid flies were observed foraging during frequency observations, accounting for 9% of all flower visits. Their foraging speed was quite variable. During abundance observations, a total of 6 muscoid flies were observed, accounting for 29% of all Diptera. The higher percentage of muscoid flies during abundance observations than frequency observations could be due to an apparent tendency for the flies to be distracted away from the flowers by the observer during frequency observations.

The muscoid flies exhibited some degree of floral constancy. While foraging, the flies made contact with the reproductive parts of the flower with their proboscis and their thorax. The variable amounts of pollen carried and variable foraging speeds indicates that their effectiveness as pollinators may vary from moderate to high. However, given their high abundance they are likely to be important pollinators at Sunset.

There is some debate as to the overall effectiveness of fly pollination, aside from Syrphidae and Bombyliidae (Kearns 1992). However, muscoid flies are frequent pollinators of forbs in salt and estuarine marshes in California (Moldenke 1976). Flies are also important pollinators especially in many areas with high altitude (Kearns and Inouye1994, Arroyo et al. 1982). Even flies that are generalist pollinators can contribute significantly to pollination (Kearns 2001). Flies such as Muscidae, also considered a "muscoid" fly, can carry pollen loads equal to that of solitary bees (Kearns 1992).

Flies in the family Milichiidae were also likely to be important pollinators at Sunset. There were 6 flies seen during frequency observations, accounting for 5% of all flower visits. There was one Milichiidae fly seen during abundance observations. These small flies may have been less visible than larger flies during abundance observations, and therefore may have been underrepresented in the abundance surveys.

The small amount of movement between plants indicates that the fly may provide limited amounts of outcrossed pollen to the flowers. Although the overall effectiveness as a pollinator may be low due to a limited amount of outcrossed pollen and a slow foraging speed, Milichiidae is still likely to be an important pollinator due to their relatively high frequency.

Although limited mention is made of Milichiidae in the pollination literature, 18 species of Milichiidae are listed as important pollinators of a Panamanian plant in the Aristolochiaceae family, *Aristolochia pilosa* (Wolda and Sabrosky 1986).

Phoridae flies are minute in size, measuring less than 2 mm. Two were observed during foraging observations, each visiting an average of 3.5 flowers per 10-minute observation. None were observed during transect surveys. No pollen was found on the insects, but since they appeared to contact the reproductive parts of the flower, they are potential pollinators. Due to their relatively low abundance and probably small amount of pollen transferred, they are probably of minor importance in pollination.

As flower visitors of *Hamamelis virginiana* (Hamamelidaceae), Phoridae flies carried up to 18 pollen grains, but the amount of pollen carried varied considerably (Anderson and Hill 2002). They were categorized as potential pollinators but probably not very effective ones.

Coleoptera: Melyridae (Dasytidae) was the only family of beetles that were frequent visitors of *C. r.* var. *robusta* at Sunset. A total of 43 Melyridae were observed during frequency observations, with each beetle visiting an average of 2.6 flowers. Melyridae accounted for 22% of all flower visits. During transect surveys, 32 beetles were observed. The beetles crawled across the inflorescence while foraging for pollen and were found with small amounts of pollen, indicating they could transmit pollen to the stigma. Since the Melyridae beetles are frequent visitors to *C. r.* var. *robusta*, move among different flowers and plants while foraging, and carry pollen, they are likely to be effective pollinators at Sunset. Melyridae beetles were also observed foraging on *Eschscholzia californica* at the site, indicating that they do not specialize exclusively on *C. r.* var. *robusta* at Sunset.

While other families of beetles such as Oedemeridae are recognized as being effective pollinators, the ability of Melyridae to pollinate effectively is debated (Proctor et al. 1996). Moldenke (1979) states that the Melyridae are probably ineffective pollinators for behavioral and morphological reasons even though they are frequent flower visitors in California. On the other hand, van der Pijl (1961) states that they are one of the primary pollinators of California poppy (*Eschscholzia californica*), as well as pollinating other Papaveraceae plants and some *Rosa* spp. Gomez and Zamora (1999) recorded that two species of *Dasytidae* deposited measurable amounts of pollen onto the stigmas of *Hormathophylla spinosa* in a single visit.

Lepidoptera: At Sunset, there was only one frequent Lepidoptera visitor: *Aristotelia argentifera*, a small moth in the Gelechiidae family. Seven moths were seen during frequency observations, each visiting an average of 2.1 flowers within a 10-minute period within the plot. When approaching patches of robust spineflower, I frequently observed large numbers of moths flying up from robust spineflower plants to take refuge in Ericameria plants occurring nearby. It is likely therefore that their visits to *C. r.* var. *robusta* flowers were underestimated.

Although no pollen was found on the moths, foraging behavior observations indicate that the moths could potentially transmit small amounts of pollen. Some movement between different plants while foraging indicates that insects could be transmitting pollen between plants. Jerry Powell, who identified the species, indicates that they could be accidental pollinators of *C. r.* var. *robusta* (personal communication 2002). Dr. Powell also indicated that they feed on Ericameria. However, given that the

Ericameria was not yet in bloom at the site, it is possible that they may be using *C. r.* var. *robusta* as an alternate source for nectar. Given their relatively high frequency at the site, it is likely that they could have some impact on pollination of *C. r.* var. *robusta*. They were also observed visiting robust spineflower in 2001, indicating they could have been pollinators during 2001 as well.

Hemiptera: The only frequent Hemiptera visitor was the genus *Geocoris* (family Lygaeidae). A partially predaceous insect, *Geocoris* is important agriculturally as a predator of other insects that cause damage to crops. I did not find *Geocoris* listed as an important pollinator in literature on pollination. However, at Sunset the insect was frequently seen foraging for nectar at flowers. Seven insects seen during frequency observations visited an average of 2.1 flowers within a 10-minute period in the plots. They accounted for 3% of flower visits. Seven insects were seen foraging at flowers during abundance observations as well. The two *Geocoris* that were examined carried small amounts of pollen. Due to their relatively high frequency at Sunset, *Geocoris* are likely to be of some importance in pollination of *C. r.* var. *robusta*. *Geocoris* bugs were also seen foraging on flowers in 2001.

The remaining insect visitors at Sunset appear to be infrequent visitors to *C. r.* var. *robusta* or to have ineffective foraging behavior, indicating that they were probably only occasional pollinators of robust spineflower. These visitors include Coccinellidae and various Hemiptera. In addition, several minute insects (less than 1 mm) also visited *C. r.* var. *robusta*.

Evaluating pollinators at Pogonip

Hymenoptera: This was the dominant pollinator order at Pogonip, accounting for 61% of all flower visits during frequency observations. Within the Hymenoptera, the Nomadini tribe of the Anthophoridae family and the Bembicinae subfamily in the Sphecidae family combined were the most frequent Hymenoptera visitors. Eighteen insects each visited an average of 5.6 flowers in a plot within a 10-minute observation period, accounting for 34% of all flower visits. All insects examined had liberal amounts of pollen on their bodies. Both Anthophoridae and Sphecidae are likely to be efficient pollinators due the rapid foraging speed and their ability to carry and transmit pollen on their setae.

In the Sphecidae family, *Sphex ichneumoneus* was also a very frequent visitor to *C. r.* var. *robusta*. Nine individuals each visited an average of 5.7 flowers in a plot within a 10-minute period, accounting for 17% of all flower visits. Due to relatively high frequency, large amounts of pollen carried, and high intra-plant movement while foraging, *S. ichenumoneus* is likely to be an important pollinator of *C. r.* var. *robusta* at Pogonip.

Larrinae wasps, a subfamily within Sphecidae, were also likely to be an important pollinator at Pogonip. There were 3 insects seen during frequency observations, accounting for 4% of all flower visits. They visited an average of 3.7 flowers in a plot per 10-minute period, indicating a moderate foraging speed. There were 6 Larrinae; representing 22% of all Hymenoptera, observed during transect surveys. The insects examined all carried pollen, and while foraging made contact with the reproductive parts of the flower.

Another Sphecidae, the genus *Ammophila,* was also likely to be an important pollinator at Pogonip. Although none were seen during frequency observations of plots, 4 were observed during transect surveys. The insects examined carried large amounts of pollen. Since the insect represented 15% of all Hymenoptera observed during transect surveys, it is likely that they are an important pollinator at Pogonip.

Within the Halicitidae family, three bees in the genus *Agapostemon* were observed during frequency observations, accounting for 9% of all Hymenoptera. Each insect visited an average of 4 flowers during a 10-minute period, accounting for 1% of flower visits. A relatively high frequency and moderate foraging speed indicates that *Agapostemon* are likely to be of some importance to pollination.

One *Bombus* sp. (family Apidae) visitor was seen during frequency observations, indicating that bumblebees could be an occasional pollinator at Pogonip.

Lepidoptera: Pollinators in the order Lepidoptera were second to Hymenoptera in terms of overall frequency at Pogonip. There were 8 butterflies observed during frequency observations, each visiting an average of 8 flowers within a 10-minute period. Although fewer in number than Diptera, they visited a greater number of flowers due to their faster foraging speed.

Acmon blue (*Plebeius acmon*), in the Lycaenidae family, was the most frequent butterfly to visit *C. r.* var. *robusta*. There were 3 observed during frequency observations, accounting for 11% of flower visits. Each Acmon blue visited an average of 11 flowers in a 10-minute period, a very fast foraging speed. There were 15 observed during abundance observations, accounting for 52% of all Lepidoptera.

In the Nymphalidae family, Buckeye (*Junonia coenia*) was somewhat frequent. There were 2 seen during frequency observations accounting for 5% of all flower visits, and 9 during transect surveys accounting for 31% of all Lepidoptera. Buckeye visited an average of 7 flowers in a plot within a 10-minute period.

In the Satyridae family, common ringlet (*Coenonympha tullia*) was another frequent Lepidoptera visitor. Two *C. tullia* butterflies were seen during frequency observations, visiting an average of seven flowers in a plot within a 10-minute period. They accounted for 5% of all flower visits. There were 5 seen during abundance observations, accounting for 17% of Lepidoptera.

Butterflies are recognized as important pollinators, in California particularly in grassland plant communities (Moldenke 1976). The adult butterflies in the families found at Pogonip feed on flower nectar from a variety of plant species (USGS 2003), and are unlikely to specialize on *Chorizanthe*. While the caterpillars can have a narrower range of host plants, none of the listed host plants includes *Chorizanthe*. However, several species of *Eriogonum* serve as host plants for caterpillars of the Acmon blue. It is possible that *Chorizanthe robusta* var. *robusta* could serve as a host plant for caterpillars as well, since it is also in the Polygonaceae family.

Diptera: Flies are also likely to be important pollinators at Pogonip. Calliphoridae and Tachinidae were frequent visitors, with 4 seen during frequency observations. These flies accounted for 7% of all flower visits. Four flies were seen during transect surveys, representing 57% of Diptera. Flies in these families were likely to be effective pollinators since they foraged quickly and carried pollen. Bombyliidae were also important Diptera pollinators at Pogonip. Three Bombyliidae were seen during frequency observations, accounting for 2% of flower visits. They visited an average of 2 flowers in plots during frequency observations, which is slower than most other insect visitors at Pogonip. Given that they were numerous and were found to have pollen on their bodies, they were still likely to have an impact on pollination of *C. r.* var. *robusta.*

Coleoptera: Three families of beetles visited *C. r.* var. *robusta* at Pogonip. There were 2 beetles in the Bruchidae family seen during frequency observations. Each beetle visited an average of 3.5 flowers in a 10-minute period, accounting for 2% of flower visits. Due to their moderate foraging speed, and to the fact that they move among flowers while foraging, they could have an impact on the pollination of *C. r.* var. *robusta.* Beetles in the Chrysomelidae family may also be of some importance in pollination. Only 1 was seen during frequency observations. However, the beetle visited 3 flowers during frequency observations, and was found with pollen on its body. Beetles in the family Dermestidae are not likely to be beneficial to pollination, due to their limited intra-plant foraging and their damaging foraging behavior.

Pollinator diversity in *C. robusta* var. *robusta*: *Chorizanthe robusta* var. *robusta* is pollinated by a wide array of pollinators at both the Sunset and Pogonip populations. The diversity at the order and family level is comparable to that of some other generalist pollination systems. A pollination study of three populations of *Hormathophylla spinosa* (Cruciferae), a plant with a generalized pollination system, identified 23 different families of pollinators within five different orders (Gomez and Zamora 1999). In another generalist pollination system, 28 different species of insects visited seven different populations of *Jasminum fruticans* (Oleaceae) (Thompson 2001).

The level of diversity in *C. robusta* var. *robusta* is also comparable to diversity in a few other Polygonaceae species with generalist pollination systems. Pollinators of *Eriogonum ovalifolium* var. *vineum* included five families in the order Diptera and Halictidae bees in the order Hymenoptera (Neel et al. 2001). For *Polygonum thunbergii* there were a total of 30 families of insects that visited the flowers (Momose and Inoue 1993).

Other species of *Chorizanthe* also appear to have numerous species of pollinators, suggesting generalist pollination systems as well. Species of wasps, bees, flies and butterflies were observed visiting the Ben Lomond spineflower, *C. pungens* var. *hartwegiana* (US FWS 2001). Pollinators of unspecified *Chorizanthe* species include wasps, beeflies and generalist bees (Moldenke 1976).

A generalist pollination system should be advantageous to edaphic plant species such as *C. r.* var. *robusta* that occur within different habitats with different pollinator assemblages. This would be especially true for plant species that occur within different climatic conditions. Kearns and Inouye (1994) determined that for *Linum lewisii*, which also grows in windy and cold environments and has different pollinator pools across its range, the ability to attract a wide array of pollinators enhanced its prospects for seed set. Other plant species occurring within different geographic areas also have generalist pollination systems with significant differences in pollinator assemblages among populations (Thompson 2001, Gomez and Zamora 1999, Kearns and Inouye 1994).

Potential competition for pollinators: There were numerous other flowering plants blooming near and among patches of *C. r.* var. *robusta* at Sunset, which may indicate the potential for some degree of competition among plant species for pollinators. However, observations of the flowering phenology of these other plant species seems to indicate that *C. r.* var. *robusta* is the dominant floral resource throughout most of its blooming period.

While I did not conduct systematic observations of pollination in other plant species, a few observations of flowers of other species revealed that some pollinators of robust spineflower also visited flowers of other species while *C. r.* var. *robusta* was in bloom. This could indicate that although the robust spineflower appears to dominate in terms of numbers of flowers while in bloom, the potential may exist some pollinator competition. as well as indicating a lack of obligate specialization on *C. r.* var. *robusta* for these pollinators.

Since there were no other flowers blooming within the patch of *C. r.* var. *robusta* at Pogonip throughout most of the flowering season, it is likely that most insects were forced to behave as specialists while foraging among robust spineflower. For strong fliers such as the Bembicinae, it is quite likely that they also foraged among flowers located some distance away. Some Anthophoridae species are specialists, but it is not known whether any of the *Nomadini* (Anthophoridae) species at Pogonip specialized on *Chorizanthe*.

Variability in the pollinator assemblage: The proportion of visits by each order of pollinators differs for the two populations studied. Many of the intra-site differences in the visitation frequency of pollinator orders are consistent with visitation rates to other plant species in grassland or coastal scrub habitat (Moldenke 1976). In Moldenke's study, the total percentage of coastal scrub species visited frequently by bumblebees was larger than the percentage of grassland species visited by bumblebees. Moldenke also found that the percentage of grassland species pollinated by Halictid bees, solitary bees or butterflies was larger than the percentage of coastal scrub species pollinated by these insects. However, the relatively high percentage of visits by muscoid flies to *C. robusta* flowers at Sunset and Pogonip is not typical of species at grassland sites and at coastal scrub sites. Similarly, the high proportion of visits by wasps to *C. robusta* flowers is not common for species at grassland or coastal scrub sites (Moldenke 1976).

The differences in visitation frequency of different insect groups at the two sites may relate to differences in habitat and climate. The Pogonip site is further inland and thus further removed from the climatic conditions along the immediate coast, which include fog, wind and cooler temperatures. Climatic conditions may relate directly to the lower percentage of Hymenoptera visitors at Sunset than at Pogonip. Small-bodied solitary bees are poikilothermic and require warmer temperatures to forage (Moldenke 1976). They are therefore likely to be less abundant at Sunset than at Pogonip. A study of two sites in Northern California, one close to the coast and one further inland, revealed that the number of bee species at the inland site was twice that of the coastal site (Dobson 1993). Large-bodied bees such as bumblebees are able to maintain their body temperatures and fly in cooler conditions, which could account for their high frequencies at Sunset.

Climatic differences are also likely to account for the larger percentage of pollinator visits by Diptera at Sunset than at Pogonip. Flies may be better able to forage under the more extreme climatic conditions at Sunset, and therefore be more abundant relative to Hymenoptera pollinators. Flies have lower energy requirements than bees and are able to take advantage of microclimates to maintain body temperatures. For these reasons, flies are able to survive and forage within a wider range of climates and habitats than bees (Kearns 1992). In several studies comparing pollinator assemblages in different climates and habitats, fly pollination increased in importance as climatic conditions became more extreme (Kearns and Inouye 1994, Arroyo et al. 1982). Lepidoptera tend to be especially numerous in grassland habitats (Moldenke 1976) which could explain the greater frequency of butterfly pollinators at Pogonip than at Sunset.

Within-habitat variations in climate may also affect the foraging patterns and distribution of different groups of pollinators at each site. The major groups of pollinators such as solitary bees and muscoid flies tolerate different climatic conditions. Particularly at Sunset, this may allow for at least some insect visitation within different microclimates throughout the entire site.

Visitation frequency and environment

Results of this study demonstrated that pollinator visitation rates vary throughout each site, and that this variation correlates with different plant community characteristics and plot locations. Visitation rates are also significantly higher at Pogonip than at Sunset.

Since seed set increases with pollinator access, seed set in *C. r.* var. *robusta* could also be affected by variability in visitation rates.

Since the number of flower visits had to be estimated during observations with many insect visitors, it is possible that inaccurate visitation estimates in plots receiving large numbers of visitors could have biased the results. However, a review of the data indicates that this is unlikely. There were a total of 14 observations where the total number of visitors was greater than 12 and may have been estimated. Of these 16 observations, 6 occurred in plots where the number of flowers was above the mean flower number of 604. Seven of the observations occurred in plots where the mean number of flowers was below 604. The number of visitation estimates in plots with an above average number of flowers was roughly equal to the number of visitation estimates in plots with a mean number of flowers below the average. Since the mean number of flowers is proportional to plot density, it is unlikely that estimates for insect visitation would have biased the results to show higher visitation rates in plots with lower density of spineflower.

Insects visited flowers of *C. r.* var. *robusta* more frequently overall at Pogonip than at Sunset. These results are consistent with results of other studies that demonstrate a lower diversity and abundance of insects at coastal sites than at sites further inland. Moldenke (1976) estimated that coastal scrub in northern California had about 50% less insect diversity than inland chaparral sites. Similarly, Dobson (1993) found that a coastal scrub site in northern California had about 50% the number of bee species as were found at a more inland chaparral site with similar vegetation. Diversity is also very high in grassland communities, and often increases in oak savannas (Moldenke 1976). Although overall insect abundance and diversity of insects at the species level were not measured in this study, it is expected that comparable differences exist in coastal versus more inland sites that could result in differences in visitation frequency.

At Pogonip, the higher visitation frequency late in the flowering season could be due to high numbers of pollinators competing over the few remaining flowers. At Sunset, there were no significant differences in visitation frequency by season. This could be due to an apparent greater availability of other floral resources adjacent to *C. r.* var. *robusta* flowers at Sunset than at Pogonip late in the season.

At both Sunset and Pogonip, visitation frequency varied among plots. In all but one plot at Pogonip, the probability of a flower being visited by a pollinator was greater than 100%. In 25% of the plots at Sunset, flowers had a less than 50% chance of being visited by a pollinator. Since self-fertilization in the absence of pollinator visitation appears to result in lower rates of seed set, the potential exists for pollinator limitation at Sunset. This could especially be true for plots that receiving relatively few visits by pollinators. Outcrossing could also be limited in plots that receive few pollinator visits. These results are consistent with the findings of Moldenke (1976), who notes that pollinators may be limiting at coastal sites.

Variability in visitation frequency is further associated with environmental conditions at each site. However, pollinator response to the environmental variables analyzed differs for the two populations of *C. r.* var. *robusta* studied. The different responses of pollinators to these environmental variables may relate to the different climatic conditions occurring at the two sites.

Insect visitation and environment at Sunset: At Sunset State Beach, the significant correlation between density of other plants and insect visitation to *C. r.* var. *robusta* both early and late in the flowering season is somewhat surprising. Also surprising is the negative correlation between spineflower density and insect visitation. These results contrast with other studies which show that insect visitation rates for a plant species tend to increase with increasing density of that species (Silander and Primack 1978, Kunin 1993).

The puzzling correlation between a high density of other species and a high rate of insect visitation to *C. robusta* flowers may relate to strong climatic factors at Sunset. Wind speeds are particularly strong along the immediate coast, with wind speeds averaging 14.3 to 15.7 mph at a height of 50 m along the immediate coastline of Monterey Bay (US DOE 2003). This contrasts with wind speeds of wind speeds averaging 0 to 9.8 mph in areas of Monterey Bay inland from the immediate coast. Plots with a high density of other species at Sunset may provide pollinators protection from wind, thereby increasing insect visitation in those plots. Robust spineflower plants are generally less than 20.3 cm tall (Reveal 1989). The average height of other plant species in 7 observation plots ranged from a low of 23.1 cm to a high of 47.8 cm, with a mean height of 37.6 cm. It is possible that a higher density of other species provided more protection from wind than a high density of spineflower, and allowed a greater diversity and abundance of insects to forage.

Although wind speed was not measured in this study, insect visitation was lowest at plots on west-facing slopes that had the greatest exposure to the prevailing ocean winds: In all plots on west-facing slopes, insects visited less than 5% of flowers in each plot during each 10-minute observation period. This could also indicate that climate may play a strong role in insect foraging at Sunset.

Other studies have documented the effects of strong wind, fog and cooler climates that are found in coastal areas on insect abundance, diversity, and activity levels of specific groups of insects (Dobson 1993, Moldenke 1976). Small bees in particular need higher temperatures for long periods to warm their bodies sufficiently so that they can fly. Dobson (1993) noted at a coastal site in Marin County that abundance and diversity of bees varied hourly based upon weather conditions.

At Sunset, the microclimate within each observation plot probably varied based on exposure, density of other plant species, as well as other factors. Since climate does affect insect behavior, the variability of microclimates at Sunset could also influence insect activity. Microclimates that are warmer and less windy may allow for a greater abundance and diversity of foraging insects.

Insect visitation and environment at Pogonip: At Pogonip, the relationships between environmental variables and the rate of insect visitation to *C. r.* var. *robusta* are quite different. Visitation is highest where there is a low density of other species within the larger area around the plant, and where plants are taller within the immediate area around the plant being visited. The different responses of pollinators to plant community characteristics at Pogonip may relate to the different climatic conditions occurring at the two sites. Since the Pogonip site is further inland, the site is less affected by the harsh climatic conditions occurring along the immediate coast. In addition, the site is smaller so there are probably fewer microclimatic differences among plant patches. Therefore, one might expect climate to play less of a role in variability of visitation rates at Pogonip and for pollinators to respond in a more typical manner to density of other plant species. Indeed, at Pogonip the higher insect visitation rates in plots with a lower density of other plant species are consistent with the results of some other studies (Kunin 1993).

The negative correlation between density of other plant species within the 6.0 m^2 plots and the rate of insect visitation to *C. r.* var. *robusta* flowers could indicate that patch size is important to insect visitation at Pogonip. Although the density of robust spineflower at 6.0 m^2 was not included the PCA, it is expected that the density of spineflower would be higher where the density of other plant species is lower. Higher density of *C. r.* var. *robusta* within the 6.0 m^2 area indicated a large patch size.

Visibility of spineflower plants to pollinators may also be important at Pogonip. Since the flower is quite small and not especially showy, a high density of other plant species within the 6.0 m^2 area surrounding the plant may obscure the visibility of *C. robusta* var. *robusta* flowers to insects. In addition, the positive correlation between plant height in the 0.4 m^2 plots and insect visitation rates could indicate that taller plants were more visible to the insects and therefore were visited more often. The negative correlation between a high density of other species in the 6.0 m^2 areas surrounding the 0.4 m^2 observation plots could indicate potential competition with other plant species for pollinators. However, in this case pollinator competition is unlikely since there were very few other plants blooming at the study site when the robust spineflower was flowering.

Results indicating that a larger patch size may increase visitation rates at Pogonip are consistent with results of other studies analyzing density and insect visitation. One study showed that an individual *Oenothera fruticosa* flower growing in a dense population received an average of 6.9 bee visits per day, whereas an individual flower growing in a sparse population received an average of only 3.6 bee visits per day (Silander and Primack 1978). Other studies have documented that pollinators may preferentially visit large patches to increase foraging efficiency (Kunin 1993).

Comparing insect visitation and environment at Sunset and Pogonip: At Pogonip, insect visitation to *C. r.* var. *robusta* increased with increasing vegetation height and decreasing density of other species within a large area surrounding the plant. This indicated that pollinators respond positively both to a larger patch size, higher density, and to increased visibility of *C. r.* var. *robusta* flowers at Pogonip. At Sunset, pollinators responded positively to a lower density of robust spineflower in the area immediately around the plant during midseason. Early and late in the season at Sunset, pollinator visitation increased in response to a higher density of other species in the area immediately surrounding the plant. Pollinator visitation also appeared to be lowest in plots that had the greatest wind exposure. At Sunset, the effects of climate may be one of the main determinants of foraging behavior.

The response of pollinators to density of *Chorizanthe robusta* var. *robusta* and density of other species may thus differ from one population to another, based in part upon climatic and environmental conditions at the population site. Depending on weather and climate conditions at each site and throughout each day, insect pollinators may have to employ different foraging strategies. Whereas density of spineflower may promote greater frequency of insect visitation at one site, microclimatic conditions that afford protect from wind and weather may increase visitation frequency at other sites. Any predictions as to response of pollinators to plant density would be difficult to make without understanding the influence of weather and other factors at the site.

Pollinator foraging ranges and habitat requirements

Information from other studies on potential foraging distances of several pollinators of *C. r.* var. *robusta* indicates that pollinator foraging ranges are likely to be quite variable. Bumblebees, which are frequent pollinators at Sunset, may fly long distances from their nests to forage for nectar and pollen and may forage among widely spaced plants and plant patches. One study documented individual *Bombus terrestris* regularly flying more than 200 meters from their nests to forage (Osborne et al. 1999). Another study documented bumblebees foraging among foxglove plants that were more than 4 meters apart (Proctor et al. 1996). Some bumblebees visited isolated plants that were more than 24 meters away from the main patch. Honeybees, also frequent visitors at Sunset, can forage several kilometers from their nests (Visscher and Seeley 1982). The foraging range of Halictid bees is likely to be more restricted than that of bumblebees or honeybees (Handel 1983). Little information is available on the foraging ranges of other pollinators of *C. r.* var. *robusta*. However, in one study a wide array of pollinators foraged primarily among plants that were less than 20 meters apart (Kunin 1993).

To the extent that the composition of the pollinator assemblage varies among sites, the pollinators at each site are likely to have different foraging ranges and may therefore have different effects on gene flow. Pollinators such as bumblebees and honeybees are capable of foraging among widely spaced patches and may facilitate gene flow among widely spaced plants. Honeybees, bumblebees and some solitary bees may also forage by trap lining, where they repeatedly visit a series of spatially separated patches of one or several plant species along a set route (Proctor et al.1996). If populations of trap-lining pollinators or pollinators with long foraging distances were to decrease, then patterns of gene flow within the population could change. If a population or colony lost connectivity with other populations due to changes in pollinator foraging, there would be the potential for inbreeding, genetic bottlenecks, and eventual extirpation of the population.

Fragmentation of populations of *C. r.* var. *robusta* that altered the distances between patches could also result in loss of gene flow within the population. Alterations to the spatial distribution of patches have the potential to alter the distribution and foraging behavior of pollinators (Fahrig and Merriam 1993). Particularly in populations with few pollinators capable of flying long distances to forage, increasing distance between patches of *C. r.* var. *robusta* could result in fewer pollinator flights between patches. This could result in loss of genetic exchange between patches and potentially increase inbreeding within isolated patches.

A review of the literature indicates that although the pollinator assemblage at both sites has diverse habitat requirements, many of the pollinator families require bare ground for nesting. Most bee species in the Halictidae and Anthophoridae families nest in the ground, either in chambers, burrows, or on level ground (Borror et al. 1989). Some bee species in the Anthophoridae family, like the genus *Ceratina* found at Sunset, excavate the pith from shrub stems and nest in the hollowed-out cores (Borror et al. 1989). Since the twigs and stems must be broken or burned before *Ceratina* can nest, these species can benefit from fires (Pascarella 2002). In the family Apidae, queen bumblebees nest in the ground, using existing holes such as those left by rodents or rotting wood (Handel 1983). In the Sphecidae family, most species nest in the ground in areas that are mostly bare of vegetation (Bohart et al. 2000). Many use preexisting holes. The genus *Steniolia* found at Pogonip nest in bare ground. The genus *Tachysphex* found at Pogonip also burrows in soil to form a nest (Porter 2003).

Other pollinator requirements include food sources for both adult and immature insects, in addition to other specialized requirements. Obtaining food may require complex interactions with other plant and insect species. Adult Acmon blue butterflies, frequent pollinators at Pogonip, feed on flower nectar. Larvae feed on leaves, flowers and developing seeds of *Eriogonum, Lupinus, Lotus and Astragalus*. Ants (USGS 2003) tend caterpillar larvae. Adult common ringlets (*Coenonympha tullia*) feed on flower nectar, and larvae feed on grasses and rushes. Adult Buckeyes (*Junonia coenia*) feed on nectar of flowers in the Asteraceae family, as well as other families. Caterpillar hosts include plants from the snapdragon, plantain and acanthus families. At Sunset, *Aristotelia argentifera* feeds on *Ericameria* (J. Powell, personal communication 2002).

Parasitic insects in the Sphecidae family found at Pogonip include *Steniolia* sp. that provision their nests with Diptera, and *Tachysphex* sp. that provision their larvae with orthopteroids (Porter 2003). Bees in the Nomadini tribe found at Pogonip (Anthophoridae) are parasitic on other insects, frequently on other species of bees (Pascarella 2002). The larvae of *Ptilodexia* sp. (Tachinidae) found at Sunset are parasites of scarab beetle larvae (J. Schweikert personal communication 2002).

The robust spineflower in the ecological community

While some pollinators of *C. r.* var. *robusta* were also observed visiting flowers of other plant species, *C. r.* var. *robusta* appears to be the dominant floral resource in the area during peak flowering. At Sunset, the peak blooming period for most other species appeared to occur either before or after the peak bloom in *C. r.* var. *robusta*. Robust spineflower also appeared to dominate in terms of overall abundance of flowers. At Pogonip, *C. r.* var. *robusta* appeared to dominate to an even greater degree. While robust spineflower was in bloom at Pogonip, no other plants were observed flowering within 15 m of the circumference of the *C. robusta* colony until July.

Observations of the foraging behavior of many pollinators at both Sunset and Pogonip reveal repeated visits to *C. r.* var. *robusta* flowers over a period of several minutes, indicating some degree of floral constancy among observed pollinators. Pollinators such as Halictidae, Anthophoridae and *Bombus* will often specialize on the dominant floral resource, and may shift floral resources as species come in and out of flower sequentially throughout the season (Moldenke 1976). Other studies have documented high levels of floral constancy among generalist pollinators such as flies while foraging in dense monospecific patches (Kearns 1992). Since *C. r.* var. *robusta* appears to be the dominant floral resource throughout much of its blooming period, it is likely that many pollinators may specialize on this species to some degree and assure some level of floral constancy. Since robust spineflower produces much of the floral resources during its blooming period, it is likely to play an important role in supporting the pollinator guilds during peak bloom. *Chorizanthe r.* var. *robusta* is likely to be a crucial food source for the insect community, helping to sustain pollinators of other rare species that occur at the site.

Pollinators and the conservation of the robust spineflower

Results of the present research indicate that frequency of visitation by insects can vary widely under certain conditions. Although the potential for pollinator limitation depends in part upon the degree to which *C. r.* var. *robusta* is capable of autogamous self-pollination, the present research does indicate that seed set is lower without pollinator visitation in the population studied. It is therefore likely that under certain conditions and within portions of some populations, reproductive output in *C. r.* var. *robusta* might be pollinator limited. Since there are few remaining populations of robust spineflower, the survival of each population may be important to the conservation of the species (US FWS 2002). Particularly for small populations, even small reductions in reproductive output have the potential to negatively affect the long-term survival. Therefore, even small reductions in seed set resulting from pollinator limitation have the potential to affect the conservation of the species. Protecting the plant-pollinator relationship in *C. r.* var. *robusta* populations could be an important component of survival and recovery of the taxon.

Aside from the potential for pollinator limitation within populations of *C. r.* var. *robusta*, pollinators are also essential for outcrossing. Although not examined within this study, outcrossing is necessary to the long-term survival of many plant species that have some degree of cross-pollination. Reduced levels of outcrossing can result in inbreeding depression within populations. In addition, reduced outcrossing can result in loss of genetic diversity, which can make populations less adaptable to environmental change. Where insect visitation is low, levels of outcrossing could be reduced.

For a species such as *C. r.* var. *robusta* that has a high diversity of pollinators and is at least partially self-compatible, pollen limitation is generally considered unlikely (Burd 1994, Proctor et al.1996). For these types of plant species, resources are considered more likely to limit reproductive output than pollination. Within the ranking system developed by Bond (1994), *C. r.* var. *robusta* would probably be considered at low risk of extinction resulting from failed pollinator mutualisms. Characteristics that could put a plant species at higher risk of extinction include dioecious flowers and self-incompatibility, reliance on a single pollinator, and propagation by seed only. Although *C. r.* var. *robusta* does reproduce by seed only, the species is self-compatible and has many pollinators.

While risks of pollen limitation may be low for a particular plant species as a whole, pollen limitation may occur under certain conditions that limit the frequency or effectiveness of pollinators. Kunin (1993) determined that for a self-incompatible species with a high diversity of pollinators, low density appeared to result in low floral constancy of pollinators. A high proportion of pollen delivered to plants was from plants of other species, resulting in reduced reproductive output in plants. Plants of this species were pollinator-limited under conditions of low density. Habitat fragmentation can also increase the potential for pollination limitation in populations of some species. A study by Jennersten (1988) indicated a strong probability of pollinator limitation for a population of the self-compatible *Dianthus deltoides* growing in a fragmented patch of habitat surrounded by agricultural fields. By comparison, another population of the same species growing in an area of contiguous natural habitat showed no evidence of pollination limitation.

The results of this research indicate that plants growing in conditions that are likely to be unfavorable to pollinator visitation receive fewer pollinator visits. For these plants, reproductive output could be limited by infrequent pollinator visits. Other research has shown that small populations, isolated populations or of patches within populations, and alteration of habitat that affects pollinator diversity, abundance or behavior could also result in pollinator limitation. New populations established as part of a recovery effort could also be vulnerable to pollinator limitation. Populations where several of these conditions occurred could be particularly vulnerable to pollinator limitation. Effects of pollinator limitation on long-term survival of the population could depend in part on the size and persistence of the seed bank, as well as the germination rate and seedling survival.

Suggestions for further research

This research has established that pollinators are important to plant fitness in C. robusta. In addition, this research has identified the most frequent pollinators at two populations, recorded visitation frequency, and evaluated some of the factors that might affect visitation rates. Additional research could help clarify the importance of insect pollinators, determine the primary factors affecting insect visitation rates, and evaluate the importance of the robust spineflower to pollinators.

1. To what degree is *C. robusta* var. *robusta* self-compatible?

Although sporophytic or gametophytic self-incompatibility mechanisms are unlikely in a plant capable of self-fertilization, late-acting partial self-incompatibility mechanisms could still exist (Proctor et al. 1996). This could include abortion of ovules fertilized by pollen from the same flower. Further research is necessary to determine whether such mechanisms exist within the robust spineflower. Experiments involving hand pollination of flowers with pollen from the same plant and hand pollination of emasculated flowers with pollen from different plants could determine the actual difference in seed set between self-pollination and cross-pollination. Knowledge of any incompatibility mechanisms could help determine the importance cross-pollination and pollinators for this species.

2. Is there any evidence of inbreeding depression in populations of *C. robusta* var. *robusta*?

The relatively high germination rate of seeds from self-pollinated flowers gives no indication of inbreeding depression. However, reduced fitness of self-fertilized progeny can manifest during other stages of plant development as well, including seedling survival and development of seedlings to maturity. Inbreeding depression could be particularly likely with smaller isolated populations. Research on genetics of the robust spineflower, or experiments that compare fitness levels of self and crossed progeny through all stages of plant development and at different populations, could determine whether inbreeding depression may be significant in any populations of *C. r.* var. *robusta*. Pollinator activity may be particularly important in populations where inbreeding depression occurs.

3. How important is climate and plant density in determining frequency of visitation?

This research suggests that climate, particularly wind speeds, may influence visitation rates more strongly than plant density at some sites. Additional research assessing the effects of wind and temperature could help determine what factors influence visitation rates.

4. Do any pollinators specialize primarily or exclusively on *C. r.* var. *robusta* flowers?

Some Anthophoridae are specialist feeders, feeding on a particular genus, family or closely related plant taxa. Further work identifying solitary bees to the species level and analyzing pollen on specimens could help to determine whether specialist bee species occur at Sunset or Pogonip.

RECOMMENDATIONS

In the draft recovery plan for *C. robusta* var. *robusta* (2000), the US Fish and Wildlife Service states that managing habitat to maintain ecosystem processes is a priority for recovery of the taxon. Research identifying requirements of *C. r.* var. *robusta* populations for long-term viability is also listed as a priority. This research has demonstrated that pollinators are important to reproduction in *C. r.* var. *robusta*, and are likely be important to the long-term survival of the variety. Following are recommendations for managing *C. r.* var. *robusta* habitat that will protect plant-pollinator interactions as an essential ecosystem process. These recommendations are intended to protect pollinators and pollinator habitat, while also considering other requirements of *C. r.* var. *robusta.*

The results of this research support several recommendations included in the Critical Habitat Designation (US FWS 2002):

1. Control invasive plant species.

Controlling the spread of non-native species within *C. r.* var. *robusta* habitat at Sunset and Pogonip can help to maintain areas of open ground required by many pollinators. In particular, many of the Hymenoptera pollinators including most Sphecid wasps, bumblebees, and Halictidae and Anthophoridae bees require bare ground for nesting. At Sunset, continuing the current program involving removal of iceplant and veldt grass will help to maintain areas of open ground as nesting habitat for pollinators (CDPR 1998). At Pogonip, where a lower density of other species was associated with a higher frequency of visitation, control of invasive non-native species may also help to maintain existing rates of pollinator visitation to *C. r.* var. *robusta*.

2. Protect the existing diversity of native plant and insect species.

At Sunset, pollinator visitation frequency appears to benefit from the presence of other plant species. In addition, all pollinators that actively forage before or after *Chorizanthe robusta* var. *robusta* has flowered need other sources of nectar and pollen. Research on insect diversity within a tallgrass prairie ecosystem indicates that maintaining a high diversity of native plant species and plant community richness is perhaps the best strategy for maintaining high insect biodiversity (Panzer and Schwartz 1998). The authors conclude that this strategy might apply to insect biodiversity in temperate regions more generally.

Protecting the diversity of native plant species should also help to meet the requirements of other insects upon which some pollinators of *C. r.* var. *robusta* depend. In the Sphecidae family, *Tachysphex* spp. provisions its nest with various genera of Diptera, and *Steniolia* spp. provisions its nest with orthopteroids. The larvae of the Acmon blue butterfly, a frequent pollinator at Pogonip, are tended by ants. At Sunset, the moth pollinator *Aristotelia argentifera* feeds on *Ericameria* spp. In addition, all pollinators that are actively foraging before or after *Chorizanthe robusta* var. *robusta* has flowered need other sources of nectar and pollen. Maintaining the existing diversity of native plant species should help to provide appropriate food sources and other requirements for a wide array of pollinators.

Controlling invasive non-native species should help to maintain high plant diversity by assisting other native plant species that may also be poor competitors. Existing disturbance regimes may also help to maintain a variety of habitats suitable to a wide diversity of plant species.

At coastal sites such as Sunset, the climate is often harsh and windy, particularly at locations exposed to coastal winds. Tolerance of harsh climatic conditions varies among different groups of pollinators. By protecting the existing high diversity of pollinators, insect visitation may be more likely to occur within the different microclimates at each site.

3. Maintain continuity between discrete patches of *C. r.* var. *robusta*.

At Pogonip, pollinators with wide foraging ranges may be more limited than at Sunset. Foraging distances of pollinators may vary among different *C. r.* var. *robusta* populations depending on the composition of the pollinator assemblage. Maintaining continuity among patches within populations could allow for the possibility of genetic exchange among separate patches, including populations where the foraging ranges of pollinators may be limited.

4. Protect the Pogonip population from trampling and foot traffic.

In 2002, the Pogonip population declined in number late in the flowering season. The area appeared to have been heavily trampled. Deer may have trampled the area at night, since several deer were observed in the area. Since the colony is small to begin with, loss of a significant number of individuals could negatively affect the population.

To protect plants from germination to seed set, it is recommended that the area be fenced off from pedestrians from April through July.

Several additional recommendations are also suggested from the results of this research:

5. Implement an insecticide-free buffer zone at Sunset.

Pesticide has the potential to cause serious disruptions to insect populations. In one study, bees in the Halictidae and Anthophoridae families along with other insects were observed dying after forests were sprayed with Matacil. Several understory plant species on which they foraged showed reduced fecundity following spraying, which was attributed to reduced pollinator visitation (Thompson et al. 1985).

At Sunset State Beach, agricultural fields surround the robust spineflower population on three sides. I observed pesticide applications to the agricultural fields adjacent to the site on three occasions. Even if drift does not occur at the site, spraying may harm pollinators of *C. r.* var. *robusta* that also forage on sprayed crops nearby. Bumblebees can forage up to 200 meters from their nests, and might forage among crop plants. Solitary bees might also forage among crop plants, since they occur in close proximity to robust spineflower plants. Small bees are particularly susceptible to the effects of pesticides (Kearns and Inouye 1997). Due to low fecundity of many bee species, it can take many years for populations that have been affected by pesticides to recover (Karron 1991).

Due to potential impact of pesticides on pollinator populations, it is recommend that a no-spray buffer zone within a 0.2-kilometer radius of *C. r.* var. *robusta* plants be implemented at Sunset to protect pollinators. This distance represents a typical foraging distance of bumblebees, which are likely to have the widest foraging distance of the most frequent pollinators. Buffer zones have been used to protect pollinators of endangered species in rangeland areas in the southwest, where pesticide spraying is prohibited within a 3-mile radius around sites where protected outcrossing species occur (Kearns and Inouye 1997).

It is also recommended that the pesticide levels within the area where *C. r.* var. *robusta* grows at Sunset be monitored after the buffer zone has been implemented, particularly after pesticide applications in nearby fields. Further studies determining whether *C. r.* var. *robusta* at Sunset show any evidence of pollinator limitation are also recommended. If pesticide levels are high at the site, or if there is any evidence of pollinator limitation, then it is recommend that the buffer zone area be increased.

6. Monitor the effects of fire on pollinators.

The use of burning to control invasive species should be monitored, since burning can harm ground-nesting pollinators including bumblebees (Lesica 1993). On the other hand, bees in the genus *Ceratina* may benefit from the effects of fire. Further research into the breeding season of ground-nesting pollinators at each site could help determine when burning might be least harmful to pollinators.

7. Recommendations for establishing new populations.

Successful pollination may be important to the long-term survival of newly established populations. New populations established as part of a restoration or recovery effort may be particularly susceptible to pollinator limitation. One study of a restored population of *Cordylanthus maritimus* var. *maritimus*, a marsh species that is self-compatible but requires insects for pollination, found that the reproductive capacity in some areas of the population was pollinator limited (Parsons and Zedler 1997). The lower abundance of important pollinators was attributed in part to the highly fragmented landscape.

Potential sites should contain a diversity of other native plant species near the site to sustain pollinators. Areas of bare ground should be present to provide suitable nesting sites for many pollinators and minimize competition from other plants. Selecting sites that are within large areas of natural habitat could allow for access by a large diversity and abundance of pollinators. Selecting sites near established populations could also help increase the probability that potential pollinators would be present, as well as providing the possibility for some degree of genetic exchange with the established population.

Conclusions

The results of this research add significantly to our existing knowledge of the pollination ecology of *C. robusta* var. *robusta*. This research demonstrates the importance of pollinators to fitness in the robust spineflower, as well as indicating potential areas of vulnerability within the pollination system. The experimental portion of this research has demonstrated that the robust spineflower is self-fertile and capable of autogamous self-pollination, as are some other annuals in the genus *Chorizanthe*. This research also documented increased levels of seed set in flowers that were accessible to pollinators, demonstrating that insect pollination confers increased levels of fitness to *C. robusta* var. *robusta* flowers. Observations indicate that the protandrous breeding system may serve to promote cross-fertilization initially, while potentially allowing for self-fertilization in flowers that have not been cross-pollinated. This is similar to the breeding system in some other annual *Chorizanthe* species.

Observations of pollinator activity indicate a diverse array of pollinators comparable to that found in some other generalist pollination systems. Correlations between pollinator frequency and environmental characteristics indicate that there may be a relationship between environmental conditions and insect visitation frequency. Interestingly, pollinators appeared to respond favorably to an increased density of other plant species along the coast, and negatively to an increased density of other plant species at the site further inland. The different responses of pollinators at the two sites to density of other plant species may relate to the different climatic conditions occurring at the two sites. The high diversity of pollinators may allow for insect pollination within a diversity of microclimates and microhabitats found at population sites.

At Sunset, the frequency with which pollinators visited flowers varied significantly among different areas at the site. This may relate to different microclimatic conditions occurring within the site. Spatial variability in pollinator visitation may indicate that some areas of the population could be vulnerable to fluctuations in pollinator levels and could potentially experience reduced levels of fitness because of reduced pollinator visitation.

Since insect pollination can increase seed set in *C. robusta* var. *robusta*, clearly pollinators are important to the species. Varying levels of pollinator visitation within the larger coastal population also indicate that some segments of the population could be negatively affected by reduced pollinator activity. The recommendations in this report are intended to protect the pollinator assemblage and pollinator habitat and to maintain existing levels of pollinator diversity and activity. The results of this research and the recommendations offered should help to protect the plant-pollinator relationship, thereby increasing the potential for survival and recovery of the robust spineflower.

Table 7. Insect visitors of robust spineflower at Sunset in 2001 and 2002.

Order	**Family**	**Subfamily**	**Genus**	**Species**	**Likely to pollinate**	**Year**	**ID***
Coleoptera	Coccinellidae				?	01,02	KM
	Dasytidae				yes	01,02	GE
Diptera	Bombyliidae		*Lepidanthrax*	sp.	yes	01,02	JS
	Bombyliidae				yes	01,02	KM
	Calliphoridae				yes	01,02	JS
	Milichiidae				yes	01,02	JS
	Phoridae				?	02	GE
	Sarcophagidae				yes	01,02	JS
	Syrphidae				yes	01	KM
	Tachinidae		*Ptilodexia*	sp.	yes	01,02	JS
Hemiptera	Lygaeidae		*Geocoris*	sp.	yes	01,02	JH
Hymenoptera	Anthophoridae	Xylocopinae	*Ceratina*	sp.	yes	02	HC
	Anthophoridae				yes	01,02	HC
	Apidae	Apinae	*Apis*	*mellifera* L.	yes	01,02	KM
	Apidae	Bombinae	*Bombus*	sp.		01,02	HC
	Brachonidae		*Chelonus*	sp.	yes	02	RZ
	Formicidae	Dolichoderinae	*Linepithema*	*humile*	no	01,02	KM
	Formicidae				?	01,02	KM
	Halictidae	Halictini			yes	01,02	HC
	Sphecidae	Sphecinae	Ammophila	*nasalis Provancher*	yes	01,02	HC

		Bembicinae	*Bembix*	*americana comata*	?	01,02	HC
		Larrinae			yes	01,02	KM
Lepidoptera	Gelechiidae	Gelechiinae	*Aristotelia*	*argentifera*	yes	01,02	JP
	Lycaenidae	Plebiinae			yes	01,02	KM
	Nymphalidae		*Vanessa*	sp.	yes	01	KM
	Nymphalidae		*Junonia*	*coenia*	yes	01	KM

* Insects identified by Dr. Helen Court (H.C.), Dr. Robert Zuparko (R.Z.), and Dr. Jere Schweikert (J.S.) of the California Academy of Sciences; Dr. Gordon Edwards (G.E.) and Dr. Jeff Honda (J.H.) of San Jose State University; Dr. Jerry Powell (J.P.) of the University of California at Berkeley; and Kathryn Murphy (author).

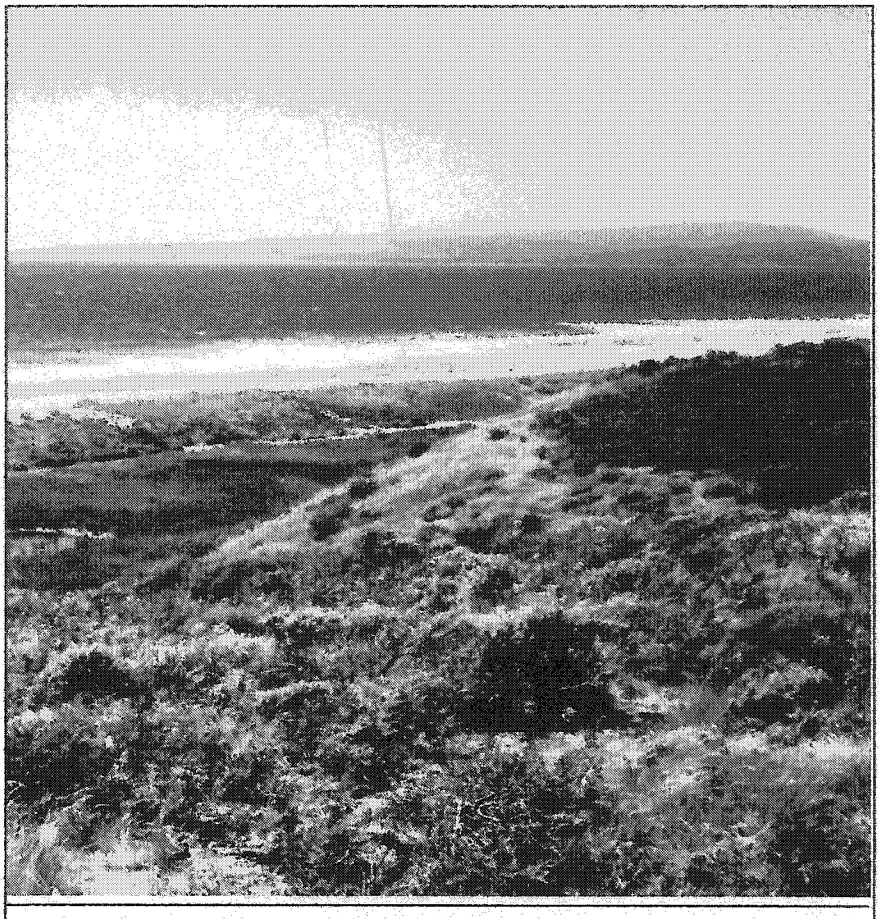
Table 8. Insect visitors of robust spineflower at Pogonip Park in 2002.

Order	**Family**	**Subfamily**	**Genus**	**Species**	**Likely to Pollinate?**	**I.D.***
Coleoptera	Bruchidae				yes	G.E.
	Chrysomelidae				yes	G.E.
	Dermestidae				no	G.E.
Diptera	Bombyliidae		*Lepidanthrax*	sp.	yes	J.S.
	Bombyliidae		*Glabellula*	sp.	?	J.S.
	Calliphoridae				yes	J.S.
	Tachinidae				yes	J.S.

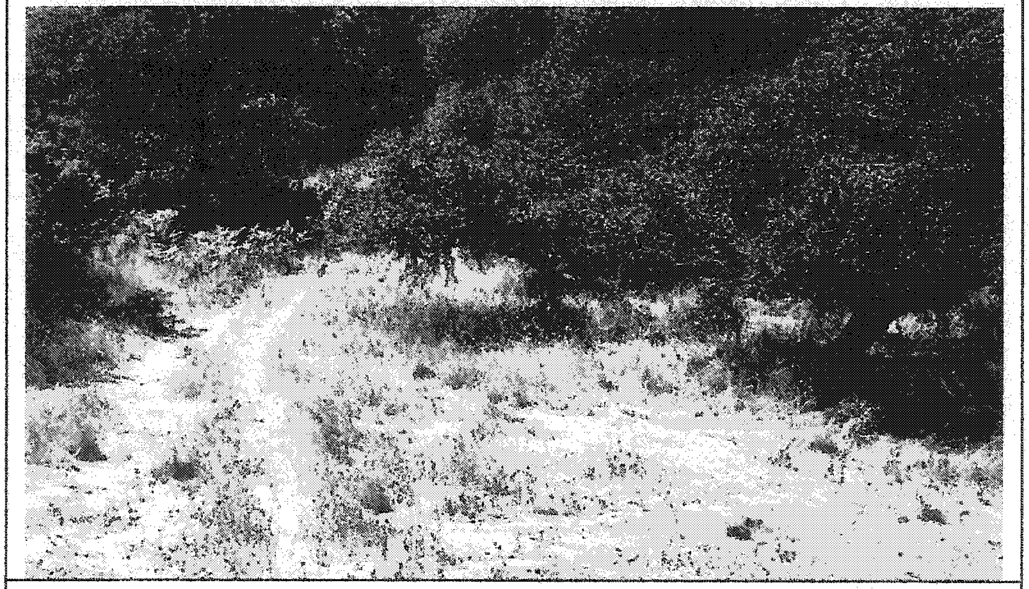
Hymenoptera	Anthophoridae	Nomadinae	*Nomadini*	sp.	yes	H.C.
	Apidae	Apinae	*Bombus*	sp.	yes	H.C.
	Halictidae	Halictinae	*Agapostemon*	sp.	yes	H.C.
	Sphecidae	Sphecinae	*Sphex*	*ichenumoneus*	yes	H.C.
	Sphecidae	Bembicinae	*Steniolia*	*elegans*	yes	H.C.
	Sphecidae	Larrinae	*Tachysphex*	sp.	yes	H.C.
Lepidoptera	Lycaenidae	Plebiinae	*Plebeius*	*acmon*	yes	K.M.
	Nymphalidae				yes	K.M.
	Nymphalidae	Nymphalinae	*Junonia*	*coenia*	yes	K.M.
	Nymphalidae	Satyrinae	*Coenonympha*	*tallia*	yes	K.M.

* Insects identified by Dr. Helen Court (H.C.), Dr. Robert Zuparko (R.Z.), and Dr. Jere Schweikert (J.S.) of the California Academy of Sciences; Dr. Gordon Edwards (G.E.) and Dr. Jeff Honda (J.H.) of San Jose State University; Dr. Jerry Powell (J.P.) of the University of California at Berkeley, and Kathryn Murphy (author).

Photos: Study sites



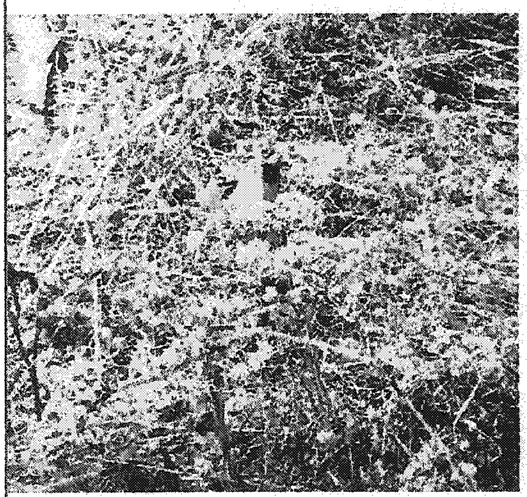
Sunset State Beach study site



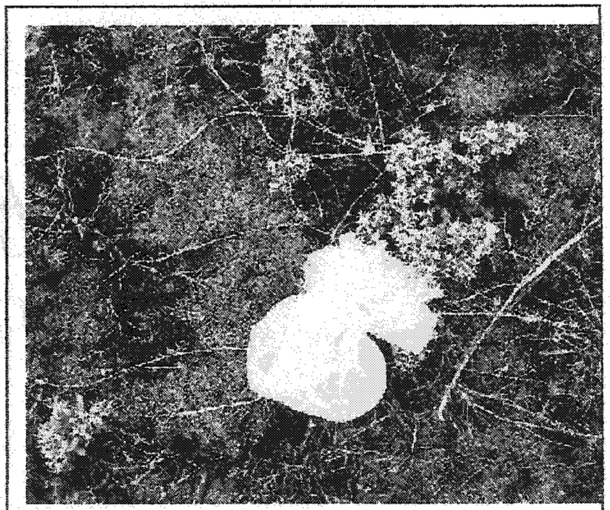
Pogonip Park study site

Photos: Methods

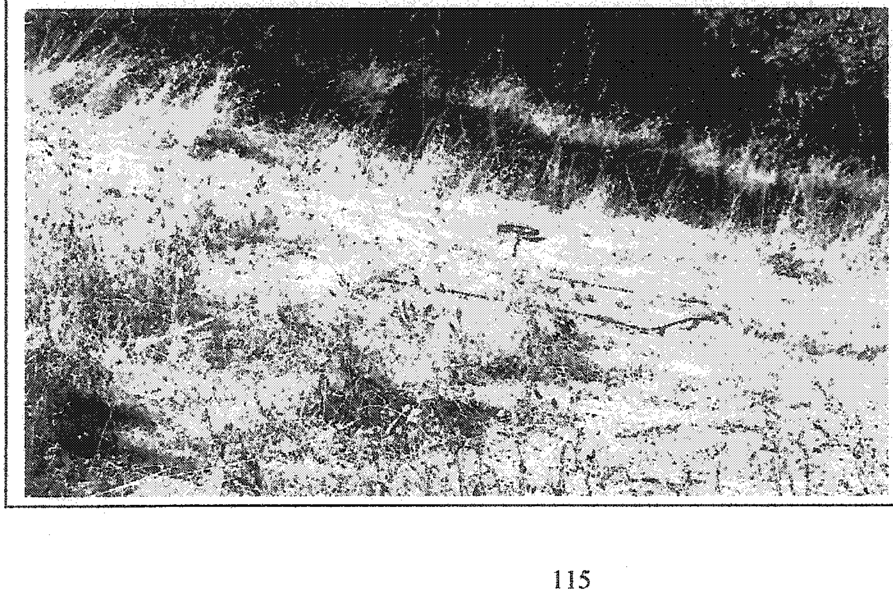
Chorizanthe robusta var. *robusta* at Sunset State Beach



Stake marking sample plot



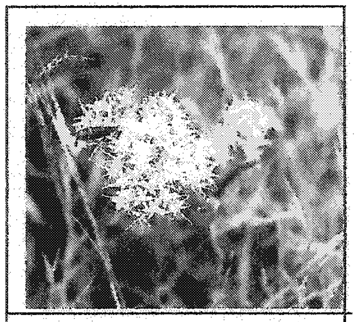
Closed bag excluding pollinators



Measuring plot attributes for correlation study at Pogonip Park

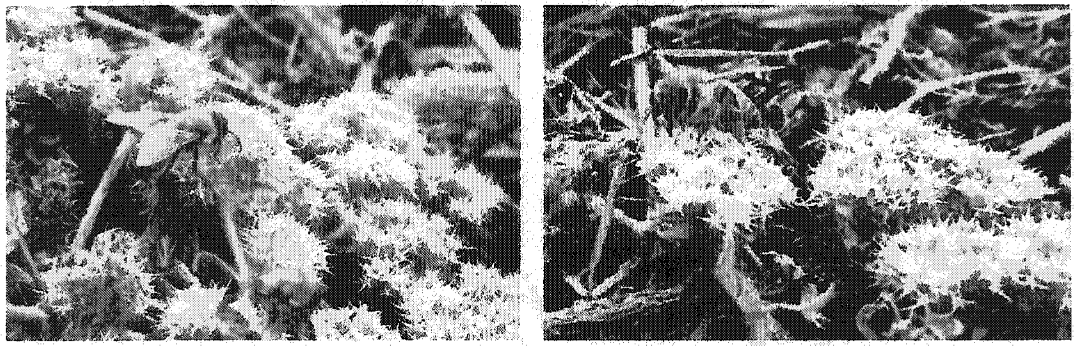
Photos: Pollinators

A. argentifera moth at Sunset



Aristotelia argentifera moth at Sunset

Dasytidae beetle at Sunset



Apis mellifera feeding on robust spineflower at Sunset State Beach

Bombus sp. at Sunset

Bombus sp. feeding on robust spineflower at Sunset State Beach

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