The Mating System of the Yreka Phlox (*Phlox hirsuta* E.E. Nelson)

Final Report

Prepared by

C. S. Ferguson, J. Filipski & K. Donham
Southern Oregon University
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Introduction

Phlox hirsuta (E.E. Nelson) (Polemoniaceae) is endemic to ultramafic soils ("serpentine") in the vicinity of Yreka in Siskiyou County, California. It is known from only five occurrences, and is listed as Endangered by both the State of California and the U.S. Fish and Wildlife Service (USFWS 2000). Information on the biology of *P. hirsuta* is needed, and research on its pollination and reproductive ecology has been identified as an important component of its recovery strategy (USFWS 2004). Detailed studies on the pollination biology of *Phlox* species are limited, and no information on any aspect of the pollination biology of *P. hirsuta* was available prior to this research.

The goal of this project was to develop a better understanding of the reproductive biology of *P. hirsuta* in order to help direct conservation and management plans for the successful recovery of the species. The major objectives of the research were to: 1) determine the mating system of *P. hirsuta* and 2) identify primary pollinators and determine their role in *P. hirsuta* seed set. Ancillary objectives of the research included characterizing the floral phenology and the pollen of *P. hirsuta,* and identifying seed viability and germination requirements of *P. hirsuta*.

Effective management of a rare plant species requires knowledge of its mating system (Kearns & Inouye 1997). Brown (1990) describes five basic types of mating systems in flowering plants: 1) predominantly selfing or autogamous, where flowers can be fertilized by their own pollen or by pollen from other flowers on the same plant; 2) predominantly outcrossing or xenogamous, where flowers can be fertilized only by pollen from a different plant; 3) mixed mating, where both selfing and

outcrossing occurs; 4) partial selfing of gametophytes, and 5) partial apomixis, where flowers produce fruit without sex through agamospermy or vegetative reproduction**.**

Most outcrossing plants rely on insects to transfer their pollen. Plant mating systems are affected by environmental factors and may vary among populations of a species, among individuals of a population, or between years in the same population (Barrett 1990). For example, a population may be primarily out crossing one season and primarily selfing the next season, if pollinators are limited.

Mating systems for members of the Polemoniaceae family are diverse (Grant 1961), including all five types described by Brown (1990). Mating systems for *Phlox* species include out crossing in *Phlox divaricata, P. bifida, P. glaberrima* (Grant & Grant 1965, Wiggam-Harper 2003) and selfing in *P. oklahomensis* (Springer & Tyrl 1989).

Animals known to pollinate *Phlox* species are also diverse. *Phlox* pollinators include butterflies, moths, flies, bees, beetles and hummingbirds (Grant & Grant 1965, Springer & Tyrl 1989, Scott 1997, Wiggam-Harper 2003).

Species Description

Phlox hirsuta is a semi-woody perennial that grows in compact clumps (5 to 15 cm high) and produces numerous showy pink to white flowers (Fig. 1). The stems and leaves are hirsute (hairy). Individual flowers consist of a tubular hirsute calyx and a salverform (trumpet-shaped) corolla tube (10 –13 mm long) (Draft Recovery Plan 2004). Nectar is produced in the base of the corolla tube (Grant & Grant 1965). Five

stamens are inserted at different levels in the corolla tube. The anthers dehisce longitudinally and produce yellow pollen. *Phlox hirsuta* pollen has not been described. The pistil is attached to the calyx and located halfway down the corolla tube and the style terminates with three stigmas where pollen is deposited. The ovary contains three carpels with one ovule per carpel. The resulting fruit is a capsule.

Study sites

All *P. hirsuta occurrences* are located near the city of Yreka in Siskiyou County, California (Fig. 2). Two of the five known *P. hirsuta* occurrences were chosen as study sites. These sites, China Hill and Cracker Gulch, have fairly dense populations of *P. hirsuta*, which was desirable for pollinator observations.

China Hill. The China Hill occurrence includes 1,000-3,000 *P. hirsuta* plants scattered over 19 ha (USFWS 2004). This site is approximately 1.6 km northeast of downtown Yreka (Fig.2). China Hill consists of exposed, open serpentine ridges and slopes ranging from 850 m to 900 m. China Hill is adjacent to an insular portion of Great Basin Province plant communities (sagebrush steppe and juniper savanna) (USFWS 2004).

A 20m x 20m plot (N = 41°44’32.9”, W= 122°36’56.8”) was established within the China Hill occurrence in March 2004. The plot is on the southwest facing side (aspect 240°) of a ridge, and adjacent to a dirt road with an elevation of 867 m. There are 195 *Phlox hirsuta* plants within the plot. The plot does not contain any trees or tall shrubs but woodlands dominated by *Juniperus occidentalis* (western juniper)

and *Ceonothus cuneatus* (buckbrush) grow on surrounding hills. Vegetative cover was estimated using a USDA Forest Service visual technique. Total vegetative cover in the China Hill plot was approximately 48%. The dominant shrubs (comprising 40% cover of the plot) are *Eriogonum sphaerocephalum* var. *halimoides* (wild buckwheat) (35%) and *Chrysothamnus nauseosus* (rabbitbrush) (5%). The dominant herbs (approximately 8% cover) are annual grasses such as *Bromus tectorum* (2-6%) and *Vulpia microstachys* (1-4%), and perennial grasses (4%) such as *Elymus multisetus* and *Festuca idahoensis.* Other commonly occurring herbs include *Eriophyllum lanatum* (1%), *Eriogonum strictum* (1%), *Phacelia corymbosa*, and *Lomatium macrocarpum.* Appendix A contains a detailed list of plants occurring at the China Hill site.

Cracker Gulch. The Cracker Gulch occurrence is 11.3 km southwest of the City of Yreka, in the Yreka Creek drainage, on the south side of Highway 3 (Fig. 2). The Cracker Gulch occurrence does not contain as many plants (estimated 200 to 300 plants) or nor occupy as much area as the China Hill occurrence. This site is characterized by a mixture of more developed serpentine soils. A detailed description of the soils at Cracker Gulch and China Hill can be found in the local USDA soil survey (Soil Conservation Service 1983) or the Draft Recovery Plan for *Phlox hirsuta* (USFWS 2004). The occurrence is on a ridge top that flattens into a long bench, and the plant community is *Pinus jeffreyi* (Jeffrey pine*)* woodland.

A 10 m x 40 m plot (N = 41°40’4.4”, W = 122°43’2.7”) was established within the occurrence April 2004. The bench has a slight convex shape and its elevation is

1306 m. The plot begins approximately 50 m from the dirt road. There are an estimated 294 small *P. hirsuta* plants in the plot.

Vegetative cover in the plot is 38% and the remainder consists of bare soil and exposed rock. The canopy cover (15%) is comprised of *Pinus jeffreyi (*Jeffrey Pine) (10%), and *Calocedrus decurrens* (incense cedar) (5%). The minimal shrub layer (2.5% cover) consists of *Cercocarpus ledifloius* and *Chrysothamnus nauseosus.* The herb layer (35% cover), while dominated by grasses, is quite diverse. *Minuartia douglasii* (3%), *Chlorogalum sp.* (1%), and *Eriogonum strictum* (2%) are some of the more common herbs. Appendix A provides a plant list of this site.

In 2005, an HOBO Micro Station® data logger was placed adjacent to the China Hill study plot in early March to monitor air temperature and wind. The data logger was mounted to a mast approximately 0.5 m above the ground (Fig. 3). The logger recorded air temperature and wind readings every 30 minutes until June 8th. The data was downloaded with a Palm Pilot using Hand Car EX® software and transferred to computer program BoxCar Pro®. The minimum, maximum, and mean wind and temperature data was determined for each day of the field study and used as an aid in comparing insect activity observations.

Methods

Phenology and Mating System(s)

In each plot at each study site, 40 randomly-selected *Phlox* plants were tagged with standard aluminum plant tags (nailed in the ground adjacent to a plant). Plants were tagged in March 2004 at China Hill, and in April 2004 at Cracker Gulch.

At both locations, 20 of the selected plants were covered with pollinator exclusion bags (bagged) and 20 of the selected plants were not covered (unbagged) and therefore accessible to potential pollinators. Pollinator exclusion bags were constructed of white nylon mesh (mesh size of 0.7mm x 0.4mm) with an elasticized band around the opening. The bags were placed over two wire loops secured in the ground to form a small dome over the plant (Fig.4). The bag openings were secured with metal stakes and rocks. Pollinator exclusion bags remained over plants during the entire bloom period, from just before initial bloom to one week after blooming ceased. In 2004, bags remained on plants at China Hill from mid-March to mid-May and on plants at Cracker Gulch from early April until late May. In 2005, the same *Phlox* plants were again used. However, at each site half the plants from the 2004 bagged treatments were unbagged, and half the plants from the unbagged treatments were bagged. This was done to minimize sampling error (minimizing the possibility that only individuals capable of a particular mating system were chosen) and to minimize bagging effects. In 2005, bags remained on plants at China Hill from late February to early June and on plants at Cracker Gulch from mid-March to early June.

Phenology, or the timing of developmental events, was monitored on the 20 bagged and 20 unbagged *Phlox* plants at each site from bud to fruit set in 2004 and 2005. The number of buds, number of flowers in anthesis (open with mature pollen released from anthers), number of capsules, and presence of pollen was recorded for each tagged plant several times per week. At each site, the beginning, ending, and peak of the flowering season were determined by calculating the percentage of total

flowers in bloom from all sampled plants, each day phenology was monitored. A seasonal phenology curve depicting peak bloom (defined as the majority of flowers in anthesis) was generated for both years. Floral damage due to herbivory and the presence of insects on or inside flowers were also recorded.

The total reproductive yield for each tagged plant was determined each season by counting the total number of flowers, fruits, and seeds produced per plant. Total number of flowers produced by a given plant was determined by counting the semi-persistent calyces. Plants were monitored for developing fruits (capsules) for several weeks after the removal of exclusion bags. The total number of fruits on the plants was counted prior to bagging them for seed collection. As fruits began to swell and turn from green to brown, small white mesh bags were fastened around individual plant stems to capture seeds as fruits dehisced. Bags were used to allow seeds to attain full maturation on the plant and to ensure that all seeds from a plant were collected and counted.

Three types of bags were used for seed collection: 1) paint strainer bags made of nylon and mesh size of 0.7mm x 0.4mm, attached to stems by cinching an inlaid cotton drawstring, 2) bridal sachet bags made of nylon and mesh size of 0.3mm x 0.3mm, attached to stems by an inlaid nylon drawstring, and 3) homemade bags made of organdy material and mesh size of 0.2mm x 0.2mm, attached to plant stems with a wire twist tie.

Seed collection bags were on plants at China Hill for approximately 35 days in May-June of each sample year. At Cracker Gulch, bags were on for approximately 25

days in May-June of 2004, and 40 days in June-July of 2005. Collected seeds were transported to the SOU lab, where they were manually cleaned and counted. Seeds from each tagged plant were placed in paper envelopes and stored in a dry, dark place at room temperature (approximately 18.3°-29.4°C) for a fourteen-week after-ripening period in 2004 and a ten-week after-ripening period in 2005.

The total number of flowers, fruits and seeds produced in each treatment (bagged and unbagged) at each site in each year were calculated as well as the percentage of fruit and seed set. The mean number of flowers, fruits, and seeds in each treatment was calculated and compared within a site for each year using a Student's T test ($p < 0.05$). The mean number of flowers, fruits, and seeds in each treatment was compared between sites in 2004 and 2005 using a Student's T test.

Hand-Pollination Experiment

In 2005, an additional 15 *Phlox* plants (outside but adjacent to the established plot) were randomly selected at China Hill for a hand-pollination experiment to better define the mating system(s) of *P. hirsuta*. The plants were tagged with aluminum tags. Each tagged plant received four treatments: 1) unbagged (open control for self-incompatibility), 2) bagged (control for self-compatibility), 3) bagged & selfed (receiving pollen from other flowers on same plant to test for geitonogamy- another form of self-compatibility), and 4) bagged & outcrossed (receiving pollen from other plants). For the three-bagged treatments, small mesh bridal sachet bags were attached to individual shoots on the plant for the duration of the bloom period (Fig. 5).

For the hand-pollination treatments a small scissors and forceps were used to transfer pollen. The corolla tube was cut lengthwise, which allowed access to the stigma. In the "bagged and selfed" treatment, the anthers in flowers were emasculated (pulled off with forceps), forceps cleaned with ethanol, and an anther with dehiscing pollen from another flower on the plant was pulled off with forceps and lightly brushed over the stigmatic lobes of the flower being hand-pollinated. The same procedure was followed for hand-pollinating the outcrossed treatment, except that dehiscing anthers from other *P. hirsuta* plants (5m-20m away) were used. Phenology (flower set) and fruit and seed set were recorded and seed collection was performed in the same manner as in the pollinator exclusion experiment.

Fruit and seed set were determined for each of the four treatments. The mean number of flowers, fruits, and seeds were compared between treatments using Analysis of Variance (ANOVA). Ninety five percent confidence intervals were calculated and compared between pairs of treatments: bagged to bagged and selfed, and unbagged to bagged and outcrossed.

Insect Observations and Potential Pollinators

During each field season, insect activity was recorded at China Hill and Cracker Gulch regularly (3-5 times/week and 1-2 times/week, respectively) throughout *P. hirsuta* bloom. The identity of the insect (Order, Family) and its behavior (flying by, basking, visiting flowers) was noted and local weather conditions. When insects were observed visiting *P. hirsuta* flowers (spending at least 5 seconds over a flower),the amount of time probing an individual flower, and the occurrence

and pattern of floral visits (consecutive visits to different flowers on one plant or consecutive visits between different *Phlox* plants or between *Phlox* and other plant species) were recorded. In addition, the presence of insects on or inside flowers were also recorded during the phenology checks.

In 2004, a total of 128 hours and 29 hours were spent observing insect activity at China Hill and Cracker Gulch, respectively. Observations were performed during four discrete 2 hour time periods: CAM - Crepuscular AM (1 hour before and after sunrise), DAM - Diurnal AM (10-noon), DPM - Diurnal PM (2-4), and CPM - Crepuscular PM (1hour before and after sunset). These time periods were used to monitor differences in the level of insect activity throughout a day and to identify potential pollinators that may exhibit periodicity in their floral visits. In 2005, a total of 180 hours and 41 hours were spent observing insect activity at China Hill and Cracker Gulch, respectively. Insect observations in 2005 were not restricted to the four 2-hour time periods and included some night observations between sunset and 10 PM using a black light (to attract night-flying insects). The intent in 2005 was to concentrate insect observations during time periods when insects were most active.

Representatives of the insects observed in the sites were collected using a standard insect aspirator or aerial net. We attempted to collect all insects visiting *P. hirsuta*. Collected insects were processed (pinned and labeled) and stored in a permanent reference collection at the Southern Oregon University (SOU) Insect Museum. In addition, flying insects were continuously monitored at each site using 16 sticky traps - 7.6 cm by 12.7 cm yellow plastic cards coated on both sides with an

odorless sticky substance (Tanglefoot®). Traps were positioned vertically in a metal holder approximately 15 cm off the ground. Traps were collected weekly and examined for insects carrying *Phlox* pollen under a stereo dissecting scope (Zeiss® Stemi 2000).

Pollen from pinned insects was removed by either dripping 70% ethanol on the specimen's mouthparts held over a watch glass or by detaching the proboscis with tweezers and placing it in a watch glass with 70% ethanol. The resulting solution in the watch glass was pipetted onto a slide and checked for pollen under a Leica® microscope. Pollen grains were compared to the pollen reference collection to determine if *Phlox* pollen was present.

The proboscis of a representative specimen of each Lepidopteran species observed probing *P. hirsuta* was uncoiled, air-dried, measured and photographed using Spot® digital image software. The probosci lengths were compared to the length of *P. hirsuta* corolla tube to determine if the insect was capable of accessing the nectar at the base of the corolla tube.

During the phenology checks in 2004, several common insects, were noted with *Phlox* pollen attached to their bodies. These insects included thrips (Order Thysanoptera) and beetles (Order Coleoptera, Families Chrysomelidae and Meloidae). Therefore, in 2005, two *Phlox* plants within the study plot at China Hill were monitored to determine if these non-lepidopteran insect visitors moved pollen within a *P. hirsuta* plant or between *P. hirsuta* plants. Powdered blue analogue dye (Shannon Luminous Materials) was applied with 30 micro liter pipettes. On the first

plant, dye was applied to a total of 9 flowers during the bloom period. A white paper-covered twist tie at the base of the stem identified the treated flowers. The entire plant was covered with a pollinator exclusion bag during the duration of the study. Over the bloom period, 36 untreated flowers were examined with a blacklight for evidence of pollen analogue. A second *Phlox* plant was left unbagged and each of the 123 flowers were treated with blue analogue dye as they bloomed. Five yellow sticky traps (7.6 cm by 12.7 cm yellow plastic cards coated on both sides with an odorless sticky substance Tanglefoot®) secured on wire holders were placed around the perimeter of the plant. The cards were changed weekly between March 30 and June 2 and examined under a stereo dissecting scope (Zeiss® Stemi 2000) for insects with dyed pollen.

Pollen Identification

Phlox hirsuta pollen and pollen from all plants blooming coincidentally with *P. hirsuta* were collected from both China Hill and Cracker Gulch. Flowers were collected, stored in paper envelopes, and transferred to the SOU research laboratory. Anthers were removed from dried flowers using forceps and suspended in a 10% glycerol solution. The glycerol solution was agitated to loosen pollen from the anthers. Pollen was then examined under a compound microscope and photographed with Spot RT ™ digital image software at 63x in Phase Contrast and Differential Interference Contrast (DIC) modes. Permanent voucher pollen slides were made using Polyvinyl-Lacto-Glycerol (PVLG) and reside at SOU. These slides were used to positively identify *Phlox* pollen on insects collected at both field sites.

Seed Viability

The viability of *P. hirsuta* seeds was assessed with two techniques: a chemical test using tetrazolium chloride (TTC) (Kearns & Inouye 1997) and a direct measurement of germination rate. Both tests were performed on *P. hirsuta* seeds collected from monitored plants at China Hill. Seeds from Cracker Gulch were only tested with TTC due to limited seed production at that site. Sample sizes depended on the number of seeds produced in each treatment.

The TTC test used 100 randomly selected seeds from the 538 seeds collected from the unbagged plants at China Hill, and 25 randomly selected seeds from the 51 seeds collected from unbagged plants at Cracker Gulch. In addition, the only seed produced by the bagged *Phlox* plants was also tested. All tested seeds were placed on Whatman® number 1 filter paper in sterile 100 cm^2 Petri dishes (25 seeds/Petri dish) and moistened with distilled water. Petri dish lids were sealed with Para film® for 24 hours at room temperature (20 C°) (imbibing period). After the imbibing period, each seed was bisected along the long axis with a razor blade and placed in dry 100 cm^2 sterile Petri dishes. The bisected seeds were covered with 0.1% TTC solution. Staining was assessed after 4 hours and again after 24 hours. Petri dishes remained open during the first 4 hours and additional TTC was added as needed. Petri dishes were covered for the 24-hour sample period. The resulting staining pattern on each seed was visually assessed (The Association of Official Seed Analysis, www.aosaseed.com). Seeds were considered viable if they were stained entirely pink-red or at least had a completely stained radicle with the majority of the embryo stained. Seeds were considered not

viable if they were entirely green and unstained, had an incompletely stained radicle, or had purple-blotchy stains (evidence of bruising/damage).

As the TTC test can sometimes yield false positive results, germination rates were also used to assess seed viability. Germination was assessed on 400 seeds collected from the unbagged *Phlox* plants at China Hill. Seeds were divided into 4 groups of 100 seeds each, and a 12-week cold/moist stratification period to break dormancy was begun. Each group of 100 seeds was dispersed in a plastic Ziploc bag containing unpacked peat moss moistened with 75 ml distilled water. Sealed bags were left at room temperature for 4 hours to allow seeds to imbibe and then transferred to lay flat on a wire rack in the laboratory refrigerator (3° - 8°C for the first week, 1°-5°C for the second week). Originally, each group was to receive a second pre-germination treatment ($KMNO_3$). However, the results did not warrant a second treatment. A germinating seed was defined as a seed with a ruptured seed coat with a visible radicle tip emerging.

In 2004, all germinated seeds were planted in a 1:1 mixture of sandy/loam and potting soil in the Southern Oregon University Greenhouse. Ninety-six seeds were sown in individual cells of six-pack trays filled with approximately 2.54 cm of soil and 241 seeds were sown 0.5 cm apart in plastic flats (12.7 X 27.9 cm) with approximately 5 cm of soil. All seeds were watered sparingly, once every 5 to 7 days and fertilized once with Osmocot®.

In 2005, pollinator excluded or bagged plants at China Hill produced a total of 14 seeds compared to the one seed produced in 2004. Germination of the 14 seeds

from the bagged plants and 14 randomly selected seeds from unbagged plants was assessed using the same protocol described for 2004.

Results

Phenology

In 2004, *Phlox hirsuta* flowering began March 22 and ended May 13 at the China Hill site. The total duration of *P. hirsuta* bloom at China Hill in 2004 was 7.5 weeks and peak bloom (defined as the majority of flowers in anthesis) occurred in mid -April (Fig. 6). In 2005, *P. hirsuta* began flowering at China Hill the second week of March and ended the last week of May. The total duration of bloom at China Hill in 2005 was 10 weeks and peak bloom occurred in mid-April (Fig. 7).

At Cracker Gulch in 2004, *Phlox hirsuta* flowering began April 2 and ended 7 weeks later on May 23 (Fig.8). Peak bloom at the Cracker Gulch site occurred in late April to early May. However, the unbagged plants exhibited two smaller peak bloom periods, first in mid- April and secondly in late- April (Fig 8). In 2005, *P. hirsuta* began flowering at Cracker Gulch in early April and ended 10 weeks later in mid-June (Fig 9). Peak bloom occurred between early and mid-May. Bagged and unbagged plants at Cracker Gulch exhibited very similar phenology curves in 2005 (Figs. 9). In 2005, peak bloom was 2 weeks later than in 2004, and the overall bloom period lasted longer.

In general, *Phlox hirsuta* (bagged & unbagged plants combined) bloomed earlier at China Hill than Cracker Gulch in both years (Figs. 10 & 11). In. 2004, *Phlox*

at China Hill reached peak bloom two weeks earlier than Cracker Gulch (Fig. 10) but bloom ended the same time at both sites. The overall bloom period for *Phlox* was longer at China Hill in 2004. In 2005, peak bloom occurred almost 5 weeks later at Cracker Gulch and bloom ended two weeks later than China Hill. The overall bloom period for *Phlox* was approximately 10 weeks at both sites in 2005.

Mating System(s): Pollinator Exclusion

In 2004, the total number of flowers produced on the 40 monitored plants (bagged and unbagged) at China Hill (2,718) was approximately ten times the total number produced on 40 plants at Cracker Gulch (265). On unbagged plants in 2004, the percentage of flowers producing fruit was 49.6% and 37% at China Hill and Cracker Gulch, respectively. In 2005, the percentage of fruit set by unbagged plants was 29% and 15% at China Hill and Cracker Gulch, respectively. In contrast , the fruit set in bagged plants was negligible. At China Hill, fruit set was 0.06% in 2004, and 0.74% in 2005. No fruits were produced in the bagged plants at Cracker Gulch in 2004 or 2005. Fruit production is summarized in Table 1. Lack of fruit production in bagged plants suggests either pollen was not transferred between flowers in those plants, or if pollen was transferred it was self- incompatible.

In contrast to fruit set, the percentage of fruits that produced seeds was high at both sites in both years. In 2004, seed set in unbagged plants was 94.7% and 78.5% at China Hill and Cracker Gulch, respectively. Comparable seed set was obtained in unbagged plants in 2005, 90.1% and 69.2%, at China Hill and Cracker Gulch respectively. Seed set was 100% from the only fruit produced in bagged plants over

both sites in 2004 and lower, 66.7%, in bagged plants at China Hill in 2005 (Table 1). In both years, all fertile *P. hirsuta* fruits produced only a single seed, even though each *Phlox* flower has the potential to produce a maximum of three seeds.

In 2004, unbagged plants at China Hill produced a mean of 57.3 flowers (range 0-118), 28.4 fruits (range of 0-116), and 26.9 seeds (range of 0-116) while bagged plants produced a mean of 78.6 flowers (range of 0-275), 0.05 fruits (range of 0-1), and 0.05 seeds (range of 0-1) (Table 2). The mean number of fruits and seeds were significantly different ($p < 0.05$) between unbagged and bagged plants at China Hill in 2004. In 2005, unbagged plants at China Hill produced a mean of 78.6 flowers (range 7-283), 23.2 fruits (range of 0-71), and 20.9 seeds (range of 0-67) while bagged plants produced a mean of 141.9 flowers (range of 5-509), 1.05 fruits (range of 0-6), and 0.7 seeds (range of 0-5) (Table 2). The mean number of flowers, fruits and seeds was significantly different ($p < 0.05$) between unbagged and bagged plants at China Hill in 2005.

The mean number of flowers, fruits, and seeds in each treatment, unbagged and bagged plants, was compared between years for China Hill. There was no significant difference ($p < 0.05$) between mean number of flowers, fruits and seeds in unbagged plants at China Hill. However, the mean number of flowers, fruits and seeds was significantly different ($p < 0.05$) in bagged plants between years

In 2004, unbagged plants at Cracker Gulch produced a mean of 8.7 flowers (range of 0 to 42), 3.25 fruits (range of 0-21), 2.55 seeds (range of 0-17) while bagged plants produced a mean of 4.60 flowers (range of 0-30), 0.00 fruits, and 0.00 seeds

(Table 2). Almost half (48%) of the forty plants monitored at Cracker Gulch in 2004 produced no flowers. Adams (1987) found 38% of *P. hirsuta* plants at a similar site (Soap Creek Ridge) produced no flowers. In 2005, unbagged plants at Cracker Gulch produced a mean of 25.60 flowers (range of 0 to 82), 3.90 fruits (range of 0-25), 2.7 seeds (range of 0-19) while bagged plants produced a mean of 43.30 flowers (range of 0-141), 0.00 fruits, and 0.00 seeds (Table 2). The mean number of fruits and seeds was significantly different ($p < 0.05$) between unbagged and bagged plants at Cracker Gulch in 2004 and 2005.

Within a given treatment, the mean number of flowers per plant varied from year to year at Cracker Gulch. However, the mean number of fruits and seeds produced within a given treatment was not significantly different ($p < 0.05$) between years.

In summary, even when *Phlox hirsuta* plants were accessible to pollinators (unbagged), fruit set was not particularly high at either site in 2004 or 2005. However, at both sites and in both years, fruit set was significantly lower in *Phlox* plants where pollinators were excluded (bagged) for the duration of the bloom period . If flowers were successfully pollinated and produced fruit there was a moderate to high likelihood that mature seeds would be produced. It appears that *P. hirsuta* requires a pollen vector to move pollen between plants to produce fruits and set seed. The pollinator exclusion data suggests that *P. hirsuta* flowers are not autogamous.

Mating System(s): Hand Pollination

Table 3 shows fruit and seed set for each treatment. Since total number of flowers on individual treatment shoots varied, percentages of flowers producing fruits and percentages of fruits producing seeds were used to calculate fruit and seed set per treatment. The highest fruit set (49.6%) occurred in flowers on shoots that were hand-pollinated with outcrossed pollen. Flowers on unbagged shoots had 26% fruit set. In contrast, flowers on shoots that were bagged or bagged and hand-pollinated with self-pollen produced no fruits. Eighty percent of fruits produced by bagged and outcrossed flowers produced seeds, and 87%, of fruits produced by unbagged flowers produced seeds. These results provide additional evidence that *P. hirsuta* is primarily outcrossing, and may even be exclusively outcrossing. In particular, self-compatibility is not supported because flowers that were hand-pollinated with pollen from flowers on the same plant did not produce seeds.

The mean number of flowers, fruits, and seeds were compared between treatments using Analysis of Variance (ANOVA). There was significant difference in fruit set between treatments ($F = 47$; $df = 3,56$; $p < 0.0001$) and in seed set between treatments ($F = 32$; $df = 3,56$; $p < 0.0001$) (Table 4).

The confidence intervals (95%) for each mean seed set were calculated and compared to determine which pairs of treatments were significantly different. The bagged and bagged & selfed treatment were not significantly different but both were significantly different from the unbagged and bagged & outcrossed treatments. The unbagged and bagged & outcrossed treatment mean seed set differed but the 95% confidence intervals associated around the means overlapped slightly suggesting that

the difference in seed set between these two treatments was only marginally significant.

Insect Observations and Potential Pollinators

In 2004, a total of 921 insects were recorded during 128 hours of observation at China Hill. Ninety-one percent of the insects observed were from three orders: Diptera (16.9%), Hymenoptera (43.6%) and Lepidoptera (30.8%). (Table 5). Other orders represented included Coleoptera, Orthoptera, Hemiptera, Odonata, and Neuroptera. Insects were present during all four discrete time periods (CAM, DAM, DPM, and CPM). When insect observations were compared across all dates in 2004, the majority of insects were observed during the DPM time period (53.5%) followed by the DAM time period (26.2%). However, more observation hours took place during DPM across all dates.

When a comparable number of observation hours in each time period were compared during *Phlox* peak bloom (April 6 to 15th), the majority of insects (39.1%) were still observed during DPM, followed by DAM (31.2%) (Table 6). Fewer numbers of insects were observed during the Crepuscular time periods (15.6% in CAM, 14.1% in CPM) (Table 6). Both air temperature and wind speed would impact insect activity. Mean air temperature for each time period from April 6 to 15th revealed the lowest mean temperature during CAM (5.37 °C) and the highest mean temperature during DPM (18.23° C). Mean air temperature during CPM (15.53° C) was slightly higher than the mean temperature during DAM (13.32°) but it also coincided with the highest mean wind speed, 18.42 km/hr, compared to 8.32 km/hr at DAM

(www.wunderground.com). Some insect families, for example, Sphingidae, were observed more frequently during crepuscular time periods.

In 2004, overall insect activity at China Hill was low, less than 8 insects recorded during an hour of observation. Insect visits to *Phlox hirsuta* flowers were rarely observed. Only 36 or 4% of the 921 insects observed at China Hill were recorded probing *Phlox* flowers. 88.9% of these visitors to *Phlox* were Lepidopterans. Most (61.1%) of these lepidopteran visitors were in the Sphingidae family (Table 7). Other lepidopteran visitors included members of the families Hesperiidae, Nymphalidae, and Papilionidae. One hymenopteran (family Apidae (*Bombus*)) was observed probing *Phlox* in 2004.

A total of 95 insects were recorded at Cracker Gulch over 29 hours of observations in 2004. No pattern of insect activity was discernable. Only two insects, one Lepidopteran (Hesperiidae) and one Hymenopteran (Apidae), were observed visiting *Phlox* at Cracker Gulch (Table 7).

In 2005, insect observations were not restricted to the four discrete time periods. Instead, most observations occurred during longer periods of time in the afternoon, early evening and night (until 10 PM). Approximately 1,600 insects were recorded during 180 hours of observations at China Hill. A total of 129 insects (8.1% of the total observations) at China Hill were observed probing *Phlox* flowers. Most (125 or 96.8%) of these probing insects were lepidopterans (Table 7), largely represented (110 or 89.4% of these observations) by the painted lady butterfly (*Vanessa cardui*, family Nymphalidae). Other insect visitors to *Phlox hirsuta* included

the lepidopteran families Hesperiidae, Sphingidae, Papilionidae, and Pieriedae, and a single hymenopteran family, Apidae (Table 7). At Cracker Gulch, 415 insects were recorded during 41 hours of observation during 2005. Only five insects were observed visiting *Phlox* (Table 7).

The proboscis lengths of the main lepidopterans observed probing *Phlox hirsuta* were measured to determine if they were long enough to reach the nectar at the base of the flower's 10-12 mm corolla tube. The insects and their proboscis lengths are as follows:

Insect	Proboscis Length
Proserpinus clarkiae	11 mm
Hyles lineate	35 mm
Vanessa cardui	10-12 mm
Unknown (Family Hesperididae, subfamily Hesperiinae)	10 mm

In 2004, all collected lepidopterans were examined for pollen and individual pollen grains on the proboscis counted. The relative pollen load was calculated as follows: low (1-20 pollen grains), medium (20-50 grains), and high (over 50 grains). All collected Sphingids, *Proserpinus clarkiae* and *Hyles lineata* carried high loads of *Phlox* pollen and no pollen from any other plant species. Two specimens of *Hesperia juba* (Subfamily: Hesperiinae) also carried *Phlox* pollen exclusively but the loads were relatively low. Other lepidopteran examined (*Erynnis sp.*, *Nymphalis californica*, *Pontia sp.*, *Pieris sp.*, and one unidentified moth) did not carry *Phlox* pollen.

In 2005, all collected Lepidopterans (100 specimens) were examined for *Phlox* pollen and thirty-five of these were confirmed to have *Phlox* pollen (Table 8). The Sphingids only carried *Phlox* pollen and always in high amounts. Almost 50% of the Nymphalids (*Vanessa cardui*) were carrying *Phlox* pollen. Pollen loads on all *V. cardui* were smaller than pollen loads on Sphingids. All Hesperiinae specimens also carried low *Phlox* pollen loads. Other Lepidopterans carrying *Phlox* pollen included *Erynnis propertius, Papilio zelicaon, Pontia sisymbrii, Pieris rapae, Cissusa discreta, Drepanulatrix unicalcararia* and *Plataea trilinearia* (Table 8). Of these, only *Papilio zelicaon* had a high pollen load.

Other Lepidopterans examined but not carrying *Phlox* pollen included one Hesperiidae, one *Nymphalis californica,* and 18 moths in the Noctuidae and Geometridae families (collected while black lighting). The only non-Lepidopteran insect carrying *Phlox* pollen was a bumblebee, *Bombus vosnesenskii.* This specimen was carrying four *Phlox* pollen grains in addition to pollen from *Lomatium macrocarpum* and *Phacelia corymbosa*. Other insects that were collected from *P. hirsuta* and not found to have *Phlox* pollen included three Dipterans, one Muscidae and two Syrphidae.

In both 2004 and 2005, few insects were observed probing *P. hirsuta* at Cracker Gulch, and consequently, few specimens were collected. No specimens with *Phlox* pollen were recorded from Cracker Gulch in 2004 and only two Lepidopterans, *Hesperia columbia* and *Euphydras editha* in 2005 (Table 8). Other Lepidopterans

examined but not carrying *Phlox* pollen included four *Nymphalis californica,* four *Euphydras editha,* one *Pontia sp.,* and two *Autographa californica*.

In 2005, two *Phlox* plants within the study plot at China Hill were monitored to determine if non-lepidopteran insect visitors moved *Phlox* pollen, dyed with blue analogue dye, within a *P. hirsuta* plant (bagged) or between *P. hirsuta* plants (unbagged). The bagged plant in this experiment produced a total of 45 flowers in addition to the flowers with dyed pollen. Zero of the 45 flowers contained pollen analogue under blacklight illumination. No fruit set occurred in this plant. The unbagged plant in this trial produced a total of 123 flowers all of which had blue analogue dye applied to them. No pollen analogue was detected on the 1,228 insects captured on sticky traps surrounding this plant.

Pollen Identification

Bloom periods for a number of plants at China Hill (Table 9) and Cracker Gulch (Table 10) show considerable overlap with the bloom period of *P. hirsuta.* Permanent voucher slides of pollen from all coincidentally blooming plants were made and reside in the SOU Insect Museum (Appendix B). These pollen slides were used to positively identify *Phlox* pollen on insects collected at both field sites

Phlox hirsuta pollen has unique morphological characteristics, which made it distinguishable from other pollen collected at both sites, except for that of *Phlox speciosa* (Appendix B). *Phlox hirsuta* pollen grains have a mean diameter of 42 μm (range of 39-45 μm), and are spheroidal, polyporate (many apertures [circular sunken pores] spaced equidistantly over the surface), and have a polygonal reticulum

(sculpturing elements arranged in a network that has gaps) that surrounds pores (Fig. 12). *P. speciosa* pollen has a mean diameter of 35.5 μm (range of 32-39 μm) (Fig. 12). In all other respects, *P. speciosa* pollen looks identical to *P. hirsuta* pollen under a light microscope. Therefore, pollen from these two species could not be consistently separated in the laboratory.

Gilia capitata and *Linanthus sp. (like Phlox*, also members of the Polemoniaceae family) are found in the plot and also bloom coincidentally with *P. hirsuta* bloom. *Gilia* and *Linanthus* pollen grains are similar in shape (spheroidal) and size (35-40 μm) to *Phlox* pollen. However, they both have distinguishable features on the grain surface. *Gilia* pollen is zonocolporate (sunken furrows and pores arranged equidistantly around the equator of the grain) and the reticulum is striated instead of polygonal (Stuchlik 1967) (Fig. 13). *Linanthus* pollen has a polygonal reticulum that is obscured by pila (bump-like apertures) or muri (thin furrows) (Stuchlik 1967) (Fig. 13).

Seed Traits and Viability

In May of 2004, ten mature *P. hirsuta* capsules were collected from plants outside of the study site at China Hill. The capsules were examined under a dissecting scope. Eight of the ten capsules contained one seed each and two were empty. *Phlox hirsuta* seeds averaged 4.5mm in length (3.5-5.5mm range) and 2.5mm in width (1.5-3mm range). A linear-shaped embryo filled the center of the oblong seed (Baskin 2001). The green embryo was surrounded by white tissue, most likely a reduced endosperm (Atwater 1980).

Seed viability was tested with Tetrazolium Chloride in 100 of the 538 seeds collected at China Hill and 25 of the 51 seeds collected at Cracker Gulch in 2004. Results of seed viability tests with TTC indicated that 66% of the *P. hirsuta* seeds from China Hill and 76% of the seeds from Cracker Gulch were viable.

The seeds in the germination treatments (a total of 400 seeds from China Hill, 100 seeds per treatment) had slightly higher germination rates than viability rates of seeds in the TZ treatments. On the 9^{th} day of the cold/moist stratification period, several seeds in all 4 bags were observed to have emerging white radicle tips. Seeds continued to germinate for several more days. On day 21 and 28, seed viability was assessed by counting those seeds that had begun to germinate. 337 of the 400 seeds (84%) collected at China Hill germinated. .

Of the 337 germinating seeds planted out in the SOU greenhouse, 266 (79%) emerged from the soil and grew into seedlings but only 199 of these survived the next 6 weeks and developed 1 or 2 sets of true leaves (Fig. 14). Seed coats stuck to the

cotyledons of many emerging seedlings, impairing their growth and causing some mortality. Damp-off disease, a common fungal pathogen affecting seedlings in greenhouses, killed 35 seedlings. In December of 2004, Marla Knight (botanist, Klamath National Forest, took the remaining 199 seedlings to a professional nursery, and care of the plants was taken over by the National Forest and the USFWS.

In 2005, small samples of seeds from bagged plants and unbagged plants at China Hill underwent the same cold/moist stratification as in 2004. Radicle tips began to emerge from seeds beginning on the ninth day of stratification (as in 2004). On the 22nd day of stratification, 42% of the seeds from bagged plants had germinated and 71% of the seeds from unbagged plants had germinated.

Discussion

Phenology

Phlox hirsuta plants at both field sites presented flowers over an extended period, ranging from 7 to 10 weeks. In most plants, flowering time is primarily determined genetically and/or by environmental cues such as day-length, temperature, rainfall, and plant size or density (Kelly & Levin 2000). The longer blooming period in 2005 probably reflected the cooler and wetter weather in that year. In both years, peak bloom occurred later at Cracker Gulch than at China Hill, suggesting that elevation, plant size and population size may influence the flowering time of *P. hirsuta* populations (Kelly & Levin 2000, Hendrix & Kyhl 2000). The total number of days of anthesis for individual *P. hirsuta* flowers was only 2 to 3 days. However, the short duration of individual flowers was countered by the sequential blooming of large

numbers of flowers on an individual plant. The total number of flowers per plant was variable, ranging from 0 to 275 flowers, and appeared correlated to the plant size as evidenced by significantly fewer flowers produced on the smaller plants at Cracker Gulch. The flowering periods of several other plants in blooming coincidentally with *P.* hirsuta also tend to flower for six weeks or longer (Tables 9 and 10) .

The long bloom period in *P. hirsuta* may serve to increase chances of visitation by pollinators, and suggests that it is typically visited by a number of different pollinators as opposed to a specific pollinator. If a plant is insect-pollinated, the bloom period needs to coincide with the availability of the appropriate pollinator (Fenner 1985). Synchronized blooming among plants of the same species may be crucial for plants dependent on outcrossing but synchronized blooming with other plant species may create competition for pollinators. Based on field observations, *P. hirsuta* competes with several other plants (*Allium sp., Lomatium macrocarpum*) for the pollen vectoring services of various Lepidopteran species (Tables 9 and 10). Thus, while the long bloom period of *P. hirsuta* is advantageous for attracting pollinators, there is likely competition for pollinators among several long-blooming species at China Hill and Cracker Gulch.

Mating System(s)

The morphology of *Phlox hirsuta* flowers suggests self-incompatibility. Although, some of the anthers are physically close enough to the stigma for self-pollination to occur, the flowers are protandrous, meaning the anthers mature prior to the stigma becoming receptive. Protandry inhibits self-compatibility because the

stigma is less likely to receive pollen from the same flower since most pollen is gone by the time it is receptive (Kearns & Inouye 1993).

Within the large plots at each study area, zero (Cracker Gulch) or very few (China Hill) seeds were set in bagged *P. hirsuta* plants (significantly fewer than in unbagged plants at both sites). This result indicates that animal pollinators probably play a significant role in *P. hirsuta* pollination, and also suggests that P. hirsuta may be largely self-incompatible.

In the hand-pollination experiment, the use of pollinator exclusion bags in combination with the transfer of pollen between flowers on the same plant addressed the potential for geitonogamy, or interflower pollination (Kearns & Inouye 1993). *P. hirsuta* flowers receiving this treatment did not produce seed. Neither did the bagged and unmanipulated flowers. However, unbagged flowers and bagged and outcrossed flowers (hand-pollinated with pollen from anthers on a different plant) both produced seeds. The results of this experiment also strongly suggest that that *P. hirsuta* is primarily self-incompatible and requires a vector to transfer pollen between plants in order to set seed.

The few seeds produced by bagged plants at China Hill during the pollinator exclusion experiment indicate that selfing could be occurring in some *P. hirsuta* plants. Brown's (1990) mating system types are believed to exist in species on a continuum. For example, some species are facultatively xenogamous, or can outcross when pollinators are available and self-pollinate when pollinators are absent (Kearns & Inouye 1993). The mating system of a plant species can vary between years or

between populations, and individuals within a population may vary in the amount of selfing or outcrossing (Lyons & Antonovics 1991). For example, plants flowering near either end of the bloom period may be predominantly selfing while plants flowering during peak bloom may be predominantly outcrossing. The low incidence of insect visitors to *P. hirsuta* flowers over two seasons suggests *P. hirsuta* may be partially self-compatible. Self-compatibility is considered an adaptation to limited or ineffective pollinators and as a means of escaping competition for pollinators (Grant & Grant 1965, Proctor & Yeo 1973).

If *P. hirsuta* were self-compatible to some degree, there still needs to be a mechanism for the transfer of pollen to the stigma within a flower or between flowers on a bagged plant. Small insects could vector pollen on plants under exclusion bags (Kearns & Inouye 1993). In this study, insects, including thrips (Thysanoptera), small beetles (Chrysomelidae), leafhoppers (Membracidae), and spiders were observed inside bagged plants. In fact, a small amount of fruit predation occurred in bagged and unbagged plants (7.5 % of the plants) at both sites.

Another possible explanation for the observation of seed production on bagged plants is that the seeds actually resulted from pollen transferred between plants by small insects. The mesh size of the exclusion bags (0.7mm x 0.4mm) was small enough to exclude most insects still allowed access to some minute insects. Thrips (Thysanoptera) were consistently noted inside bagged plants. Thrips were observed on petals, inside the corolla tube and moving between flowers on monitored plants. In 2004, 260 thrips were counted on monitored *Phlox* plants at China Hill. Although not

normally considered pollinators, thrips were noted with pollen grains adhered to hairs on their bodies (Fig.15). Thrips are small (1.5 – 3mm) and can only carry a small number of pollen grains per individual, but may be effective pollinators if their densities are high enough. In addition, thrips are able to fly and were consistently present on the sticky traps deployed weekly within the plots. Thrips have been implicated in the pollination of a number of plants including *Lantana camara* (Verbenaceae) (Norton 1986), *Castilla elastica* (Moraceae) (Sakai 2001), *Macaranga hullettii* (Euphorbiaceae) (Moog *et al.* 2002) and *Arabidopsis thaliana* (Brassicaceae) (Hoffman 2003). However, in the small trial experiment using analogue dye on a *Phlox* plant, none of the 545 thrips examined on sticky traps surrounding the treated plant carried any *Phlox* pollen. In spite of the fact that some insects were present on bagged plants, very few seeds were set inside bagged plants supporting self-incompatibility in *P. hirsuta*.

Potential Pollinators of *Phlox hirsuta*

P. hirsuta appears to require a pollen vector to set seed. Animals, wind, and water can move pollen. Wind contributed to selfing in *Phlox oklahomensis* (Springer & Tyrl 1989), which grow in similar environmental conditions as *P. hirsuta*. It is doubtful that wind serves as an effective pollen vector to induce pollination in *P. hirsuta* because in spite of consistent afternoon winds at both sites, seed set was still low. In addition, pollen of wind-pollinated plants has a smooth, dry surface while entomophilous or insect-pollinated plants have sticky, highly ornamented pollen

(Proctor & Yeo 1973). *Phlox hirsuta* pollen grains have sticky oil bodies on their surface.

Based on experimental results, field observations, pollen type, and inspection of insects for pollen, insects are most likely the pollen vectors for *P. hirsuta*. Most other *Phlox* species outcross and are pollinated by insects, in particular lepidopterans (Grant 1961, Plitman & Levin 1990). However, few detailed pollination studies have been done on *Phlox* species with the exception of Springer & Tyrl (1989) and Wiggam-Harper (2003). *Phlox divaricata* was shown to be primarily xenogamous with some capacity for autogamy, and a Sphingid moth, *Hemaris diffinis* , was found to be its most effective pollinator (Wiggam-Harper 2003). In contrast, autogamy was determined to be the primary mating system of *Phlox oklahomensis* (Springer & Tyrl 1989). Insect visits to *P. oklahomensis* were rare, but its pollen was found on a *Hemaris diffinis specimen*.

The results from this study indicate that lepidopterans are the primary pollinators of *Phlox hirsuta.* Lepidopterans collected with *Phlox* pollen grains on their probosci included representative species from seven families including butterflies in Hesperiidae, Nymphalidae, Papilionidae, Pieridae and moths in Geometridae, Noctuidae, and Sphingidae. *Phlox hirsuta* appears to have a facultative plant-pollinator relationship, meaning it is not dependent on one insect species for pollination.

Sphingids were the second most frequent visitors to *Phlox hirsuta* and some were noted visiting *P. hirsuta* plants consecutively, a behavior that maximizes pollen

transfer. This behavior in combination with consistent high pollen loads on Sphingid specimens suggests they are effective and consistent pollinators of *P. hirsuta*. The number of Sphingids observed visiting *P. hirsuta* in this study might not accurately portray their true visitation rates. Sphingids beat their wings rapidly while feeding, which makes them more difficult to see. In addition, they are most active during crepuscular time periods when light (and thus visibility) is limited.

The most frequent visitor to *P. hirsuta* in 2005 was *Vanessa cardui* (Nymphalidae). However, a population explosion of *V. cardui* in 2005 may have been responsible for the large number of sightings on *Phlox* that season. Some *V. cardui* visited *P. hirsuta* plants consecutively but the majority visited *Phlox* sporadically and interchangeably with a number of other plant species in coincident bloom. This feeding strategy was confirmed from the analysis of different pollen sources on collected specimens. In all cases, *Phlox* pollen loads were lower (range of 4-50 pollen grains) on V. cardui than those on Sphingids. In sum, based on observations of insect behavior (but not frequency of visits) and pollen loads, *V. cardui* appear to be a less effective and inconsistent pollinator of *P. hirsuta* than Sphingid moths.

Members of the subfamily Hesperiinae (Skippers) appear to be occasional pollinators of *P. hirsuta*. Although several specimens were observed probing *P. hirsuta* and most of those collected were carrying *Phlox* pollen, the pollen load was very low (range of 2-10 pollen grains). However, it is possible that these specimens were carrying more pollen grains elsewhere (e.g., on their faces) since their proboscises were shorter than the corolla tube of *P. hirsuta*. Other insects were

observed probing *P. hirsuta* or carrying *Phlox* pollen (Table 7 & 8) on occasion and thus considered incidental pollinators. It should be noted that the pollen load estimates were based on measurements of the number of grains from each proboscis, and it is possible that some loss of pollen grains occurred during the uncoiling of the proboscises (Levin & Berube 1972). However, these potential losses were likely to have been relatively consistent among specimens.General insect activity at the sites between the two years was variable and likely reflects variable weather conditions experienced at both sites over the bloom period. Temperature fluctions of 25° C per day and 4 km/hr changes in wind speed were common at the China Hill weather monitoring site. Temperatures were generally lowest sunset to sunrise, and winds were highest in the afternoon and evening. Daily and yearly fluctuation in insect visitors has been noted on other *Phlox* species growing in similar environmental conditions (Springer & Tyrl 1989, Wiggam-Harper 2003).

Fruit and Seed Set

Fruit set in *P. hirsuta* plants was relatively low (from 15 – 49%) compared to other studies of other Phlox species (Table 1). Adams (1987) observed over 90% fruit set on P. speciosa at China Hill. Fruit set in open-pollinated *P. oklahomensis* was 81.4% (Springer & Tyrl 1989). Low fruit set suggests *P. hirsuta* may be pollen-limited and/or resource-limited.

Seed set in bagged flowers hand-pollinated with outcrossed pollen was 17% higher than in unbagged, naturally pollinated flowers. These results suggest *P. hirsuta* flowers have the potential to produce more seeds than are produced under natural

conditions and may be limited by the amount of pollen vectored by insects. Additionally, pollen quality may affect the number and quality of seeds produced (Zimmerman & Pyke 1988). Therefore, reduced seed set in *P. hirsuta* may be due to pollinator scarcity, pollinator inefficiency (i.e., not carrying a sufficient pollen load, or not visiting consecutive plants of same species), or poor pollen quality. If pollen limitation is responsible for low seed set, hand-pollination may result in as much as a tenfold increase in seed set (Burd 1994). Wilson et al (1979) tested pollen limitation in *Phlox divaricata*, and found 58% of the plant's flowers set seed in nature and 82% of the flowers set seed when hand-pollinated.

Regardless of pollen limitation, seed production may also be influenced by the availability of other resources to individual plants or populations (Zimmerman & Pyke 1988). Hand pollinating can result in seed set of 80-100% if the plant species is not resource limited. Low seed set (50%) in bagged and outcrossed *P. hirsuta* flowers indicates it may also be limited in the amount of resources it can allocate to reproduction. Resource limitation is a common occurrence in nutrient-poor soils such as serpentinite (Gordon & Lipman 1926, Mason 1946, Walker 1954). For example, *Cochlearia pyrenaica*, a serpentine endemic species, set more fruit and seed when major nutrients were added (Nagy & Proctor 1997).

Pollen

Erdtman (1966) describes Polemoniaceae pollen as variable, ranging from porate (pores) to forate (furrows) in aperture type and suboblate to spheroidal in shape. Characteristics of *Phlox* pollen include polyporate apertures (pores scattered over the

surface), reticulate sculpture, pore numbers in approximately equal numbers, and an average size of 40 μm (Taylor & Levin 1975). Pollen of *P. hirsuta* could not be distinguished from pollen of another *Phlox* species, *P. speciosa*. *Phlox speciosa* is present at both study sites but only in small numbers on the edge of the *P. hirsuta* occurrences. However, most insects were collected in solid (not intermixed) patches of *P. hirsuta*, and it was inferred that *Phlox* pollen found on collected insects was *P. hirsuta* pollen. None of the lepidopterans believed to be pollinators of *P. hirsuta* were observed visiting *P. speciosa.* However, more studies are needed to verify this. Scanning electron microscopy (SEM) could be used to elucidate the differences between pollen of the two *Phlox* species.

Taylor & Levin (1975) illustrated how pollen size among species of the Polemoniaceae influences what animals can carry it. According to their data, *P. hirsuta,* with a mean diameter of 42 μm, falls within the category that is most likely to be pollinated by a moth, bee, bee fly, or butterfly. Potential pollinators within the Polemoniaceae family include butterflies, moths, bees, beetles, flies, hummingbirds, and bats (Taylor & Levin 1975). The diversity of pollinators may reflect the morphological diversity of pollen in the *Phlox* family. Other floral traits, besides pollen size, affect which animals vector pollen. Taylor's data shows that butterfly mediated pollen are the smallest size. There does not appear to be a statistical correlation between pollen sculpturing types and mating systems. For example, *P. drummondii* and *P. cuspidata* have similar pollen sculpture yet the former is self-incompatible and Lepidopteran pollinated and the latter is self-compatible (Taylor &

Levin 1975). Thus, it cannot be inferred that *P. hirsuta* and *P. speciosa* employ the same mating system or the same pollen vectors.

Seed Traits, Viability, and Germination

Like seeds of many Polemoniaceae species, the seeds of *P. hirsuta* are "non-endospermic" (characterized by reduced or no endosperm, 2-8 mm length, large cotyledons, and an embryo that occupies most of the seed) (Atwater 1980). The seeds are protected by a thick woody coat, which is readily permeable to water but remains semi-permeable or impermeable to other chemical substances. In addition, seeds in this group may contain chemical inhibitors, which play an important role in controlling seed germination in the field.

The results of the 2004 TTC and germination tests suggest that a high percentage of seeds produced by *P. hirsuta* plants are viable, and confirm that fertilization occurred under natural conditions. The TTC test indicated that 66-75% of the seeds produced by *P. hirsuta* plants were viable. Viability appeared to be higher at Cracker Gulch than at China Hill, but the small sample size at Cracker Gulch may limit the usefulness of this comparison.

TTC tests often suggest higher viability rates than germination tests due to unknown germination requirements, seed dormancy and other difficulties with germination in the lab (Kaye 2005, Grabe 1970). However, the test may have underestimated the viability of *P. hirsuta* seeds in this study.

The results of the germination treatment indicate that *Phlox hirsuta* seeds possess a nondeep physiological dormancy, which is broken by warm summer

temperatures (Baskin 2005). Herbaceous perennials from the temperate steppe/grassland zone (such as China Hill) are known to have seeds that are dormant at maturity (Baskin & Baskin 2001). Most of these perennials have seeds that require cold stratification (due to physical dormancy) while others (*P. hirsuta*) germinate after dry storage at room temperatures, indicating that after-ripening occurred to break the nondeep dormancy (Baskin & Baskin 2001). Seed storage in paper bags at room temperature (20°C or higher) for two to six months simulates an after-ripening period (Drake *et al*. 1998).

Phlox hirsuta seeds appear to come out of dormancy during the warm, dry summer and require subsequent cool temperatures and moist conditions for germination. In the field, P. hirsuta seeds most likely germinate in autumn after the onset of the rainy season Baskin (2005) found a similar dormancy and germination pattern in *Phlox bifida.* However, neither *Phlox drummondii* nor *Phlox maculata* seeds were found to be dormant (Atwater 1980). When stored at 15-20°C and treated with fungicide, these species germinated within 21-28 days. Seeds from other species of *Phlox* possess deeper dormancy. For example, *P. diffusa*, a high elevation species, had a germination rate of just 30% after 16 weeks of cold stratification (Kaye 1997). *Phlox oklahomensis* was found to require at least two weeks of cold/moist stratification to achieve a maximum germination rate of 60% (Springer & Tyrl 1989).

The fact that *P. hirsuta* germinated at a low temperature of 3° C suggests, in its natural habitat, this species germinates in fall or early winter. This might explain why no one has observed seedling establishment in the field (Adams 1987, USFWS 2004).

Alternatively, seedlings may not be observed because of seed/seedling predation, lack of overwintering survivors, or other factors. Enforced dormancy, where viable seeds do not germinate because of some limitation in the environment (Roberts 1972), may play a role in limiting P. hirsuta germination in the wild. Future studies to elucidate the factors influencing *P. hirsuta* seed establishment and its reproductive fitness would be worthwhile.

Conclusions

Phlox hirsuta appears to have a predominantly outcrossing mating system, and relies on a number of Lepidopterans to vector pollen between plants. Fruit and seed set in *P. hirsuta* were relatively low, indicating that pollen and/or resources may be limited. In addition, individual flowers produced only one seed, although they are capable of producing three seeds. Fertilized fruits yielded a high percentage of viable seed, which germinated readily in the lab. Further studies could reveal whether factors such as low pollen load delivery, seed/seedling predation, or limited resources negatively impact seed production and/or germination in the field.

The effective population size of a population is the size at which reproductive fitness is ensured through sufficient pollination, fertilization, and a diverse gene pool (Bond 1994). Plants that rely on outcrossing usually require a larger effective population size to attract sufficient pollinators and to preserve genetic diversity. As *P. hirsuta* is predominantly xenogamous, the conservation of large populations or several smaller subpopulations should be a management priority. Small populations of

outcrossing plants are more susceptible to inbreeding depression (Kearns & Inouye 1997).

The fact that *P. hirsuta* has a facultative plant-pollinator relationship is advantageous in terms of increasing its chances of being visited and pollinated. However, the low frequency of insect visits to *P. hirsuta* may be indicative of pollinator scarcity. A scarcity of pollinators may result in more seed set through self-pollination, which results in less vigorous offspring (Kearns & Inouye 1997). In addition, xenogamous plant species that are infrequently visited by pollinators have been shown to experience a marked decline in pollination when their habitat becomes fragmented (Rathcke & Jules 1993). For effective management of *P. hirsuta*, the habitat of its pollinators (including the plant species that are hosts to larval stages) should be conserved.

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TABLES

Table 1. Total number of flowers and percentage of fruits and seeds produced by 20 *P. hirsuta* plants in unbagged and bagged treatments at China Hill and Cracker Gulch sites in 2004 and 2005.

Year/Site/treatment	Flowers	Fruit Set (%)	Seed Set per plant (%)	Seed set per fruit (%)
2004 – China Hill				
unbagged	1146	49.6	46.9	94.7
bagged	1572	0.1	0.1	100.0
2004 – Cracker Gulch				
unbagged	174	37.0	29.3	78.5
bagged	91	0.0	0.0	0.0
2005 – China Hill				
unbagged	1572	29.0	26.5	90.1
bagged	2838	0.7	0.5	66.7
2005 – Cracker Gulch				
unbagged	512	15.0	0.1	69.2
bagged	866	0.0	0.0	0.0

Table 2. Mean number of flowers, fruits, and seeds produced per *P. hirsuta* plant in unbagged and bagged treatments at China Hill and Cracker Gulch sites in 2004 and 2005.

Year/Site/treatment	Mean no. Flowers	Mean no. Fruits	Mean no. Seeds
2004 – China Hill			
unbagged	57.3	28.4	26.9
bagged	78.6	0.05	0.05
2004 – Cracker Gulch			
unbagged	8.7	3.2	2.5
bagged	4.6	0.0	0.0
2005 – China Hill			
unbagged	78.6	23.2	20.9
bagged	141.9	1.05	0.7
2005 – Cracker Gulch			
unbagged	25.6	3.9	2.7
bagged	43.3	0.0	0.0

Table 3. Total number of flowers and percentage of fruits and seeds produced by 15 *Phlox hirsuta* shoots in unbagged, bagged, bagged and selfed and bagged and outcrossed treatments at China Hill in 2005.

Treatments	Flowers	Fruit set (%)	Seed set (%)
Unbagged	173	26.0	86.6
Bagged	160	0	0
Bagged and Selfed	112	0	0
Bagged and Outcrossed	133	49.6	80.0

Table 4. Mean number of flowers, fruits and seeds produced by 15 *Phlox hirsuta* shoots in unbagged, bagged, bagged and selfed and bagged and outcrossed treatments at China Hill in 2005.

Treatments	Flowers / shoot	Fruit set / shoot	Seed set / shoot
Unbagged	11.5	3.0	2.6
Bagged	10.7	0	0
Bagged and Selfed	7.5	0	0
Bagged and Outcrossed	8.9	4.4	3.5

Table 5. Numbers of dipterans, hymenopterans, lepidopterans, and other insect taxa observed at China Hill during crepuscular a.m. (CAM), diurnal a.m. (DAM), diurnal p.m. (DPM), and crepuscular p.m. (CPM) in 2004.

Taxon	CAM	DAM	DPM	CPM	Total
Diptera	28	45	58	25	156
Hymenoptera	6	131	212	53	402
Lepidoptera	29	55	167	33	284
Other	2	11	56	10	79
Total	65	242	493	121	921

Table 6. Numbers of dipterans, hymenopterans, lepidopterans, and other insect taxa observed at China Hill during the peak bloom period (6-15 April) in 2004. Time periods are Crepuscular a.m. (CAM), diurnal a.m. (DAM), diurnal p.m. (DPM), and crepuscular p.m. (CPM).

Taxon	CAM	DAM	DPM	CPM	Total
Diptera	28	32	27	8	95
Hymenoptera	6	52	51	20	129
Lepidoptera	26	31	60	20	137
Other	0	5	12	6	23
Total	60	120	150	54	384

Table 7. Taxa and number (in parentheses) of insects observed probing *P. hirsuta* flowers at China Hill and Cracker Gulch in 2004 and 2005.

China Hill – 2004	Cracker Gulch - 2004	China Hill - 2005	Cracker Gulch - 2005
Lepidoptera	Lepidoptera	Lepidoptera	Lepidoptera
Hesperiidae	Hesperiidae	Hesperiidae	Hesperiidae
Erynnis sp. (1)	Unident. Hesperiinae (1)	Unident. Hesperiinae (5)	Unident. Hesperiinae (1)
Unident. Hesperiinae (1)	Hymenoptera	Nymphalidae	Nymphalidae
Nymphalidae	Apidae	*Vanessa cardui* (110)	*Vanessa cardui* (1)
Unident. Melitaeini (1)	*Bombus* sp. (1)	Papilionidae	Sphingidae
Vanessa cardui (5)		*Papilio zelicaon* (2)	Unident. taxon (1)
Papilionidae		Pieridae	Hymenoptera
Papilio zelicaon (2)		*Colias* sp. (1)	Apidae
Hyles lineata (1)		*Pontia* sp. (1)	*Bombus* sp. (1)
Sphingidae		Sphingidae	Diptera
Proserpinus clarkiae (15)		*Hyles lineata* (4)	Syrphidae
Unident. taxa (6)		*Proserpinus clarkiae* (2)	Unidentified taxon (1)
Hymenoptera		Hymenoptera	
Apidae		Apidae	
Bombus sp. (4)		*Bombus fervidus*. (1)	
		Bombus sp. (3)	

Table 8. Species and number (in parentheses) of lepidopterans observed carrying *P. hirsuta* pollen at China Hill and Cracker Gulch in 2004 and 2005.

China Hill - 2004	Cracker Gulch – 2004	China Hill - 2005	Cracker Gulch – 2005
Hesperiidae *Hesperia juba* (2) Sphingidae *Proserpinus clarkiae* (5) *Hyles lineata* (2)	(No lepidoptera with pollen)	Geometridae *Plataea trilinearia* (1) *Drepanulatrix unicalceraria* (2) Hesperiidae *Erynnis properties* (1) *Hesperia juba* (2) *Hesperia columbia* (1) *Atalopedes campestris* (1) Noctuidae *Cissusa discreta* (1) Nymphalidae *Vanessa cardui* (14) Papilionidae *Papilio zelicaon* (1) Pieridae *Pieris rapae* (1) *Pontia sysymbrii* (2) *Pieris rapae* (1) Sphingidae *Proserpinus clarkiae* (3) *Hyles lineata* (2)	Hesperiidae *Hesperia columbia* (1) Nymphalidae *Euphydras editha* (1)

Table 9. Bloom periods of plants in coincidental bloom with *P. hirsuta* at China Hill.

	2004		2005	
Plant name	Start Date	End Date	Start Date	End Date
Achillea millefolium	4/14	5/30	5/9	6/10
Allium siskiyouense	3/25	4/17	3/17	5/7
Amsinckia intermedia	4/10	5/30	4/10	5/17
Arabis puberula	4/8	4/18	3/17	5/21
Astragalus purshii var. tinctus	4/8	4/22	3/17	5/7
Castilleja hispida	4/10	5/30	3/31	5/31
Ceonothus cuneatus	4/4	4/22	3/17	5/12
Chrysothamnus nauseosus	5/6	5/30		
Clarkia gracilis	4/20	5/30	4/26	6/10
Claytonia exigua			2/23	4/26
Claytonia lanceolata			2/23	3/31
Collinsia parviflora			3/17	4/21
Crocidium multicaule	3/30	4/13	2/23	4/21
Dichelostemma capitatum	4/4	4/30	3/23	4/21
Epilobium sp.	4/6	4/24	4/14	5/30
Erigeron bloomeri var. bloomeri	4/25	5/30	4/24	6/10
Eriogonum sphaerocephalum	4/25	5/30	4/28	6/25
Eriophyllum lanatum	4/17	5/30	4/14	6/10
Eschscholzia californica	4/8	5/30	4/10	6/10
Fritillaria pudica			2/23	3/23
Gilia capitata	4/20	5/30	4/21	6/10
Lasthenia californica	4/10	4/20	5/17	5/30
Lewisia rediviva	4/20	5/4	4/26	5/17
Linanthus sp.			4/19	5/26
Lithophragma parviflorum	4/6	4/18	3/11	5/17
Lomatium macrocarpum	4/1	4/28	3/11	5/10
Lupinus argenteus	4/1	5/30	4/14	5/26
Minuartia douglasii	4/20	5/30	4/26	6/10
Orobanche uniflora	5/2	5/30	4/10	6/10
Phacelia corymbosa	4/6	5/30	4/19	6/10
Phlox hirsuta	3/22	5/13	2/23	6/2
Phlox speciosa	4/1	5/4	3/5	5/31
Plagiobothrys sp.	4/6	5/20	4/2	6/10
Sisrynchium douglasii			2/23	4/19
Viola beckwithii	3/15	4/1	2/23	4/2

Table 10. Bloom periods of plants in coincidental bloom with *P. hirsuta* at Cracker Gulch.

	2004		2005	
Plant name	Start Date	End Date	Start Date	End Date
Achillea millefolium	5/1	5/30	5/15	7/25
Allium siskiyouense	4/20	5/11	4/28	5/30
Arabis puberla	4/6	5/2	3/17	5/21
Calochortus sp.	4/29	5/20	5/15	6/8
Castilleja hispida	4/26	5/30	4/18	6/20
Ceonothus cuneatus			4/20	5/30
Cercocarpus ledifolius	4/20	5/2	4/1	4/28
Chlorogalum sp.	4/20	5/11	4/18	5/30
Claytonia exigua			4/18	5/30
Collinsia parviflora			4/28	5/15
Chrysothamnus nauseosus	5/6	5/30		
Epilobium sp.	4/6	4/24	4/28	6/15
Eriogonum strictum			6/4	7/25
Eriophyllum lanatum			5/15	6/20
Erysimum captitatum	4/29	5/30	5/15	6/15
Fritillaria pudica			3/17	4/18
Gilia capitatum			5/15	6/20
Lithophragma parviflorum	4/22	5/2	4/10	5/30
Lomatium macrocarpum			4/10	6/20
Lomatium triternatum			5/10	6/20
Minuartia nuttallii	4/20	5/30	5/19	6/30
Penstemon sp.	5/11	5/30	5/30	7/25
Phacelia corymbosa			5/10	5/30
Phlox gracilis			3/17	4/18
Phlox hirsuta	4/2	5/23	4/1	6/15
Phlox speciosa			4/28	6/15
Plagiobothrys sp.	4/26	5/20	4/28	6/20
Thysanocarpus curvipes	3/22	4/2	4/1	5/1
Viola purpurea	4/20	5/11	3/17	5/30

Figures

Figure 1. Phlox hirsuta flowers.

Figure 2. Location of known occurrences of *Phlox hirsuta* (Yreka phlox), Siskiyou County, California (USFWS 2004).

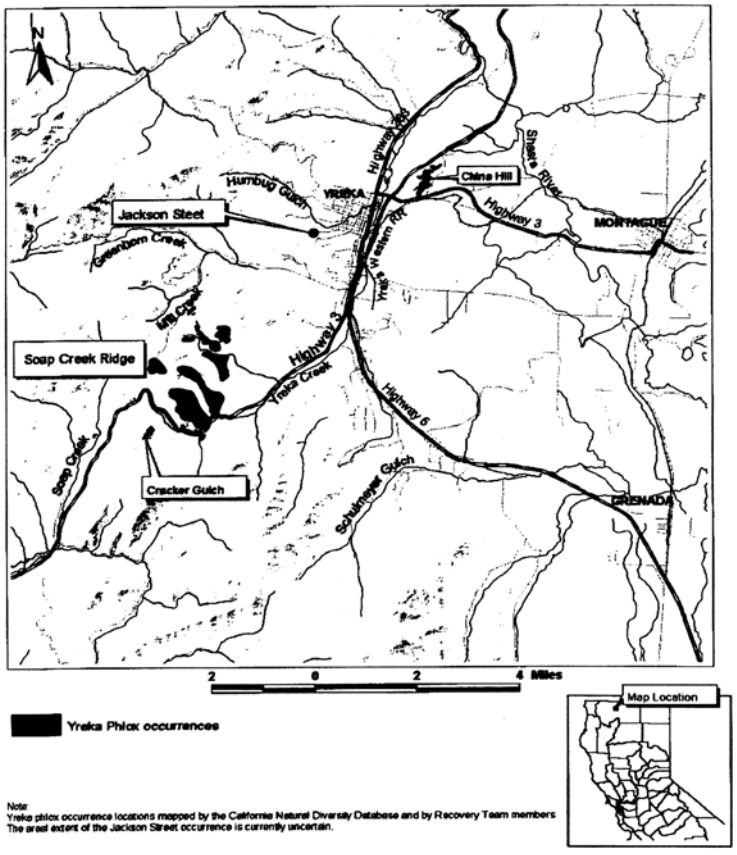


Figure 3. HOBO Microstation weather data logger at China Hill.

Figure 4. Pollinator exclusion bag over *Phlox hirsuta* plants at China Hill.

Figure 5. *Phlox hirsuta* plant at China Hill used for hand pollination experiments.

Figure 6. Percentage of *Phlox hirsuta* plants in bloom on bagged and unbagged plants at China Hill in 2004.

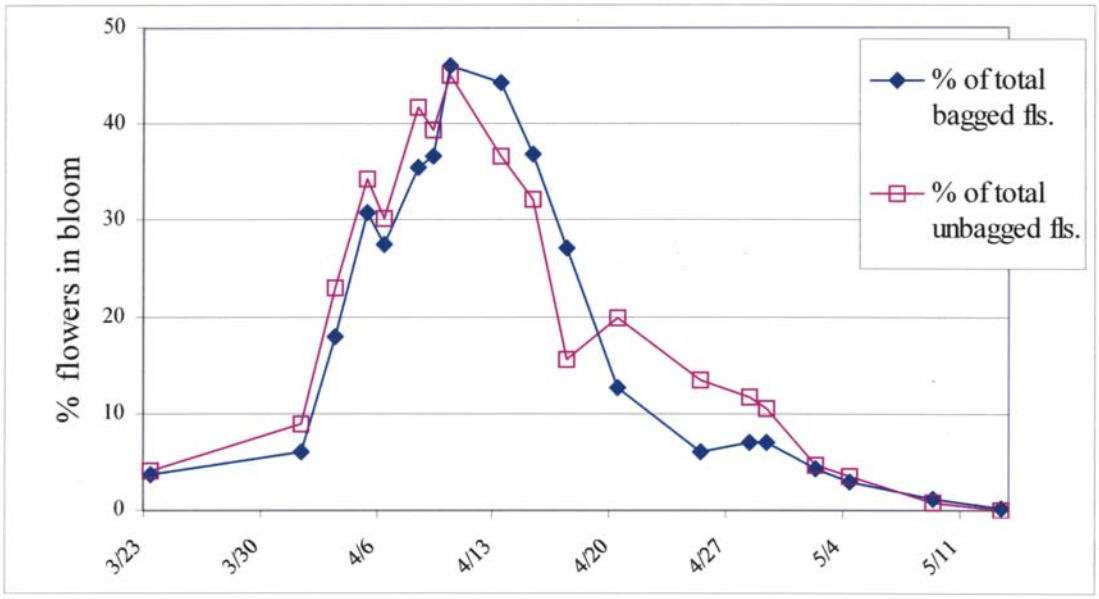


Figure 7. Percentage of *Phlox hirsuta* plants in bloom on bagged and unbagged plants at China Hill in 2005.

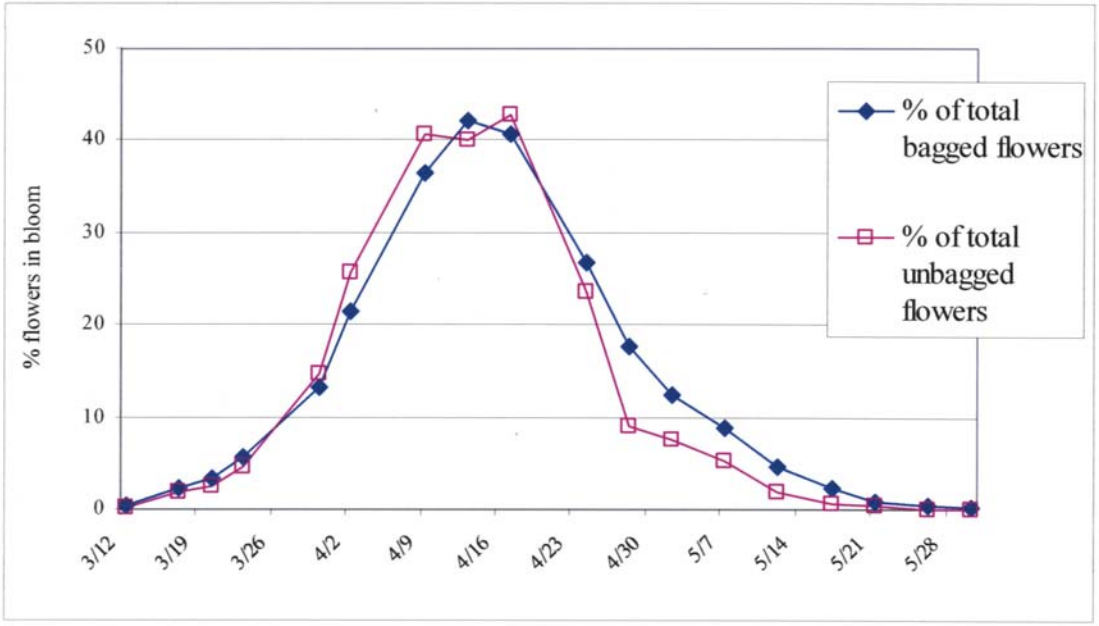


Figure 8. Percentage of *Phlox hirsuta* plants in bloom on bagged and unbagged plants at Cracker Gulch in 2004.

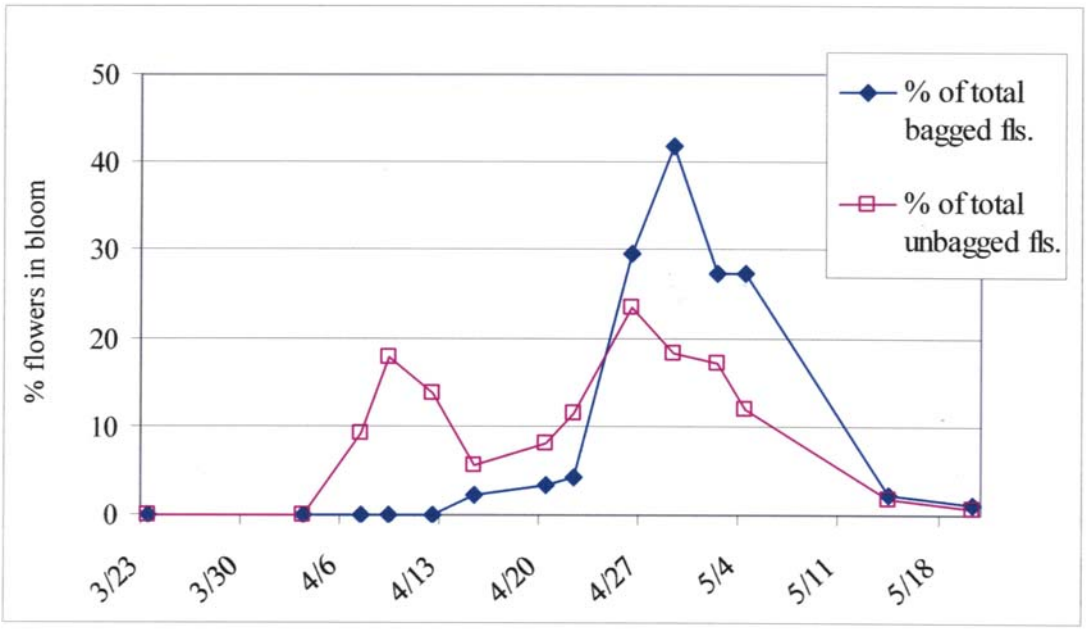


Figure 9. Percentage of *Phlox hirsuta* plants in bloom on bagged and unbagged plants at Cracker Gulch in 2005.

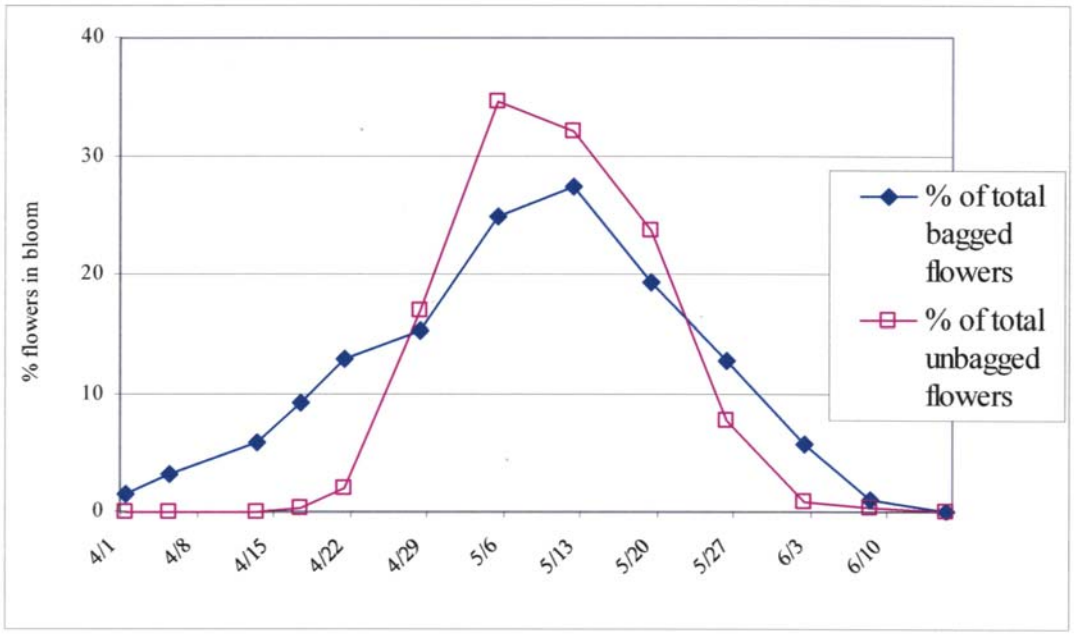


Figure 10. Percentage of bagged and unbagged *Phox hirsuta* flowers in bloom combined at China Hill and Cracker Gulch in 2004.

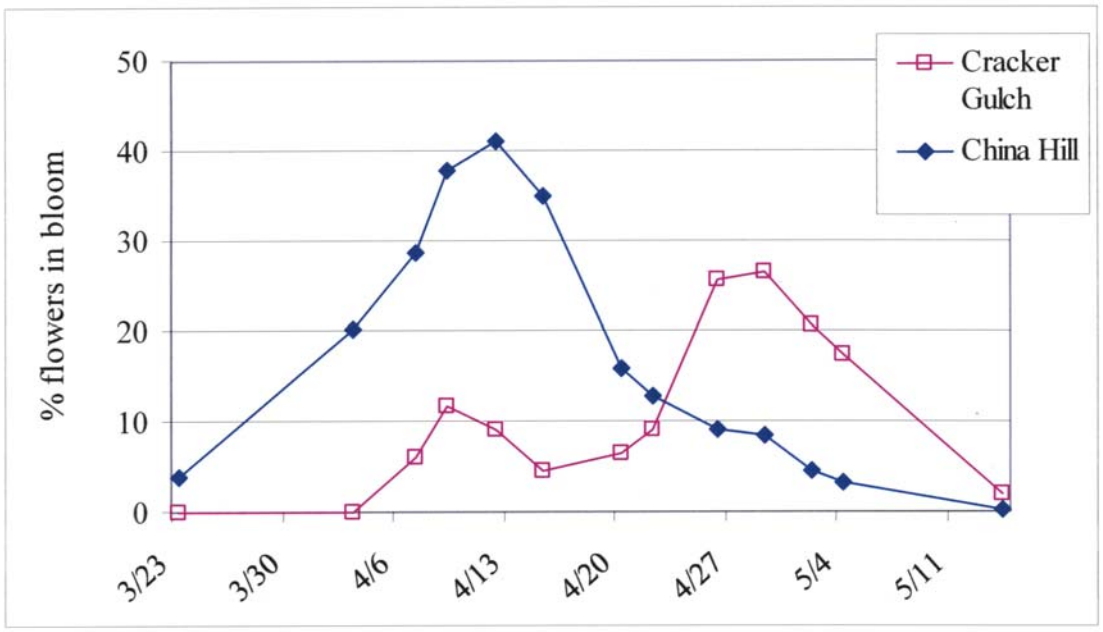


Figure 11. Percentage of bagged and unbagged *Phox hirsuta* flowers in bloom combined at China Hill and Cracker Gulch in 2005.

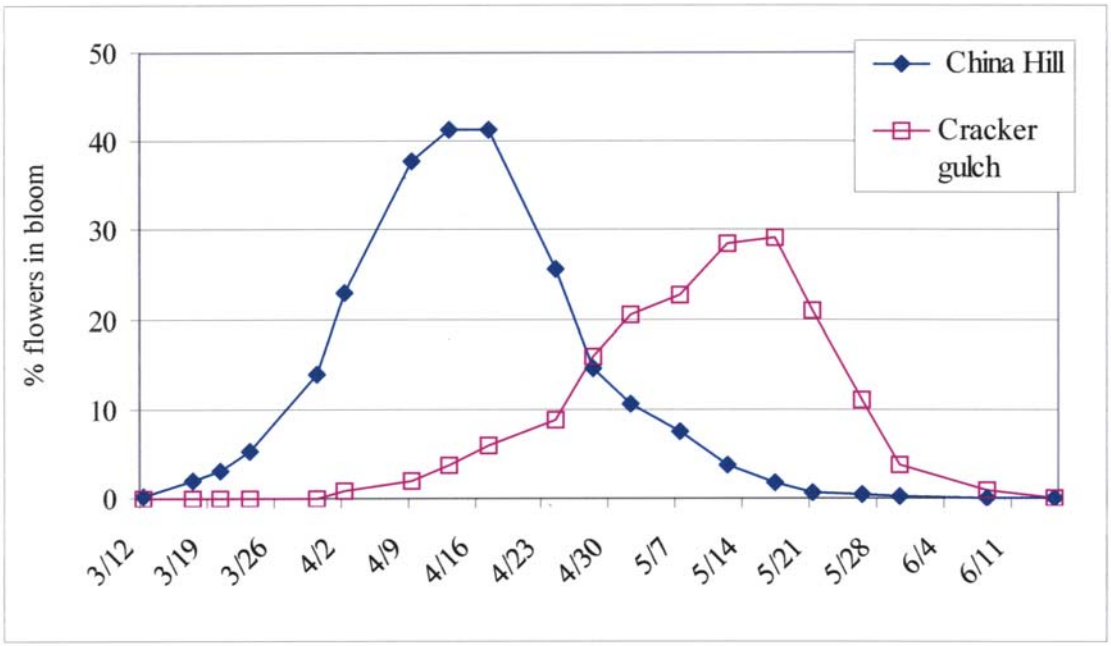


Figure 12. Pollen grains of *Phlox hirsuta* and *P. speciosa* as seen under a light microscope at 63X.

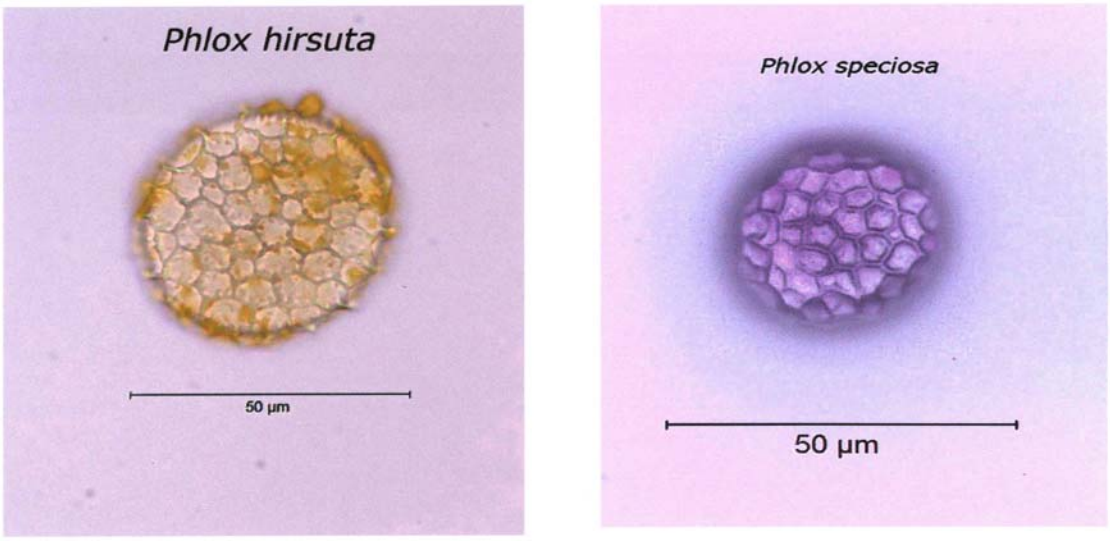


Figure 13. Pollen grains of *Gilia capitata* and *Linanthus* sp. as seen under a light microscope at 63X.

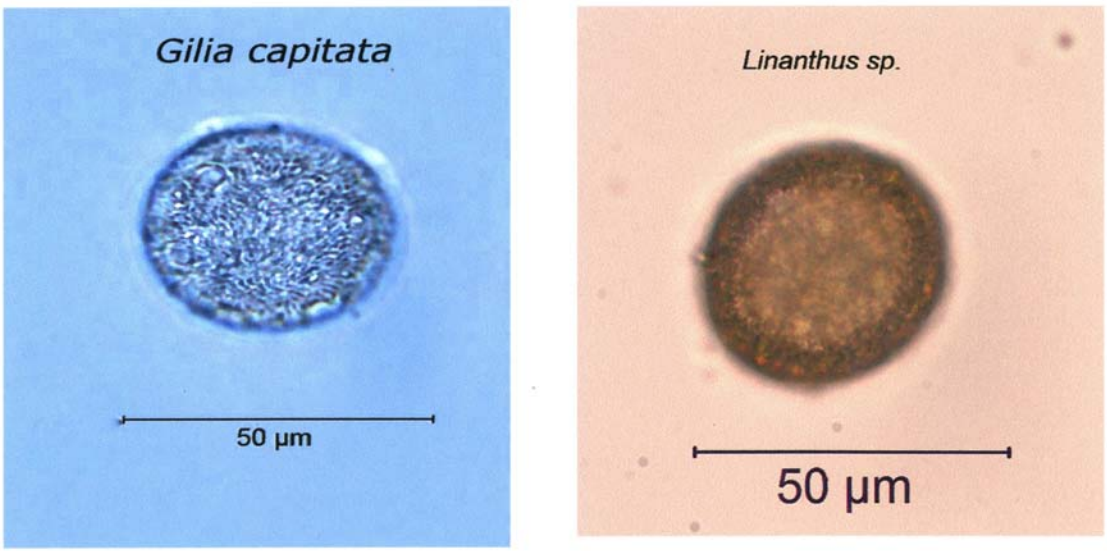
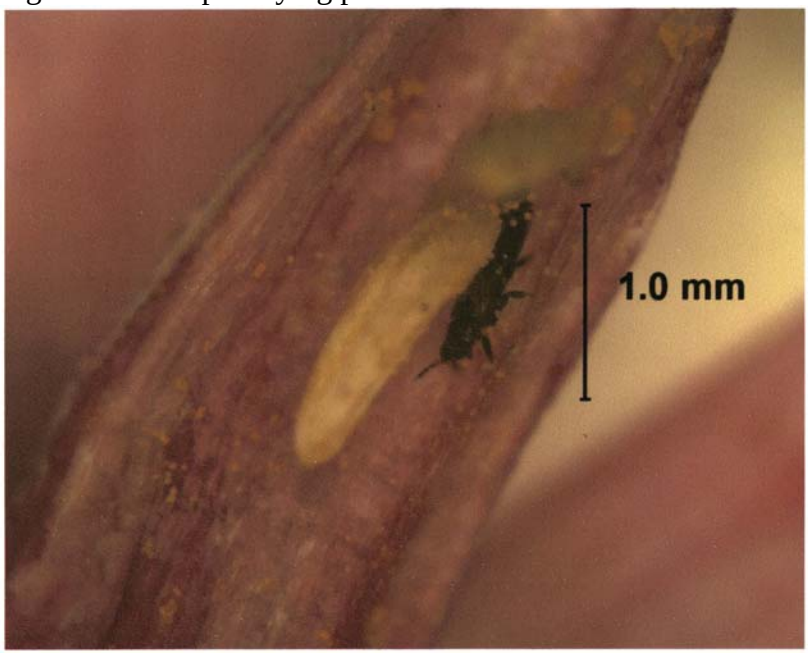


Figure 14. Phlox hirsuta seedlings in the Southern Oregon University greenhouse. Photo by Dr. Carol Ferguson.

Figure 15. Thrip carrying pollen inside *Phlox hirsuta* corolla.



APPENDIX A

PLANT COMMUNITIES

China Hill Plant Community

(*= in plot)

Trees
Juniperus occidentalis

Shrubs
Ceonothus cuneatus
*Chrysothamnus nauseosus**
*Eriogonum sphaerocephalum var. halimoides**

Herbaceous plants
Achillea millefolium
Allium sp.(siskiyouense or *falcifolium)**
Amsinckia intermedia
*Arabis puberula**
*Astragalus purshii var. tinctus**
Blepharipappus scaber
Castilleja hispida
Clarkia gracilis
Claytonia exigua
Claytonia lanceolata
Collinsia parviflora
Crepis occidentalis
*Crocidium multicaule**
Dichelostemma capitatum
*Epilobium sp.**
Erigeron bloomeri var. bloomeri
*Eriogonum strictum var. proliferum**
*Eriophyllum lanatum var. achillaeoides**
*Eschscholzia californica**
*Fritillaria pudica**
*Gilia capitatum**
Lasthenia californica
*Lewisia rediviva**
Linanthus sp. (ambiguous?)
Lithophragma parviflora
*Lomatium macrocarpum**
Lupinus argenteus
*Minuartia douglasii**
*Orobanche uniflora**
*Phacelia corymbosa**
*Phlox hirsuta**
Phlox speciosa
*Plagiobothrys sp.**
Sisyrinchium douglasii
Swertia albicaulis
*Thysanocarpus curvipes**
Uropappus lindleyi (or *Microseris sp.?)*
*Viola beckwithii**

Grasses
Achnatherum thurberianum
*Bromus madritensis ssp. rubens**
*Bromus tectorum**
Elymus elymoides
*Elymus multisetus**
*Festuca idahoensis**
*Poa bulbosa**
*Poa sp.**
*Vulpia microstachys**

Cracker Gulch Plant Community
(*= in plot)

Trees
*Calocedrus decurrens**
Juniperus occidentalis
*Pinus jeffreyi**
Pseudotsuga menziesii
Quercus garryana

Shrubs
Ceonothus cuneatus
*Cercocarpus ledifolius**
*Chrysothamnus nauseosus**

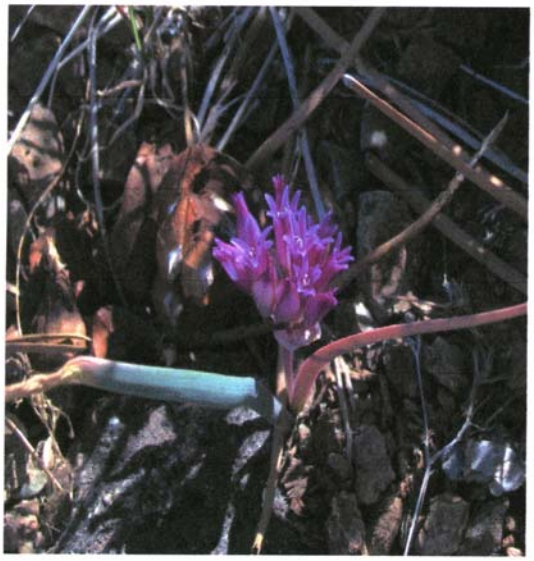
Herbaceous Plants
*Achillea millefolium**
Allium sp.(siskiyouense or *falcifolium)**
*Arabis puberula**
*Calochortus tolmei**
Castilleja hispida
*Chlorogalum sp.**
Claytonia rubra
Collinsia rattanii
*Epilobium sp.**
*Eriogonum strictum**
*Eriophyllum lanatum**
Erysimum capitatum
Fritillaria affinis
*Fritillaria pudica**
Fritillaria recurva
*Gilia capitata**
Lithophragma parviflora
Lomatium macrocarpum
Lomatium triternatum
Lupinus sp.
*Minuartia nuttalli**
*Penstemon sp.**
*Phacelia corymbosa**
Phlox gracilis
*Phlox hirsuta**
Phlox speciosa
*Plagiobothrys sp.**
Plectritis macrocera
Thelypodium sp.
*Thysanocarpus curvipes**
*Viola purpurea**

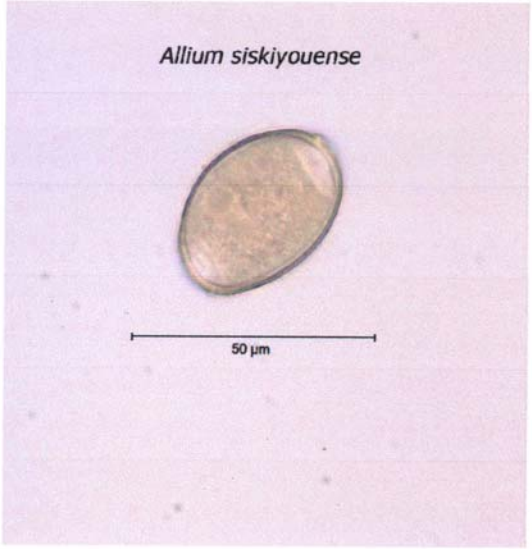
Grasses
Agropyron spicatum
*Bromus tectorum**
*Elymus glaucus**
*Festuca idahoensis**
Poa sp.
Vulpia microstachys var. pauciflora

APPENDIX B

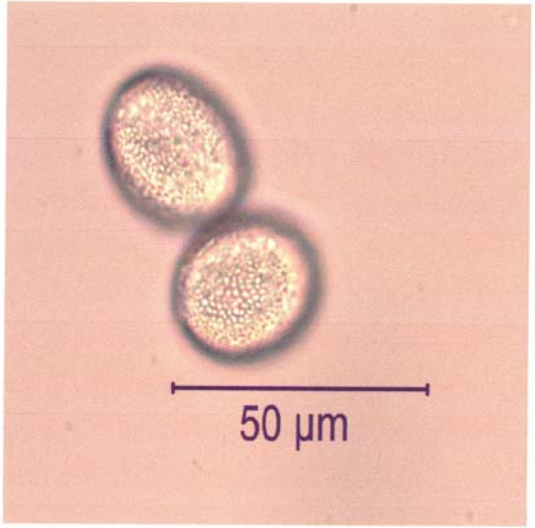
POLLEN REFERENCE COLLECTION

1) *Allium siskiyouense*

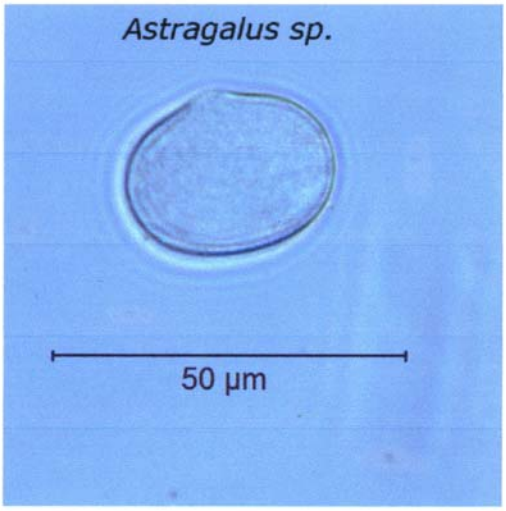




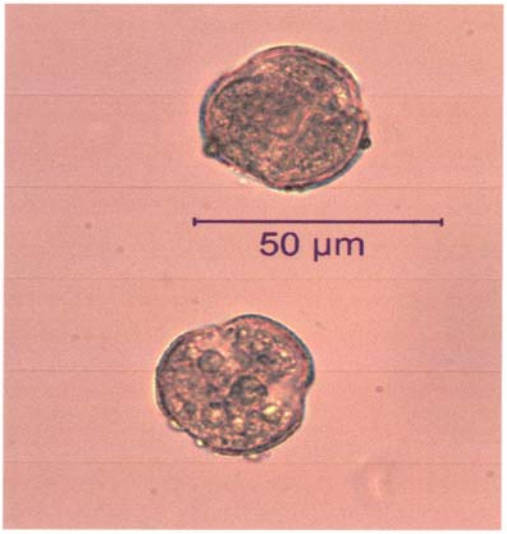
2) *Arabis puberula*



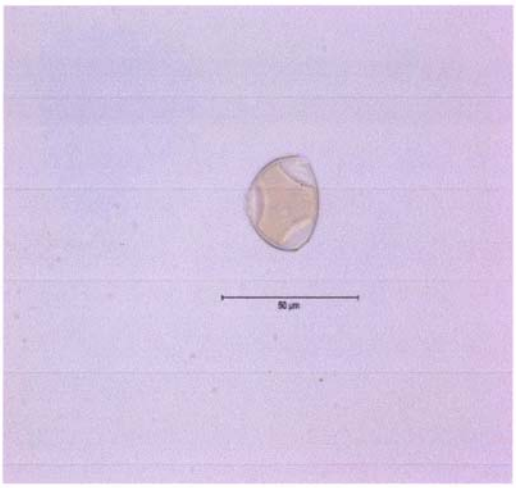
3) *Astragalus purshii var. tinctus*



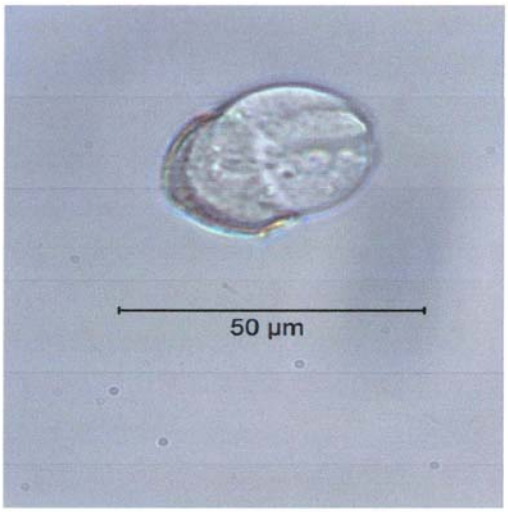
4) *Castilleja hispida*



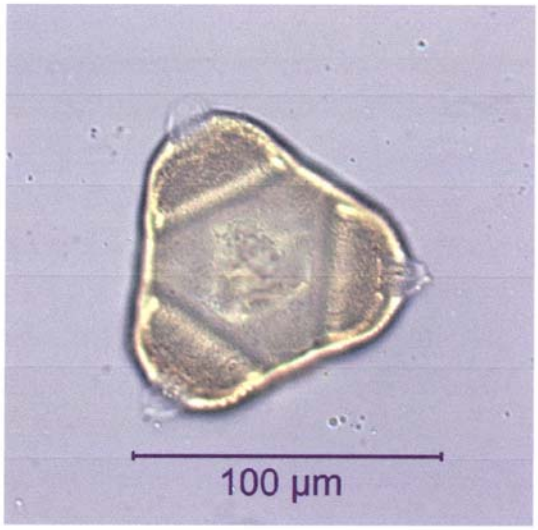
5) *Ceonothus cuneatus*



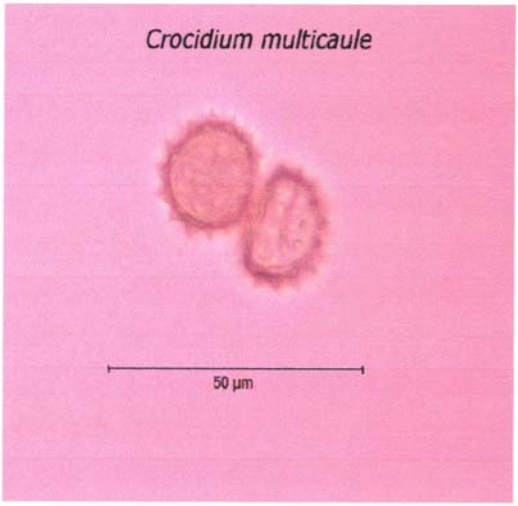
6) *Chlorogalum sp.*



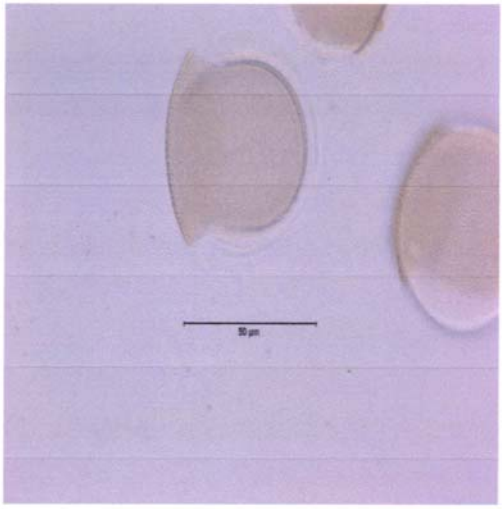
7) *Clarkia sp.*



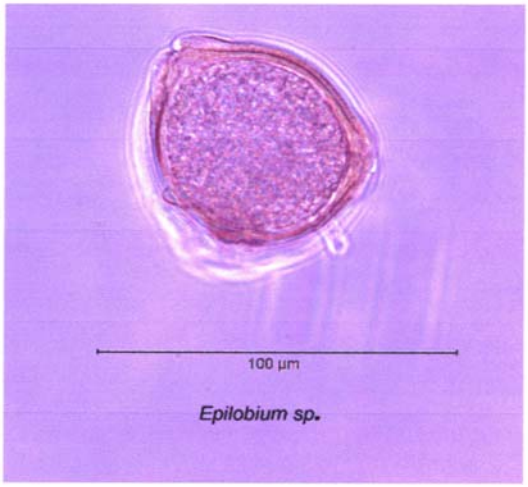
8) *Crocidium multicaule*



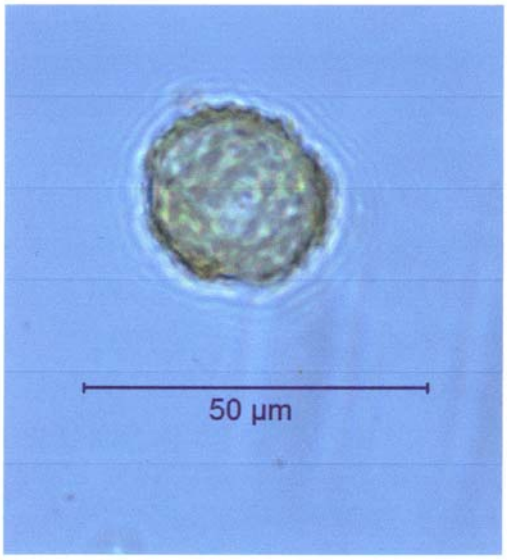
9) *Dichelostemma capitatum*



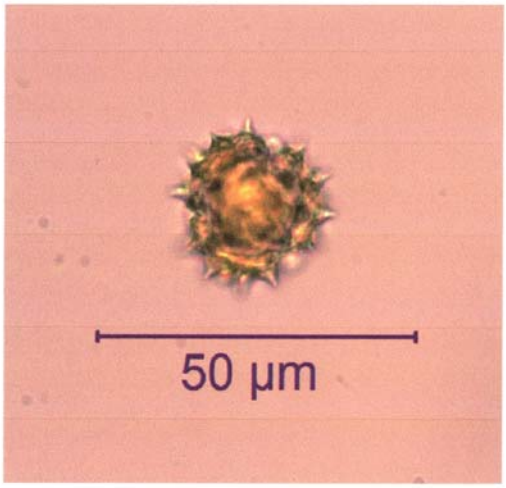
10) *Epilobium sp.*



11) *Erigeron bloomeri*

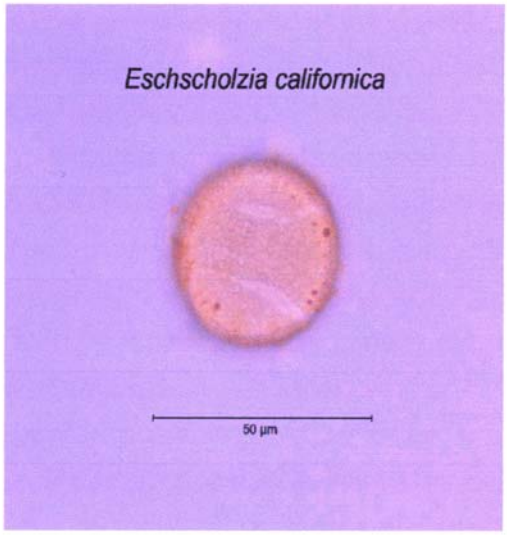


12) *Eriophyllum lanatum*

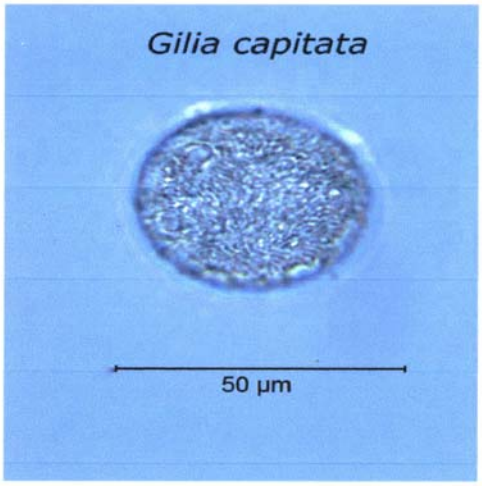


13) *Eriogonum sphaerocephalum*

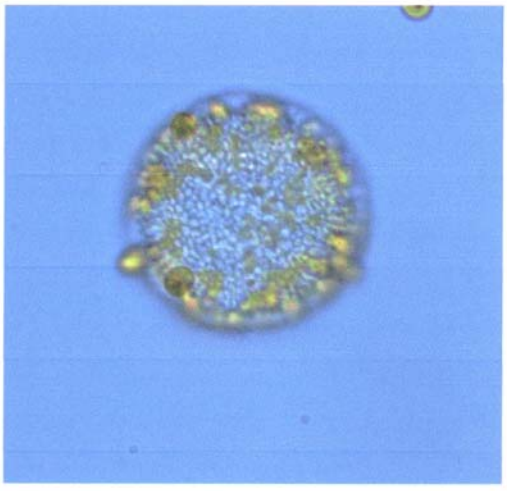
14) *Eschscholzia californica*



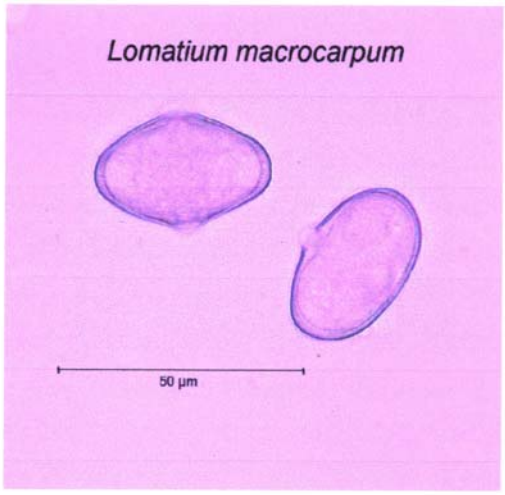
15) *Gilia capitata*



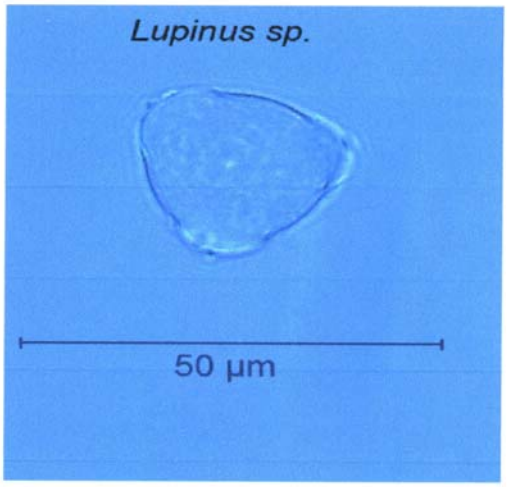
16) *Linanthus sp.*



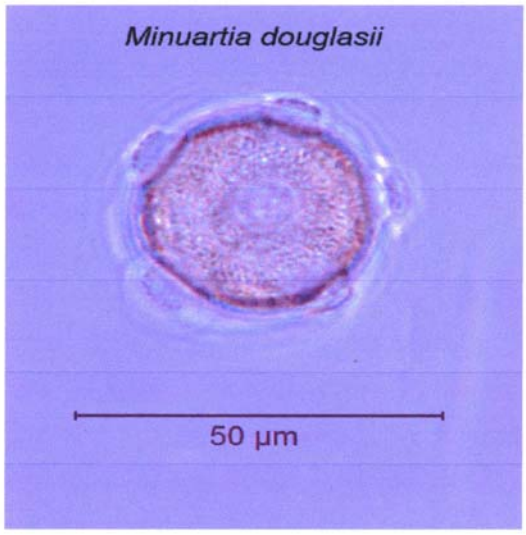
17) *Lomatium macrocarpum*



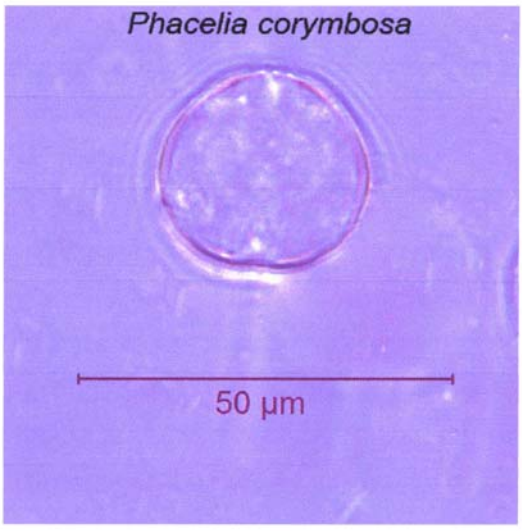
18) *Lupinus argenteus*



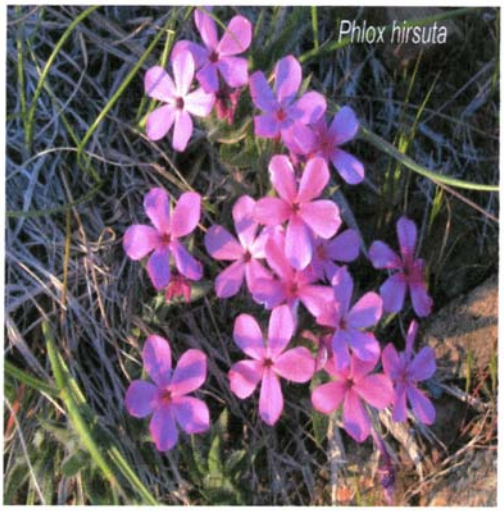
19) *Minuartia douglasii*

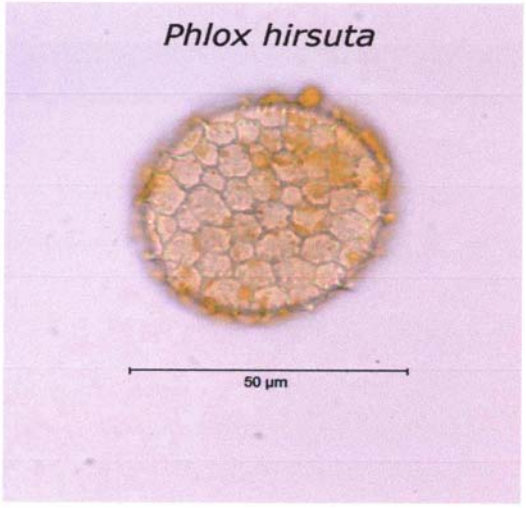


20) *Phacelia corymbosa*

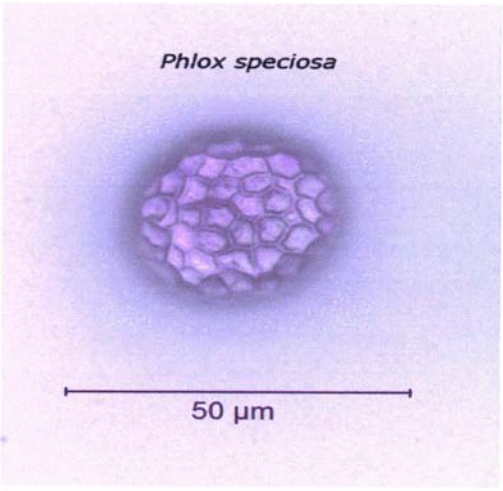


21) *Phlox hirsuta*

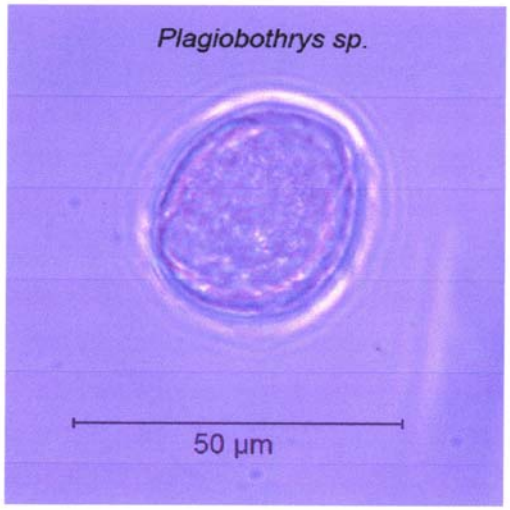




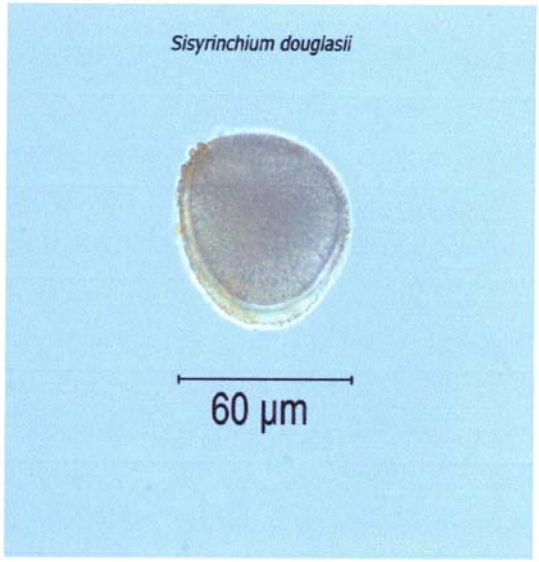
22) *Phlox speciosa*



23) *Plagiobothrys sp.*



24) *Sisrynchium douglasii*



25) *Viola beckwithii*