RESEARCH ARTICLE

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Penstemon grandiflorus Nutt. Reproductive Ecology: Prediction of Pollinator Limitation from Experiments and Field Studies

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ABSTRACT: Suspecting that *Penstemon grandiflorus* Nutt. might be susceptible to pollinator limitation of seed set, I studied reproductive ecology of this short-lived perennial herb in south-central Minnesota, USA. Seed production, fruit production, and percent seed germination were reduced when insect visitation to *P. grandiflorus* was limited. Approximately six visits per flower were required for maximum seed set. Most seeds were produced by flowers opening during the first four days of the blooming season. Visitation rates to natural plant populations showed high variation within and among years and within and among sites during nine site-year combinations. Analysis of weather records combined with visitation observations indicated that insect visits to plants in natural populations usually exceeded the number required for maximum seed production. Small and large populations, whether recently planted or long established, received ample visitation over the plant's short blooming period during most years. Comparison of observed natural visitation levels with levels experimentally determined to be adequate indicates that *P. grandiflorus* is unlikely to experience pollinator limitation of seed set in south-central Minnesota, USA, during most years.

Penstemon grandiflorus Nutt. Ecología Reproductiva: Predicción de Limitación de Polinizadores en Experimentos y Estudios de Campo

RESUMEN: Sospechando que *Penstemon grandiflorus* Nutt. podría ser susceptible de limitación de polinizador para las semillas, examiné la ecología reproductiva de esta hierba perenne de corta vida en el centro sur de Minnesota, USA. La producción de semillas, y el porcentaje de germinación estuvieron reducidos cuando la visita de insectos a *P. grandiflorus* estuvo limitada. Aproximadamente seis visitas por flor fueron requeridas para la producción máxima de semillas. La mayoría de las semillas fueron producidas por las flores que abrieron durante los primeros cuatro días del pico de la temporada. La tasa de visitas de las poblaciones naturales de plantas mostraron alta variación dentro y entre años y dentro y entre sitios durante nueve combinaciones de año y sitio. Los análisis de registros de clima combinados con observaciones indicaron que la visita de insectos a las plantas en poblaciones naturales usualmente exceden el número requerido para la máxima producción de semillas. Poblaciones grandes o pequeñas, sin importar si se implantaron recientemente o han estado establecidas por años, recibieron numerosas visitas durante el corto período pico de florecimiento durante la mayoría de los años. La comparación de los niveles observados de visitas naturales con los niveles de las visitas experimentales determinaron ser adecuados indicadores que es poco probable que *P. grandiflorus* experimente limitaciones de polinizador para la producción de semillas en el centro sur de Minnesota, USA, durante la mayoría de los años.

Index terms: insect visitation, pollinator limitation, *Penstemon grandiflorus*, prairie, reproductive ecology

INTRODUCTION

Failure of plant-pollinator mutualisms has become a concern of conservationists as natural habitats are increasingly fragmented by human activities (Rathcke and Jules 1993, Kearns and Inouye 1997, Allen-Wardell et al. 1998). Occasional inadequate pollination is a natural consequence of the unreliability of insect visitation in variable environments (Thomson 2001, Williams et al. 2001) and results in decreased seed set, and in some cases, production of inviable seeds (Johnston 1991, Petanidou et al. 1995). If pollinator populations decline, pollinator limitation may become chronic (Thomson 2001), leading to reduced fitness of plant populations over the long term, especially in populations of limited genetic diversity (Hendrix and Kyhl 2000). Reduction in plant populations can reduce both specialist and generalist pollinator populations, possibly cascading into pollinator limitation of additional plant species.

To determine whether pollinator limitation of seed set is occurring in a particular species, and what its consequences might be, one must understand plant reproductive ecology, the behavior of insect visitors, and insect-plant interactions. I suspected that *Penstemon grandiflorus* Nutt. (large beardtongue) might be susceptible to pollinator limitation because it is largely self-incompatible, has specialized polli-

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Natural Areas Journal 22:220–229

nators and a short blooming season, and is short-lived (Crosswhite and Crosswhite 1966, Davis et al. 1991). Throughout its range, *Penstemon grandiflorus* has never been common over large areas, and only a small percentage of its habitat has escaped destruction in central Minnesota, USA. These features are common to plants at risk of pollinator limitation (Burd 1994, Bond 1995, Tepedino et al. 1997). This species has been planted in many prairie reconstructions, but its pollinators may not have colonized these areas.

The goal of this study was to determine whether *Penstemon grandiflorus* receives adequate pollination in a variety of sites and years in south-central Minnesota. Pollination is adequate if additional insect visits above the observed level do not result in increased seed set. (If additional insect visits do result in additional seeds, pollinator limitation of seed set is occurring, as defined by Zimmerman and Pyke [1988]). I determined the number of visits per flower needed for maximum seed set in test plots and natural areas, and observed the number of visits actually received by flowers in natural populations. I tested the following hypotheses: (1) Seed production of *P. grandiflorus* flowers increases with increasing insect visitation to a plateau beyond which additional visits have no effect; (2) seed germination is reduced when insect visits per flower drop below a certain level; and (3) visitation rates in natural populations are frequently lower than those needed for maximum seed set, especially in small populations and in prairie reconstructions.

If the level of visitation required for maximum seed production is known, together with the actual visitation levels which occur, one can predict which populations, if any, may be subject to pollinator limitation of seed production.

Natural History of *Penstemon grandiflorus* and Its Insect Visitors

Penstemon grandiflorus (Scrophulariaceae) grows naturally in sand prairies and oak savannas in the central portion of the North American Great Plains; its native habitat has always been restricted and is now being increasingly reduced and fragmented by human activities. A short-lived perennial with basal leaves forming a rosette, it has been planted in many midwestern prairie reconstructions for the sake of its lovely flowers. Plants may flower during the second year of life. Mature plants produce from one to six flowering stems with from 6 to 60 or more flowers per stem. The flowers are pink, bell-shaped, about 4 cm in length, and borne in tiers along the flowering shoot, which can be up to 1.2 m tall. Buds in the middle tiers open first, then lower ones, and the two buds at the tip of the stem are the last to open. The flowers are horizontal; as the fruits develop they gradually turn upward and split open to release the seeds when mature. Flowers that fail to develop into fruits shrivel and remain attached to stems. There are no specialized seed dispersal adaptations. Vegetative reproduction is minimal (Davis et al. 1991).

On Minnesota prairies, *Penstemon grandiflorus* blooms in late May or early June, a time when few other plants are in flower. Weather is changeable at this time, including warm sunny days and chilly, rainy weather. The flowering period is short, usually about two weeks total at any single site. Little has been published on insect visitation to this species, but some *Osmia* Panzer species (Hymenoptera: Megachilidae) specialize on its congeners (Crosswhite and Crosswhite 1966, Tepedino et al. 1999), and some *Penstemon* species, particularly large-flowered ones like *P. grandiflorus*, have a wide variety of flower visitors (Lawson et al. 1989, Clinebell and Bernhardt 1998, Tepedino et al. 1999). Clinebell and Bernhardt (1998) found no seeds were produced by *P. grandiflorus* flowers on stems from which insects were excluded. In a two-year pilot study I obtained identical results using individually bagged flowers. Other flowers on the same stems were exposed to insect visits and produced from 45 to 142 seeds per fruit (n=14 in 1990 and n=9 in 1991, at the Cedar Creek Natural History Area, Anoka, Minn.).

METHODS

Phenology of Populations and Individual Flowers

I counted stems, flowers, and flower clusters in bloom at each site visit. At the test plot in 1997, each flower was individually marked and the dates it opened and dropped were recorded. Flower longevity was calculated as the days between flower opening and corolla drop for each flower.

Insect Visitation Rates and Seed Set in the Test Plot

1997 and 1998 experiments were done in a test plot in St. Paul, Minnesota, containing mature *P. grandiflorus* plants of nursery origin. Plants were randomly assigned to insect visitation levels of 0, half day, full day and unlimited visits in 1997 and 0, 1, 2, ... 14 visits and unlimited visits in 1998. Unmanipulated plants were present to attract insect visitors and serve as pollen sources. Flower visitors were excluded from entire flowering stems with bridal veil net bags put in place before the flowers opened and removed after all flowers fell, except during the experimental exposure periods. In 1997 every flower was marked on the base of the corolla with acrylic fabric paint on the day that it opened so each one could be monitored for longevity.

All plants were clipped to only one stem to achieve better control over pollinator visitation. I tested the effects of clipping on fruit and seed production in 1998. Number of seeds per fruit was not significantly different between stems of clipped plants (109.9 ± 24.0 SD) and matched stems of unclipped controls (96.2 ± 28.8 SD), (*t*-test, two-tailed, equal variances df= 12, $t = 1.02$, $P = 0.32$).

In 1997, 32 plants were each assigned to one of four groups: zero insect visitation, half day or full day exposures to visitation, and unlimited visitation. Flower number was not significantly different among the groups (SAS GLM, df = 31, F= 1.25, $P > 0.312$). Insect visitation was observed for 0.5 h out of every hour the stems were exposed to visitation, giving an approximate visitation level of 1.3 visits per flower for the half-day exposure group and 10.9 visits per flower for the full-day exposure group. Number of seeds per fruit were compared using the Nonparametric Multiple Comparisons Test (Sokal and Rohlf 1981). The SAS NPAR1WAY nonparametric analysis procedure (SAS/STAT 1990) was used to develop the relationship between visitation and seeds set per fruit. *T*-tests were used to compare seed production between the 10.9 visits (full-day exposure) group and the unlimited exposure group. When variances were unequal based on the F-max test (Sokal and Rohlf 1981), a heteroscedastic *t*-test was performed.

In 1998, 82 plants in the test plot were randomly assigned to one of 14 controlled exposure groups (1–14 visits per flower), zero exposure, unlimited exposure, and unclipped groups. Plants were systematically exposed to insect visitation during a two-day period starting at 1400 h on 28 May. I removed all the pollinator exclusion bags (except the zero exposure group) and counted pollinator visits until the entire group had received an average of one visit per flower (827 visits to the patch then containing 827 flowers). Then the first exposure group, one visit per flower, was rebagged. The remaining exposed flowers were counted, insect visits were monitored, and a second group of flowers was rebagged as the two visits per flower group once the flowers had received an average of one additional visit, and so on. This procedure was continued until flowers in the final group had received an average of 14 visits each. Stems in the unlimited exposure group continued to receive visitation until the last flowers dropped, 10 days after the controlled visitation experiment.

In 1997 I collected stems and fruits from populations exposed to natural visitation at BEL and GRO (see Table 1 for site descriptions) to compare seed production and germination with the test plot plants.

Insect Visitation Rates and Seed Set in Natural Populations

At CC and LLRP on 10 and 11 June 1993, I clipped all but four flowers with extended stigmas per stem on stems that had been bagged in bud. Flowers were exposed to visitation for one, two, or three days at each site except for the zero exposure group (bagged for the entire time) and the unlimited exposure group (never bagged). Exposure to visitation was interrupted by two rainy days at each site; plants were left bagged until the weather cleared. Visitation rates were estimated from observations on three days at each site for a total of 20 15-minute observations at CC and 24 observations at LLRP. Visitation to the unlimited exposure group was not estimated.

Number of seeds produced per fruit by treatment was log-transformed to improve normality and homoscedasticity. Exposure groups were compared using Tukey's Studentized Range Test, and the relationship between visitation and number of seeds per fruit was developed using the General Linear Models Procedure.

Estimation of Visitation Rates and Number of Visits per Flower in Natural Populations

Insect visitation rates were observed in native and reconstructed prairie sites (Table 1). All sites are within the natural range of the species (Great Plains Flora Association 1986) and have sandy soil. The study sites are surrounded by farms, woodlots, and suburban housing developments.

Table 1. *Penstemon grandiflorus* (PGR) study sites in Minnesota, USA. Clusters were separated by at least 30 m.

Site	Location	Type	Dates of Observations	No. PGR Stems	PGR Dispersion
Afton State Park ASP	Washington County	tallgrass reconstruction planted 1982	1990	19	single cluster
Belwin Outdoor Education Center BEL	Washington County	sand prairie	1997	880	single cluster
Cedar Creek Natural History Area CC	Anoka County	oak savanna	1990	210	1 large and 8 small clusters
			1991	331	
			1993	300	
Crow Hassan Park Reserve CHR	Hennepin County	sand prairie reconstruction planted 1980	1990	100	
			1991	130	3 clusters
Cottage Grove Ravine Regional Park GRO	Washington County	degraded oak savanna	1997	33	4 clusters in woods
Long Lake Regional Park LLRP	Ramsey County	sand prairie reconstruction planted 1987	1993	1350	single cluster
Test Plot	Ramsey County	test garden	1997	40	single cluster
			1998	90	

I counted flower entries by insects for 15-minute periods, then calculated number of visits per flower. To estimate the proportion of visits that occurred at different times of day, and to determine the best times to observe visits, I observed visitation from 0700 h to 2000 h for three days in 1990, one day each during the peak blooming season at ASP, CC, and CHR. In 1991, 1993, and 1997, I made two or three 15-minute visitation rate observations per hour of as many plants as I could easily observe at one time, usually no more than 15 stems, between 0900 h and 1600 h. I recorded date, time, temperature, cloud cover, and wind speed during each observation. In 1990, I observed visitation under a variety of weather conditions and later restricted observations to sunny days with the temperature at least 19° C, when the majority of insect visits occurred. Mean number of visits for each site and year were calculated based on daily visitation rates.

Mean visitation rates were used to calculate visits per flower per hour for each hour of the day. These values were multiplied by the proportion of daily visits made during that hour to calculate total visits for each day. To calculate how many visits whole plants received during the two-week blooming period, I multiplied the daily total by the number of warm sunny days that occurred during the blooming season for each site and year, using field notes supplemented by historical climate records from the University of Minnesota Soils, Weather and Climate Department website (*http://www.soils.edu*). Three to ten days were sunny and warm enough for insect visitation during the nine two-week blooming seasons observed between 1990 and 1997.

Seed Collection and Germination Tests

Stems were collected in late August of each year when the fruits were mature. I recorded the number of fruits, aborted flowers (those not developing into fruits), and missing or damaged fruits, and counted the seeds in each fruit. Seeds from all fruits on the stem were mixed and 100 seeds were tested for germination (if less than 100 seeds were produced, all were tested). Seeds were moist-stratified at 5° C for one month, then exposed to natural light in a cool greenhouse. Germinated seeds were counted and removed regularly for six weeks in 1997. Some seeds became moldy near the end of the six weeks 1997. In 1998 I pre-treated seeds with Captan® fungicide before the germination tests. Germination was slow and low in 1998, possibly due to the fungicide, so I observed the seeds for 18 months. Percentage germination was analyzed in the same way as seeds per fruit.

Table 2. Mean (± SD) number of flowers per stem, seeds per fruit, percent of total flowers aborted, and percent seed germination for plants exposed to unlimited visitation at three Minnesota *Penstemon grandiflorus* sites in 1997. Values with the same letter are not significantly different at the *P*=0.05 level by the GLM procedure (SAS). Stems collected from BEL and GRO were matched for number of flowers. See Table 1 for site abbreviations.

Site	No. Stems	No. Flowers per Stem	No. Seeds per Fruit	% Flowers Aborted	% Seed Germination
test plot	8	26.9 (7.2)a	93.0 (8.1)a	41.0 (10.7)a	58.0 (24.7)a
BEL	24	16.7 (8.7)b	55.5 (28.8)b	42.7 (16.5)a	27.6 (20.3)b
GRO	13	16.4 (8.7)b	59.6 (46.4)ab	43.4 (13.5)a	43.2 (26.2)ab

RESULTS

Flowering Phenology

Most flowers opened within a two-week period, with the peak flowering occurring during the first few days of the flowering period (Figure 1). Flowers opened in groups of two or four, with large plants having up to 12 flowers open on a single day. Plants in the test plot produced significantly more flowers per stem in 1997 than those in two native sites (GRO and BEL)

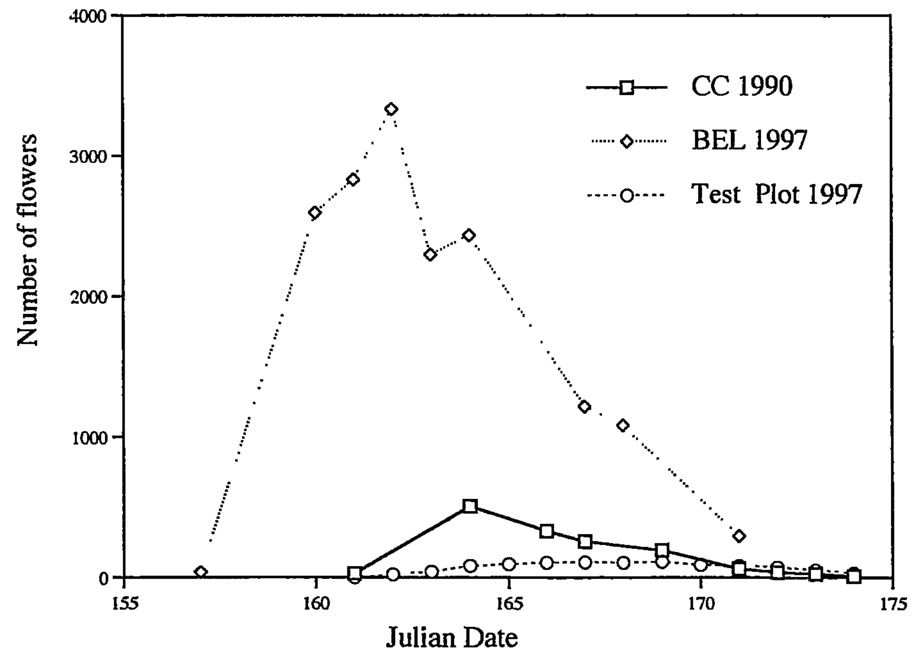

Figure 1. Number of flowers open by date. Julian date 165 is 14 June. See Table 1 for site abbreviations.

Table 3. Visitation rates per flower per 15 minutes and visits per flower per day, by sites and years at Minnesota *Penstemon grandiflorus* (PGR) sites (means ± SD).

Site	Year	No. Observations on Warm Sunny Days	Visits per Flower per 15 Minutes	Total Visits per Flower per Day	Total Sunny Days During PGR Blooming Season
ASP	1990	29	0.8 ± 0.6	27.4 ± 5.1	7
BEL	1997	69	0.7 ± 0.5	21.6 ± 6.9	9
CC	1990	52	1.2 ± 1.3	33.8 ± 18.2	7
CC	1991	35	0.5 ± 0.5	7.8 ± 4.9	10
CC	1993	29	0.4 ± 0.5	4.0 ± 5.5	3
CHR	1990	7	2.6 ± 1.0	52.9 ± 32.8	7
CHR	1991	35	1.0 ± 0.6	30.4 ± 9.9	9
GRO	1997	34	0.9 ± 0.9	46.2 ± 15.8	9
LLRP	1993	8	0.1 ± 0.3	3.75 ± 1.0	5

(Table 2). Plants that were clipped or bagged and did not produce seeds during the normal blooming season usually produced additional buds in July.

Flower Visitors

Penstemon grandiflorus flowers were visited by queen bumblebees, especially *Bombus fervidus* (Fabricius) and *B. auricomus* (Robertson); anthophorids, especially *Synhalonia* Patton spp.; megachilids, especially *Osmia* spp.; halictids, including *Augochlorella* Sandhouse spp.; Lepidoptera; syrphid flies; and occasional ruby-throated hummingbirds (*Archilochus colubris* [L.]). Nectar robbing occurred in some sites. Newly planted test plots were visited largely by bumblebees and halictid bees; plants in established reconstructions and native sites also received visits from other solitary bees.

Visitation Rates

During a typical observation period a single bee visited many flowers in a single cluster over a few minutes, then left the cluster. This pattern often produced a high variation in visitation rates on the same day for the same flower cluster. For example, visitation rates per flower to a cluster of 32 flowers at CHR were 0.5, 1.5, 1.1, and 1.8 during four 15-minute observations on 5 June 1991. Visitation rates were highly variable within and among days on the same site and season: for example, mean visitation rates per flower per 15 minutes were 2.4, 1.5, 1.1, 0.9, 0.4, and 0.3 on six observation days at CC in 1990.

Visitation rates ranged from 0 to 4.3 visits per flower per 15-minute sampling period. Visitation was markedly reduced at temperatures below 20° C, even on sunny days, and flowers were not visited while rain was falling. Visitation rates increased with temperature between 20°C and 34° C (Figure 2). Insects made 18% of their visits between 0700 h and 0900 h, 64% of visits between 1000 h and 1600 h, 7% between 1600 h and 2000 h, and less than 1% of visits before 0700 h and after 2000 h. Total visits per flower per day on warm sunny days ranged from 3.6 ± 1.0 SD to 52.9 ± 32.6 SD (Table 3).

Visitation rates varied among sites and years. Plant populations of intermediate size tended to receive more visits per flower than large or small populations. Plants in the small populations at GRO and ASP did not receive significantly fewer visits per flower than those in the large populations at LLRP and BEL (Figure 3). No consistent differences in visitation rates were found in native populations (CC, BEL, GRO) compared to those in reconstructed prairies (ASP, CHR, LLRP). Visi-

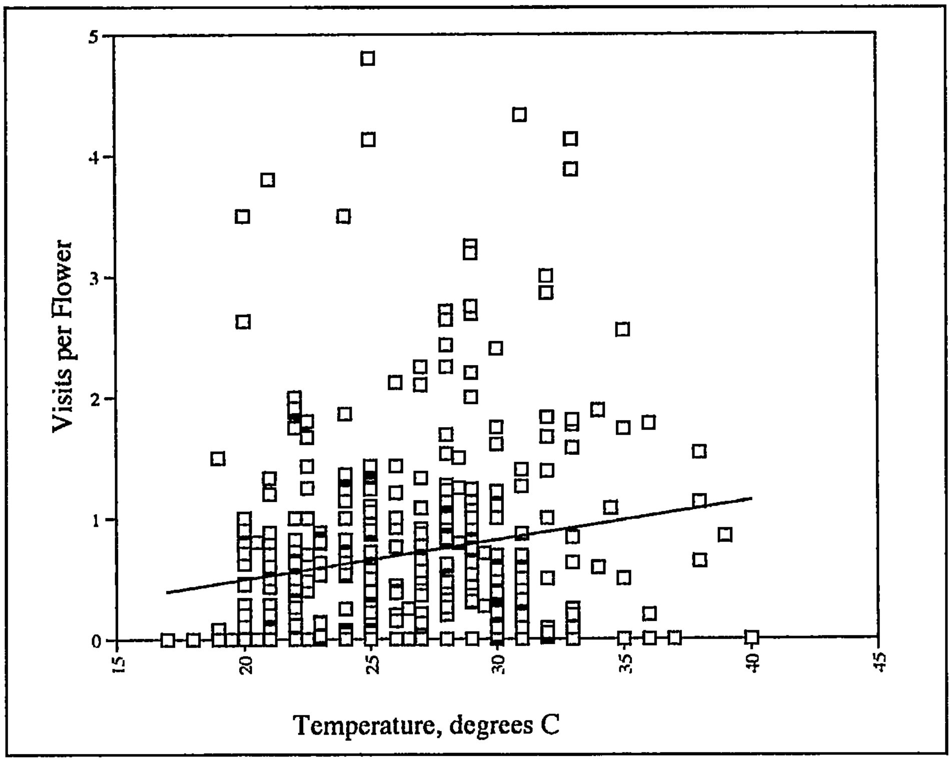

Figure 2. Effect of temperature on visits per flower per 15-min observation for all sites and years. N = 379; $y = 0.033x$; $r^2 = 0.032$; $P < 0.01$.

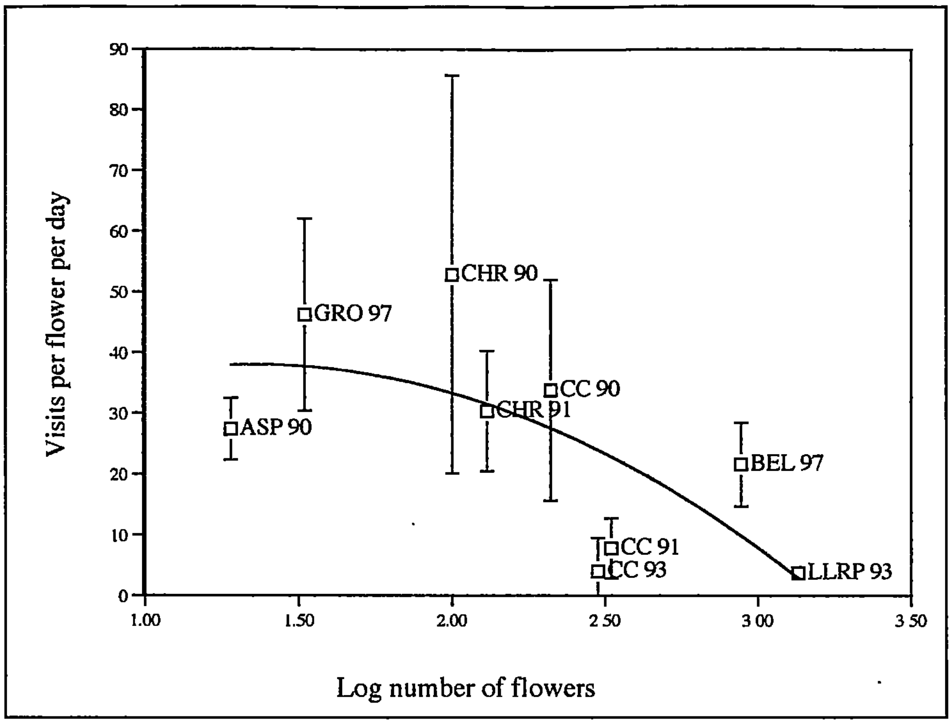

Figure 3. Effect of plant population size on visits per flower per day (means ± SD); $y = -11.119x^2 + 17.612$; $r^2 = 0.460$; $P < 0.05$.

Table 4. Insect visitation, percent flower abortion, and number of seeds per fruit in Minnesota *Penstemon grandiflorus* sites in 1993 and 1997 (means ± SD). 1993 stems were clipped to four flowers each. Within groups, values with the same letter are not significantly different at the 0.05 level by the GLM procedure, values log-transformed in 1993, nonparametric multiple comparisons test in 1997. Results of the GLM procedure for each group on the seeds-per-fruit variable:
CC: df = 4, SS= 162.9, F=42.0, $P < 0.001$
LLRP: df = 4, SS = 50.56, F = 12.56, $P < 0.001$
test plot 1997: df =3, SS = 55.16, F=12.85, $P < 0.001$

Sites, Dates, and Groups	No. of Stems	Mean % Flower Abortion	Mean Number of Seeds per Fruit
CC 1993			
zero visitation	13	98.0 ± 9.4 (a)	0.05 ± 0.18 (a)
1.8 visits	14	9.7 ± 19.9 (b)	54.4 ± 33.0 (b)
2.6 visits	16	19.6 ± 18.7 (b)	50.6 ± 29.0 (b)
9.5 visits	10	23.5 ± 32.7 (b)	66.2 ± 45.0 (b)
unlimited visitation	13	5.5 ± 12.3 (b)	111.5 ± 24.0 (c)
LLRP 1993			
zero visitation	4	75.0 ± 35.3 (a)	3.8 ± 5.0 (a)
2.6 visits	30	21.0 ± 24.6 (b)	54.7 ± 42.0 (b)
5.3 visits	9	27.7 ± 31.3 (b)	49.1 ± 32.0 (b)
7.8 visits	13	3.8 ± 9.4 (c)	104.7 ± 28.0 (c)
unlimited visitation	14	2.5 ± 6.6 (c)	104.0 ± 34.0 (c)
Test plot 1997			
zero visitation	8	65.3 ± 17.8 (a)	21.7 ± 17.9 (a)
1.3 visits	8	65.0 ± 12.5 (a)	62.1 ± 42.0 (ab)
10.9 visits	8	65.7 ± 16.0 (a)	95.7 ± 28.9 (b)
unlimited visitation	8	41.0 ± 10.7 (b)	93.6 ± 8.0 (b)

tation rates at CHR were significantly higher than those at CC in both 1990 ($X^2 = 9.21$, df = 1, $P = 0.0024$ using the Wilcoxon rank sum test) and 1991 ($X^2 = 37.3$, df = 1, $P = 0.001$).

Effects of Insect Visitation

Flower Abortion Rates

Flower abortion rates decreased with increasing visitation levels in 1998 up to approximately six visits per flower ($r^2 = 0.47$ for the log relationship y= -31.72 log[x]+ 83.50, $P < 0.01$) (Figure 4). In the group that received 6–14 visits per flower, an average of 52% of flowers aborted; some of these had received no visits because they opened after the plants were rebagged. Total seeds produced by plants at each treatment level was more closely related to number of seeds per fruit than to flower abortion rate (Figure 4). In the test plot in 1997, the unlimited visitation group had the lowest abortion rate: other differences were not statistically significant (Table 4).

Flower abortion rates in 1993 field tests (where stems were clipped to four flowers each) also decreased with increasing insect visitation (Table 4). Flower abortion rates of unbagged stems were similar at BEL, GRO, and the test plot in 1997 (Table 2) and 1998 (40% abortion ± 16.2 SD).

Flower Longevity

Flowers exposed to unlimited visitation remained on the plant only 2.5 days (N = 204, SD = 0.86). Flowers on stems that were bagged (zero exposure group) remained on the plant for a mean of 4.1 days (N = 166, SD = 1.13), significantly longer (2-tailed heteroscedastic *t*-test t= 9.40, df =1, $P < 0.001$). Flowers producing the most seeds were the earliest to fall (Table 5).

Seed Germination

Percent germination increased with insect visitation (Figure 5) in both 1997 and 1998. Seed germination of the unlimited exposure group in 1997 was 58% ± 25.1 SD, not significantly different from the 10.3-visit group. Likewise, germination by the unlimited exposure group in 1998 was not

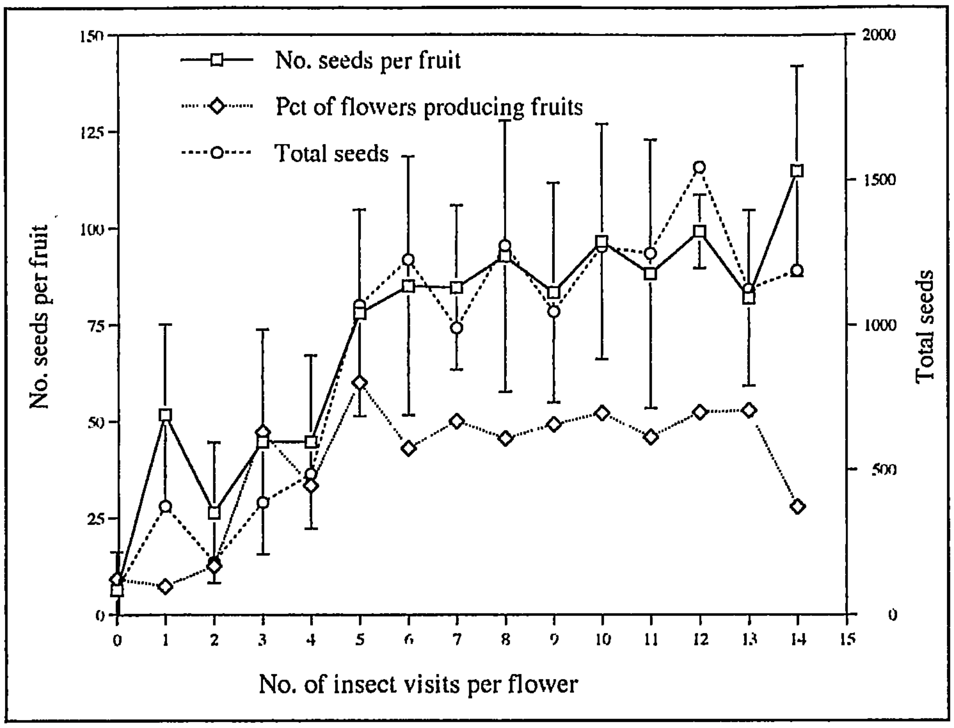

Figure 4. Effect of insect visitation on number of seeds per fruit, percent of flowers producing fruits, and total seeds per stem produced in 1998. Values are means ± SD. Percent of flowers producing fruit is the inverse of the flower abortion rate. Percent of flowers producing fruits ranged from 7.2 to 52.6 .

significantly higher than germination by the 13- or 14-visit group (29.3% ± 13 SD vs. 26.3% ± 13 SD). Seeds from natural populations at BEL and GRO in 1997 tended to show lower seed germination than seeds from the plants in the test plot in 1997 (Table 2).

Seed Production

Seed production measured as number of seeds per fruit increased with visitation (Table 4). Variance in seed production within treatment groups was high. Additional visits above 7.8 per flower (LLRP) or 10.9 per flower (test plot 1997) did not lead to increased seed production, whereas at CC additional visits above 9.5 per flower significantly increased seed production.

Table 5. Seed production and flower lifespan, from open to drop, unlimited exposure group, *Penstemon grandiflorus* test plot 1997 (means ± SD). r^2= 0.85, $P < 0.05$, for linear model y= -18.277x +111.59 .

Flower Lifespan (days)	No. Seeds per Flower	No. Flowers
1	102.2 ± 54.2	11
2	71.8 ± 52.3	83
3	42.6 ± 51.0	75
4 or 5	38.8 ± 49.9	35

In 1998 in the test plot the number of seeds per fruit was also highly correlated with visitation rate (Figure 4). Analysis of residuals indicated that a quadratic model would give the best fit. For the whole data set, r^2= 0.86 for $y = -0.598x2 + 11.858x - 0.118$, $P< 0.01$. I performed a split regression to determine the minimum visits needed for maximum seed production: using 0 to 6 visits, a linear relationship was found where r^2=0.86, $y=8.197x +2.490$, $P<0.01$. The values for 7 to 14 visits gave $r^2 = 0.03$, $y=-0.489x + 59.954$, which is not significant. This indicates that visitation significantly increases seed production up to six visits, whereas additional visits above this level do not increase seed production. Including number of flowers per stem in the regression as a measure of the size and resource level of stems did not improve the fit of the model.

Seed Production and Day of Opening

For each stem, the flowers that opened on the first day of bloom in 1997 set 40% of the total seeds produced by the stem; flowers opening during the first four days produced 89% of the total. Flowers opening eight or more days after the first flower on the stem produced no seeds. Seeds per flower decreased dramatically from days 1 and 2 through day 8 (Figure 6).

In the test plot as a whole in 1997, seed production was highest by flowers that were open on the second day of bloom, and declined thereafter. The first two days of bloom in the plot accounted for 47% of the total seeds, the first four days of bloom accounted for 82% of the seeds produced, and flowers opening during the last four days of the 13-day season produced only 0.5% of the total seeds (Figure 6).

Seed Production after the Experiment

In 1998 the unlimited exposure stems produced on average 500 more seeds per stem than stems in the 13- and 14-visit exposure groups, due to increased fruit production: 79% of the flowers in the unlimited exposure group developed into fruits, compared to 57% of flowers in the 13- and 14-visit exposure groups combined (heteroscedastic t-test, 1-tailed, df = 10, t = 2.75, P=0.02). Likewise in 1997, stems in the unlimited exposure group produced on average almost 500 more seeds per stem than the 10.9 visits per flower group: 57% of flowers in the unlimited exposure group developed into fruits compared to 34% in the 10.3-visits-per-flower group (heteroscedastic t-test, 1-tailed, df= 14, t=4.05, $P < 0.01$). There were no significant differences in number of seeds per fruit between the unlimited exposure groups and the highest controlled exposure groups in either year.

Frequency of Pollinator Limitation in Field Sites

In the test plot, approximately six visits to

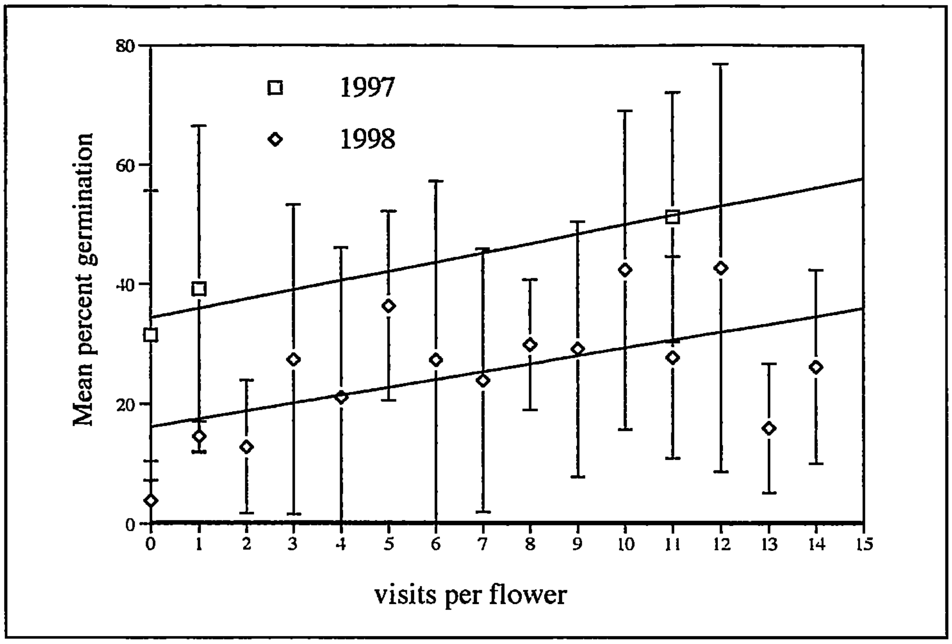

Figure 5. Effect of visits per flower on percent seed germination (means ± SD). Seeds from plants with unlimited visitation had 58.0 ± 24.7% germination in 1997, and 25.2 ± 13% germination in 1998. Regression lines are shown; $y = 1.553x + 34.448$; $r^2 = 0.904$, $P < 0.01$, for 1997; $y = 1.316x + 16.266$; $r^2 = 0.302$, $P < 0.05$, for 1998.

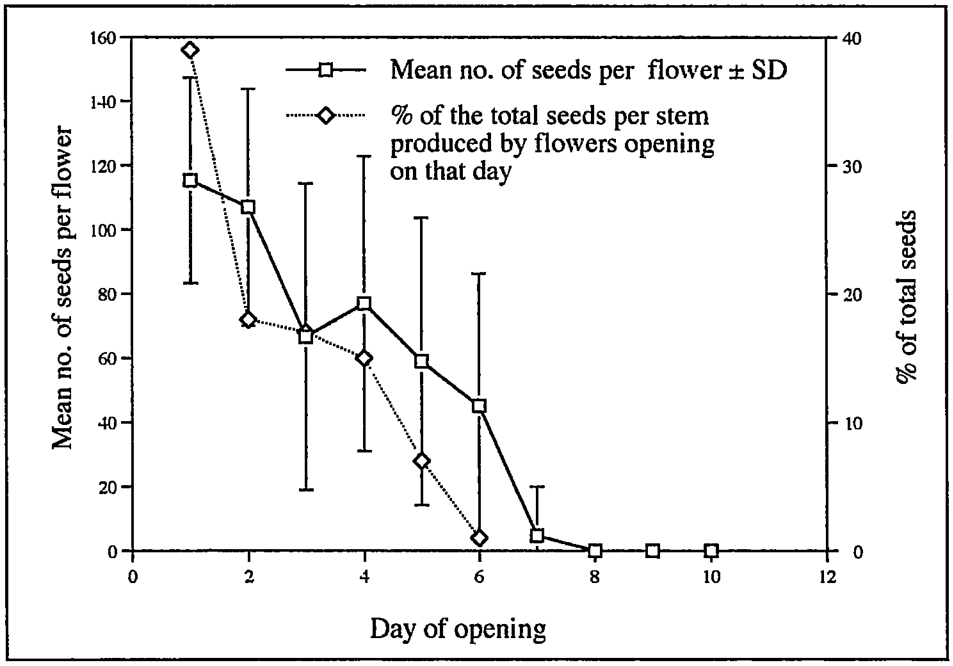

Figure 6. Day of opening and seed production, 1997, test plot. Flowers opening on day 1 were the first to open on the stem.

flowers during a single day resulted in full seed production, fruit production, and seed germination for flowers open on that day. Flowers opening during the first four days of bloom produced almost 90% of the total seeds. Therefore any plant that received approximately six visits per flower per day for four days could produce at least 90% of the seeds its resources would permit. Seven of the nine site-year observations greatly exceeded this combination of visitation level per day and number of warm sunny days during the blooming season (Table 3). CC in 1993 did not have enough sunny days and LLRP in 1993 had relatively low insect visitation over the blooming season based on a combination of low visitation rates and a lack of sunny days. CC in 1993 was the only experimental site and year where three days exposure to visitation was inadequate for maximum seed production.

DISCUSSION

Penstemon grandiflorus populations concentrate their flowering into a short period, are heavily visited by insects, and complete most of their seed set within a few days. Approximately six insect visits are required for maximum production of viable seeds by individual flowers. Additional visits do not increase seed production, whereas fewer visits result in reduced fruit production, reduced seed production per fruit, and reduced seed germination.

Contrary to expectations, visitation rates to individual flowers were generally higher than those needed for maximum seed production, for plants in large and small populations and in native and reconstructed prairies. Reduced visitation over the blooming season occurred on sites with low visitation rates in rainy springs.

Variation in responses to controlled visitation rates among plants in the different sites and among individuals in the test plot may be influenced by insect behavior and plant genetics. The effectiveness of insect visits may vary due to variation in pollen loads brought by individual insects and species (Herrera 1987, Tepedino et al. 1999). The mobility of pollinators within and among patches and inflorescences can influence the effectiveness of their visits. If a plant population is highly inbred, seed production may be reduced when pollinators with limited mobility transfer pollen mainly among close relatives (Aspinwall and Christian 1992, Wolf and Harrison 2001). In such cases, plants may require more visits to set seed than those in highly heterozygous populations (Krannitz and Maun 1991).

What Limits Seed Production by *P. grandiflorus*?

In general, seed production is limited by pollinator activity, plant resources, or the number of ovules in flowers (Zimmerman and Pyke 1988). Seed production by *Penstemon grandiflorus* flowers at the higher visitation levels was not pollen limited, as indicated by the fact that additional insect visits did not increase seed production. Ovules did not appear to be limiting because many flowers failed to develop into fruits: in particular, the top two flowers on the stem never produced any seeds (pers. obs.). The experimental stems were not resource limited at one day's exposure: similar stems with unlimited exposure were able to produce additional seeds from flowers that opened later. Overall, the pattern of declining seed production by later flowers compared to earlier ones suggests that each plant allocates a certain level of resources to seed production, then inhibits the development of further seeds.

Conservation Implications

Penstemon grandiflorus capitalizes on occasional bouts of high levels of insect visitation to produce nearly maximum seed set even when visitation occurs during only part of the plant's short blooming period. It is well adapted to the widely fluctuating visitation levels occurring during the uncertain spring weather of the midwestern United States, responding to high visitation with rapid seed set, and responding to low visitation by some self pollination, extended longevity of unvisited flowers, and production of new buds when no seeds have been produced previously.

Seed production is essential for *P. grandiflorus* survival because individuals are relatively short-lived and do not reproduce vegetatively. The strategy of highly attractive flowers and concentrated blooming by individual plants and groups within local populations seems to be successful in allowing full seed production in most sites and years. The risk of pollinator limitation is reduced by allocating a high proportion of resources to showy flowers (including many that will not set seed) that attract pollinators. This high allocation can be maintained for only a short period, but availability of efficient pollinators that have few other plant species to visit makes a short bloom period adequate. Pollination by common generalists as well as specialists helps to ensure adequate pollination in this species.

Annual, seasonal, and local variation in populations of bees and their abundance at flowers have been demonstrated in many studies (Herrera 1988, Cane and Payne 1993, Williams et al. 2001). Populations and percent of ramets flowering vary from year to year among wild plant species (Hendrix and Kyhl 2000). Visitation rates to plants vary among sites and years (Parker 1997). Consequently, pollen limitation varied among times, sites, and years in the studies reviewed by Burd (1994).

There appear to be differences in visitation levels to flowers among the site-year combinations once time of day and weather are accounted for. These differences are not related to *P. grandiflorus* population sizes or to native versus reconstructed prairie status. Differences in visitation rates may be related to insect population densities, behavior, and mobility on and off the sites. Bumblebees in particular are highly mobile, repeatedly revisit the same sites over a few days, and are generally faithful to rich floral resources (Heinrich 1979). Bumblebees visited the test plot as soon as flowers opened, although bees had not been seen there earlier in the season. The mobility of specialist bees has not been as well studied, but they have become established on prairie reconstructions where their plant species have been reintroduced (Reed 1995).

To predict and identify pollinator limitation for sites and plant species, we need to understand plant reproductive ecology and responses to visitation over a range of visitation levels. It is important to determine insect visitation levels during the period when seed set occurs. Seed viability can be tested and may give clues to levels of inbreeding in fragmented populations. Pollinator ecology is also important. In particular, we need to know whether pollinators complete their life cycles near the plants they visit or come in from other areas, which plant species and populations support pollinators throughout their life cycles, and what other habitat features pollinators require (Cane 2001). Landscape-level management may be required to ensure that plant and pollinator populations are maintained as land surrounding protected areas is increasingly altered by human activities.

ACKNOWLEDGMENTS

I thank Mark Davis, who first introduced me to this lovely plant; the many site managers who helped me locate *P. grandiflorus* populations; Dave Andow, Marla Spivak, Roger Moon, Theresa Dzuik, Arathi, Ian Burns, Diane Larson, and Nancy Shellhorn for all their advice and help; Bill Miller for reading the manuscript; insect taxonomist John Haarstad; and the anonymous reviewers. This study was funded in part by a grant from the Minnesota Department of Natural Resources Nongame Wildlife Division.

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LITERATURE CITED

Allen-Wardell, G., and 22 others. 1998. The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. Conservation Biology 12:8-17.

Aspinwall, N., and T. Christian. 1992. Pollination biology, seed production, and population structure in queen-of-the-prairie, *Filipendula rubra* (Rosaceae) at Botkin Fen, Missouri. American Journal of Botany 79:488-494.

Bond, W.J. 1995. Assessing the risk of plant extinction due to pollinator and disperser failure. Pp. 131-146 *in* J.H. Lawton and R.M. May, eds., Extinction Rates. Oxford University Press, Oxford.

Burd, M. 1994. Bateman's Principle and plant reproduction: the role of pollen limitation in fruit and seed set. Botanical Review 60:83-139 .

Cane, J.H. 2001. Habitat fragmentation and native bees: a premature verdict? Conservation Ecology (online) 5:3. [www.consecol.

org/vol5/iss1/art3]

Cane, J.H., and J.A. Payne. 1993. Regional, annual and seasonal variation in pollinator guilds. Annals of the Entomological Society of America 86:577-588.

Clinebell, R.R., II., and P. Bernhardt. 1998. The pollination ecology of five species of *Penstemon* (Scrophulariaceae) in the tallgrass prairie. Annals of the Missouri Botanical Garden 85:126-136.

Crosswhite, F.S., and C.D. Crosswhite. 1966. Insect pollinators of *Penstemon* Series *Graciles* (Scrophulariaceae) with notes on *Osmia* and other Megachilidae. American Midland Naturalist 76:450-467.

Davis, M.A., J. Villinski, K. Banks, J. Buckman-Fifield, J. Dicus, and S. Hofman. 1991. Combined effects of fire, mound building by pocket gophers, root loss and plant size on growth and reproduction in *Penstemon grandiflorus*. American Midland Naturalist 125:150-161.

Great Plains Flora Association. 1986. Flora of the Great Plains. University Press of Kansas, Lawrence. 1402 pp.

Heinrich, B. 1979. Bumblebee Economics. Harvard University Press, Cambridge, Mass. 245 pp.

Hendrix, S.D., and J.F. Kyhl. 2000. Population size and reproduction in *Phlox pilosa*. Conservation Biology 14:304-313.

Herrera, C.M. 1987. Components of pollinator "quality:" comparative analysis of a diverse insect assemblage. Oikos 50:79-90

Herrera, C.M. 1988. Variation in mutualisms: the spatio-temporal mosaic of a pollinator assemblage. Biological Journal of the Linnean Society 35:95-125.

Johnston, M.O. 1991. Pollen limitation of female reproduction in *Lobelia cardinalis* and *L. siphilitica*. Ecology 72:1500-1503.

Kearns, C.A., and D.W. Inouye. 1997. Pollinators, flowering plants, and conservation biology. BioScience 47:297-307.

Krannitz, P.G., and M.A. Maun. 1991. An experimental study of floral display size and reproductive success in *Viburnum opulus*: importance of grouping. Canadian Journal of Botany 69:394-399.

Lawson, H.R., V.J. Tepedino, and T.L. Griswold. 1989. Pollen collectors and other insect visitors to *Penstemon haydenii* S. Wats. Pp. 233-235 *in* T.B. Bragg and J. Stubbendieck, eds., Proceedings of the Eleventh North American Prairie Conference. University of Nebraska, Lincoln.

Parker, I.M. 1997. Pollinator limitation of *Cytisus scoparius* (Scotch broom), an invasive exotic shrub. Ecology 78:1457-1470.

Petanidou, T., J.C.M. den Nijs, and J.G.B. Oostermeijer. 1995. Pollination ecology and constraints on seed set of the rare perennial *Gentiana cruciata* L. in The Netherlands. Acta Botanica Neelandica 44:55-74

Rathcke, B.J., and E.S. Jules. 1993. Habitat fragmentation and plant-pollinator interactions. Current Science 65:273-277.

Reed, C.C. 1995. Insects surveyed on flowers in native and reconstructed prairies (Minnesota). Restoration and Management Notes 13:210-213.

SAS/STAT.1990. Statistics Program. SAS Institute, Cary, N.C.

Sokal, R.R., and F.J. Rohlf. 1981. Biometry. 2nd Ed. W.H. Freeman and Co., San Francisco, Calif. 859 pp.

Tepedino V.J., S.D. Sipes, J.L. Barnes, and L.L. Hickerson. 1997. The need for "extended care" in conservation: examples from studies of rare plants in the western United States. Acta Horticulturae 437:245-248.

Tepedino, V.J., S.D. Sipes, and T.L. Griswold. 1999. The reproductive biology and effective pollinators of the endangered beardtongue *Penstemon penlandii* (Scrophulariaceae). Plant Systematics and Evolution 219:39-54.

Thomson, J. D. 2001. Using pollination deficits to infer pollinator declines: can theory guide us? Conservation Ecology (online) 5:6. [http://www.consecol.org/vol5/iss1/art6]

Williams, N.M., R.L. Minckley, and R.A. Silveira. 2001. Variation in native bee faunas and its implications for detecting community changes. Conservation Ecology (online) 5:7. [http://www.consecol.org/vol5/iss1/art7]

Wolf, A.T., and S.P. Harrison. 2001. Effects of habitat size and patch isolation on reproductive success of the serpentine morning glory. Conservation Biology 15:111-121.

Zimmerman, M., and G.H. Pyke. 1988. Reproduction in *Polemonium*: assessing the factors limiting seed set. American Naturalist 131:723-738.