

Pollination Ecology of the Rare Orchid, *Spiranthes diluvialis*: Implications for Conservation

KIM PIERSON,¹ VINCENT J. TEPEDINO,² SEDONIA SIPES,³ and KIM KUTA⁴

¹Payette National Forest, McCall, Idaho

²USDA ARS Bee Biology and Systematics Laboratory, Utah State University, Logan

³Department of Entomology, Cornell University, Ithaca, New York

⁴Stokes Nature Center, Logan, Utah

Abstract: We examined the pollination ecology of *Spiranthes diluvialis* Sheviak, Ute ladies-tresses, a federally listed, threatened orchid species known only from small, isolated populations in the western United States. The pollinator composition, male and female reproductive success, and demography of *S. diluvialis* populations were examined in 1995, 1997, and 1999. *Spiranthes diluvialis* sets fruit only when visited by pollinators and observations indicate that native bees, predominantly bumblebees (*Bombus* spp.), are the most important pollinators. Comparisons of male and female reproductive success were made between populations and years. Significant declines in fruit production and pollinia removal that occurred in 1999 in the Diamond Fork population may be related to changes in pollinator composition. Significant increases in fruit production were recorded in the Brown's Park population, an area in which native pollinators were released in 1999. Management plans to conserve this threatened orchid must provide for the pollinators, their nesting habitat, and pollen-producing plants (*S. diluvialis* provides no pollen to pollinators). We identified important pollen sources for pollinators in the Diamond Fork population and supplied several types of semi-natural nesting materials to promote nesting by *Anthophora terminalis*, another species thought to be an important pollinator. Successful preservation of this threatened orchid requires a community-level conservation plan.

Evolutionary ecologists have hypothesized that as an outcrossing common plant species becomes rare, its attraction to pollinators and its dependence on conspecifics for sexual reproduction also declines (Tepedino 1979, Kruckeberg and Rabino-witz 1985). Such rare species are expected to be characterized by high levels of self-compatibility, autogamy, and possibly even agamospermy (Tepedino 1979). However, recent studies of the reproductive biology and pollinator composition of more than two dozen rare plant species in the western United States (Tepedino 2000) have provided compelling evidence that rare plant species are as dependent upon pollinators as common plants are, and that native bees are their primary pollinators.

Spiranthes diluvialis (Orchidaceae), a federally listed, threatened orchid species known only from small, isolated riparian populations in the western United States, provides a unique opportunity to examine such questions about rare plant pollination ecology. The floral morphology, presence of pollinia, and lack of pollen rewards for insect visitors combine to facilitate studies of pollination. Flowers of *S. diluvialis* are arranged spirally on a single spike, with flowering commencing at the

bottommost flower and proceeding upward sequentially. Like most orchid species, *S. diluvialis* flowers have male and female parts fused into a column, with the stigmatic surface on the ventral side of the column and the single anther containing pollinia (packages of pollen) on the dorsal side. Each flower has a pollinarium composed of two joined pollinia attached to a viscidium, which when touched releases a sticky substance that promotes its removal. The large size of the pollinarium makes removal easy to census. Pollinia are bright yellow and can be detected on an insect's body from up to 2 m away (Sheviak 1984, Sipes 1995).

Previous studies of *S. diluvialis* have shown it to depend upon insect pollinators for sexual reproduction (Sipes and Tepedino 1995). Flowers are neither autogamous nor agamospermous. Primary pollinators are bumblebees (*Bombus* spp.) and solitary bees of the genus *Anthophora*, which carry the pollinia glued to their mouthparts, which they incidentally pick up while foraging for nectar (Sipes and Tepedino 1995). Of these species, *A. terminalis* Cresson was shown in 1995 to be a more effective pollinator than other insect visitors (Sipes and Tepedino 1995): Fruit production in areas where

A. terminalis was present displayed a three-fold increase over areas where it was absent.

All bee species have high nutritional and energetic needs. Adults primarily use nectar as fuel, but they collect pollen and nectar to feed their progeny. Because *S. diluvialis* offers only nectar as a reward to its pollinators, bees must visit other flowering species to collect pollen. These supplementary pollen sources must be identified to encourage their continued presence in *S. diluvialis* habitat.

In addition to pollen and nectar, bees require particular kinds of nesting materials in a matrix of appropriate habitat. Bees in general use a range of nesting habitats, but the behavior of individual species is quite stereotypical. The nesting requirements for important pollinators of *S. diluvialis* must be determined to enhance management efforts to augment nest sites as well as to support pollinator transplantation. Preservation of habitat for pollinator nesting is vital to the preservation of *S. diluvialis*.

Our objectives were (1) to further survey the insect species that pollinate *S. diluvialis* in Diamond Fork Canyon, Utah and to compare these with pollinators observed in previous studies (Sipes and Tepedino 1995, Kuta et al. 1997); (2) to compare the efficiency of pollinators observed visiting *S. diluvialis*; (3) to determine the primary

pollen source for *A. terminalis* and other important pollinators; (4) to describe patterns of male and female reproductive success as measured by pollinia removal and fruit set; and (5) to characterize the nesting requirements for pollinators of *S. diluvialis* and to determine if semi-natural habitats and nesting materials can be tailored to promote nesting of these pollinators.

Methods

The main study area was located in Diamond Fork Canyon, Utah County, Utah, where 36 colonies of *S. diluvialis* occur along the Diamond Fork River. In July of 1999 we selected colonies 1, 14, 21, and 35 for most of our experiments (Figure 1). These sites were selected because of population sizes and accessibility, and because they allowed comparison with results from Sipes and Tepedino (1995) and Kuta et al. (1997). Colonies 4, 9, 15, and 25 were also visited to obtain estimates of final reproductive success. Two additional visits were made to the Brown's Park population located on Forest Service (USDA) land along the Green River in Daggett County (see Figure 1).

We analyzed the data on two spatial scales. First, data from Diamond Fork colonies were pooled for comparison with Brown's Park. Second, data from the Diamond Fork colonies were compared among demes.

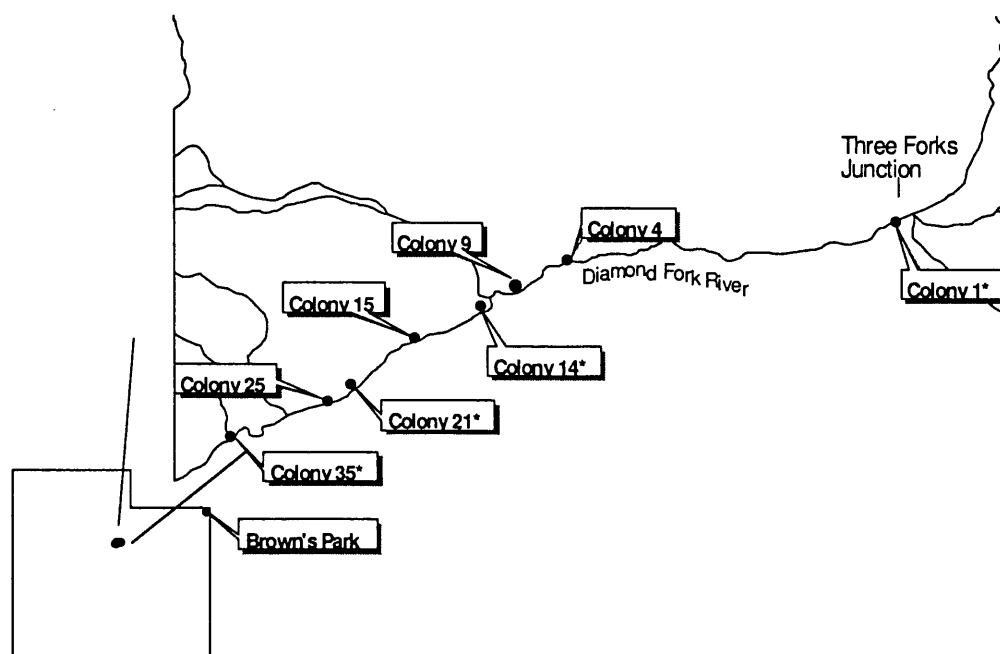


Figure 1. Distribution of *Spiranthes diluvialis* colonies in Diamond Fork Canyon and Brown's Park, Utah. Colonies used in pollination observation studies and reproductive trend studies indicated by an asterisk.

Pollinator Observation and Collection

Observations of floral visitors to *S. diluvialis* were made in colonies 1, 14, 21, and 35 throughout the flowering season. To estimate insect visitation rates and to determine pollinator species, we observed large patches of flowering *S. diluvialis* individuals for several 30-minute intervals daily between 0700 and 1800 hours. During observation intervals we recorded the taxon of the visitor (when possible), its foraging behavior on the inflorescence (acropetal or non-acropetal movement), the number of inflorescences visited, and whether or not the visitor carried pollinia of *S. diluvialis*. Several specimens of each taxon observed visiting *S. diluvialis* were collected for mounting and identification.

To test for the incidence of night pollination, 10 *S. diluvialis* individuals were marked and caged prior to floral bud initiation. Plants were caged during the day (dawn until dusk) and uncovered in the evening for the duration of flowering. Flowers were examined each morning for pollinia removal and pollen deposition. Plants remained caged for several weeks following these experiments to allow fruit development.

Comparison of Pollen Sources

A pollen reference collection was prepared by collecting pollen from all co-blooming plant species in colonies 1, 14, 21, and 35. Pollen from each species was stained on microscope slides with basic fuchsin gel (Beattie 1971).

Potential pollen sources for *S. diluvialis* pollinators were sampled by capturing insects visiting flowers of contemporaneously blooming plant species during scheduled intervals, and dabbing their bodies with a fuchsin-infused cube of gelatin that was then melted onto a microscope slide. Several transects were made through each slide, and pollen was identified and counted by comparison with the reference collection.

Nesting Requirements

We compared the efficacy of three types of semi-natural nesting materials in promoting the establishment of *A. terminalis*. Cut lengths of *Populus angustifolia* James logs (120 cm in length, 25 cm in diameter) were used to simulate downed wood. Twenty-five holes (15 cm deep, 8 mm diameter) were drilled in each end of the log. Elderberry twigs (*Sambucus* sp.) drilled with 8 mm diameter holes were used in short bundles (6 twigs per bundle, 15 cm in length) and as solitary uprights

(45 cm in length). Open areas and ecotone areas (defined as the junction of open and wooded areas) were selected for placement of materials. Five logs were placed in each selected colony (two in the open, three in the ecotone). Elderberry bundles were attached with heavy gauge wire to shrubbery in the ecotone and to upright sticks in the open where they were placed along transects at 5 m intervals. Single upright elderberry twigs were driven approximately 10 cm into the ground and placed along transects at 10 m intervals beginning in the open and continuing through the ecotone. Nesting materials were placed in colonies 1, 14, 21, and 35 in Diamond Fork Canyon and at the Brown's Park population.

All semi-natural nesting materials were collected following the completion of the *S. diluvialis* flowering season. All nesting materials except the logs were x-rayed to examine the nesting inhabitants.

Anthophora terminalis individuals found nesting in elderberry twigs in 1998 were released in the bundles at the Brown's Park population. These individuals were collected from the Brown's Park population in 1998 and were returned to the population in 1999.

Reproductive Success and Trends

The final reproductive success of individuals from colonies 1, 4, 9, 14, 15, 21, 25, and 35 was measured during the height of fruit set (September 13–23, 1999). To randomly select *S. diluvialis* individuals, a transect tape was placed through the colony and the closest individual to each meter mark was identified (Sipes and Tepedino 1995, Kuta et al. 1997). For each individual, we recorded the following information: the height of the inflorescence, the number of flowers per inflorescence, the presence or absence of pollinia for each flower, the production of fruit, the gender of those flowers that remained open, and the distances to the three nearest blooming conspecific neighbors. Final male and female reproductive success can be measured because floral parts and unremoved pollinia persist during fruit production.

The Brown's Park population was visited during the fruiting stage of *S. diluvialis* (Sept. 25, 1999). A transect was placed through the center of the colony and individuals were selected as above. We recorded inflorescence height, number of flowers, fruit set, pollinia removal, and the distance to the three nearest conspecific neighbors.

The number of flowers per inflorescence, inflorescence height, percent fruit set, and percent

pollinia removal were compared between the Diamond Fork colonies and the Brown's Park population for 1995 (Sipes and Tepedino 1995), 1997 (Kuta et al. 1997), and 1999 by using the Kruskal-Wallis non-parametric comparison (PROC NPAR1WAY, SAS Institute 1999). Data from the Diamond Fork colonies were pooled for comparisons between populations and years. The Diamond Fork data were also analyzed separately by colony to compare reproductive trends between colonies and between years (PROC NPAR1WAY, SAS Institute 1999). When significant differences between years occurred, post hoc Wilcoxon tests for unequal variances were used to explore these differences (PROC, NPAR1WAY WILCOXON, SAS Institute 1999).

Regression analyses (PROC REG, SAS Institute 1999) were used to determine if fruit set or pollinia removal were related to inflorescence height, number of flowers per inflorescence, or nearest neighbor distance. Data from the Diamond Fork colonies were pooled to compare years. These Diamond Fork data were also analyzed separately by colony to compare reproductive trends between colonies and between years. We used paired t-tests to test the null hypothesis that percent fruit set equaled percent pollinia removal within populations and colonies (PROC TTEST, SAS Institute 1999).

Results

Pollinator Observation and Collection

As in prior studies (Sipes and Tepedino 1995, Kuta et al. 1997), visitors to the flowers of *S. diluvialis* were seldom observed (Table 1). In more than 43 hours of survey, only 54 insects were seen visiting *S. diluvialis* flowers. Most visits were between 0900 and 1600 hours and the mean visitation rate for all visitors pooled was 0.41 (± 0.65 std dev) visits per plant per hour, and 2.86 (± 4.11 std dev) visits per plant per day.

Previously in Diamond Fork Canyon, Sipes and Tepedino (1995) found *A. terminalis* to be the most abundant and efficient pollinator of *S. diluvialis*. They also recorded *A. bombooides* and *A. urbana* as visitors. In 1999, however, we observed only two *Anthophora* individuals (both *A. terminalis*) within the entire Diamond Fork population. As in other years and at other *S. diluvialis* populations, we found bumblebees (*Bombus* spp., Table 1) to be the most abundant pollinators (Sipes and Tepedino 1995). Of these, *B. griseocollis* appeared to be the most abundant pollinator of *S. diluvialis*. We

Table 1. Insect visitors of *Spiranthes diluvialis* in 1999. All observations are from Diamond Fork Canyon.

Taxon	No. of observ.	Pollinia carried	Foraged bottom-up
Hymenoptera			
<i>Anthophora terminalis</i>	2	yes (2)	yes (2)
<i>Apis mellifera</i>	13	yes (6)	yes (3)
<i>Bombus appositus</i>	2	yes (1)	yes (1)
<i>Bombus griseocollis</i>	12	yes (6)	yes (6)
<i>Bombus bifarius</i>	3	yes (3)	yes (2)
<i>Bombus rufocinctus</i>	3	yes (2)	yes (2)
<i>Bombus occidentalis</i>	2	yes (1)	yes (1)
<i>Colletes</i> sp.	1	no	no
<i>Halictus</i> sp.	1	no	no
<i>Osmia</i> sp.	1	no	yes (1)
<i>Vespula maculata</i>	2	no	no
<i>Vespula</i> sp.	1	no	no
Lepidoptera			
Unidentified skipper	1	no	no
Diptera			
Syrphidae	4	no	no

also found much higher visitation by honeybees, *Apis mellifera*, than in previous years.

Insects were scored for acropetal foraging behavior, number of conspecifics visited after first perceived *S. diluvialis* visit, and the presence or absence of pollinia on the mouthparts. The two *A. terminalis* individuals, all *Bombus* spp., and some honeybees carried pollinia and foraged acropetally, indicating that they were accomplishing cross-pollination (Table 1). Other insect visitors at Diamond Fork did not pollinate the flowers (Table 1). Among these were unidentified wasps, syrphid flies, one *Osmia* bee species, halictid bees, and a butterfly. During these visits, nectar appeared to be appropriated without pollination.

Bumblebees exhibited greater fidelity to *S. diluvialis* flowers than did honeybees. On average, bumblebees visited 4.83 (± 2.58) successive *S. diluvialis* spikes until visiting a co-blooming species; honeybees visited an average of 2.25 (± 1.38) spikes. This difference was significant ($t = 3.03$, $df = 15$, $p < 0.01$).

The number of pollinia on bee bodies was also compared between bumblebees (mean = 1.55) and honeybees (mean = 1.50). No significant difference was observed ($t = 0.13$, $df = 10$, $p = 0.44$).

We collected 21 specimens of the insect species visiting *S. diluvialis* (relatively few were collected to minimize the impact on pollinators). Although none of these specimens had full pollen loads when collected, we were able to sample small

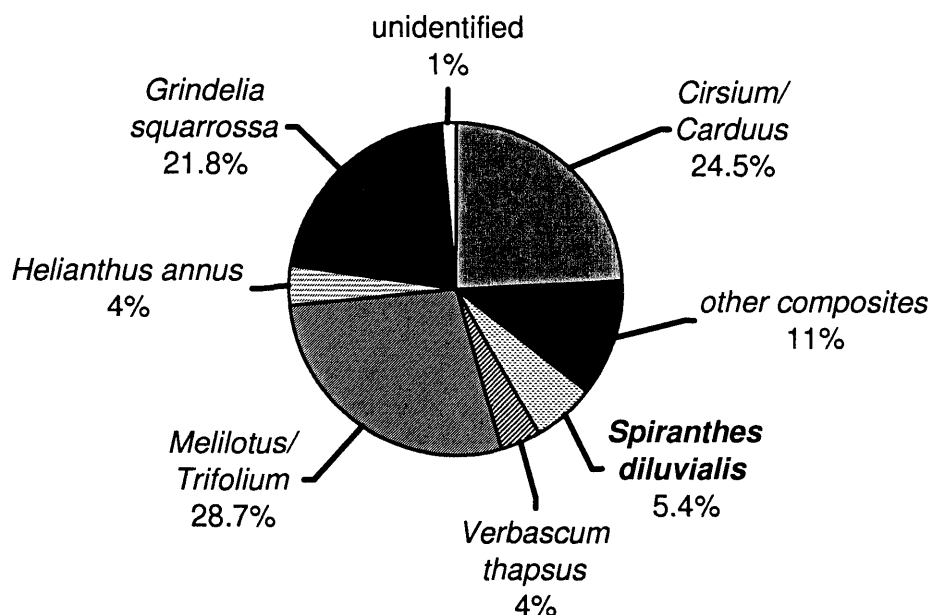


Figure 2. Identity of pollen grains from all *Spiranthes diluvialis* visitors (n = 21).

amounts of the pollen they carried. Figure 2 summarizes the average pollen load from all flower visitors of *S. diluvialis*. Most of the pollen from these bees was from *Cirsium/Carduus* (24.5%), *Melilotus/Trifolium* (21.7%), and *Grindelia squarrosa* (21.8%). Loose pollen grains from *S. diluvialis* were found on most bees. The average percent of *S. diluvialis* pollen carried was much less in 1999 (5.4%) than in 1995 (15.9%).

Nocturnal Pollination

No incidence of night pollination was observed. No pollinia were removed from the flowers of individuals uncaged at night, nor was any visible pollen observed on their stigmata. To test for the possible presence of unseen pollen, plants remained caged for several weeks following this experiment, but no fruits developed. Thus, *S. diluvialis* is not being visited on a regular schedule by nocturnal pollinators.

Comparison of Pollen Sources

The co-flowering species most abundant in *S. diluvialis* populations included *Aster hesperius*, *Carduus nutans*, *Cirsium arvense*, *Cirsium vulgare*, *Grindelia squarrosa*, *Helianthus annuus*, *Melilotus officinalis*, *Solidago occidentalis*, and *Verbascum thapsus*. Several small flowered species were also locally abundant but may not have provided the copious amounts of pollen required by many bee species. These species included *Gayophytum diffusum*, *Descurania sophia*, *Sisyrinchium idahoensis*,

and *Veronica anagallis-aquatica*. In total, we identified 31 native species and 20 exotics.

We observed these plant species to determine which were used for pollen by *Anthophora*, bumblebees, honeybees, and other insect visitors. Table 2 summarizes the co-occurring species visited by *S. diluvialis* pollinators. Not all *S. diluvialis* pollinators were observed on surrounding vegetation.

Table 2. Pollen sources for insect visitors of *Spiranthes diluvialis* in 1999. All observations are from Diamond Fork Canyon. Exotic species denoted by an asterisk.

Taxon	No. of Observ.	Additional Species Visited
<i>Apis mellifera</i>	15	<i>Aster hesperius</i> , <i>Grindelia squarrosa</i> , * <i>Carduus nutans</i> , * <i>Cirsium arvense</i> , * <i>Medicago sativa</i> , * <i>Gutierrezia sarothrae</i>
<i>Bombus appositus</i>	3	* <i>Carduus nutans</i> , * <i>Cirsium arvense</i> , * <i>Cirsium vulgare</i>
<i>Bombus griseocollis</i>	9	<i>Aster hesperius</i> , <i>Carduus nutans</i> , * <i>Cirsium arvense</i> , <i>Prunella vulgaris</i> , <i>Helianthus annuus</i> , <i>Grindelia squarrosa</i> , * <i>Melilotus officinalis</i>
<i>Bombus bifarius</i>	3	* <i>Cirsium arvense</i> , * <i>Carduus nutans</i>
<i>Bombus rufocinctus</i>	1	* <i>Melilotus officinalis</i> , * <i>Carduus nutans</i>
<i>Bombus occidentalis</i>	1	* <i>Carduus nutans</i> , * <i>Cirsium arvense</i>

Nesting Requirements

Despite the diversity of semi-natural nesting materials employed at Diamond Fork Canyon to attract *A. terminalis* and other bees, few nests were made. Indeed *A. terminalis* produced only one nest, in an elderberry upright in the ecotone, and it contained only two very unhealthy looking cells. In general, elderberry uprights and bundles were not much used in either population. The use of nesting material by *A. terminalis* was higher at Brown's Park, where one elderberry upright (9 cells) and one twig bundle (4 cells) were obtained. In contrast, many nests were constructed in logs by the bees *Megachile pugnata*, *M. inermis*, and *M. relativa*, and *Isodontia* and other unidentified wasps. Unfortunately, none of these species were pollinators of *S. diluvialis*.

Reproductive Success and Trends

Patterns of reproductive success convey important information about the population dynamics of both Diamond Fork and Brown's Park populations of *S. diluvialis*. Female reproductive success, as measured by percent fruit set, was significantly greater in the Diamond Fork population than at Brown's Park in all years measured (Table 3, Figure 3; Sipes and Tepedino 1995, Kuta et al. 1997). Within the Diamond Fork population, percent fruit set varied significantly among years (Table 3). A significant increase in percent fruit was observed between 1995 and 1997 (Wilcoxon

$Z = 3.92$, $p < 0.0001$), but this was followed by a significant decrease in 1999 (Wilcoxon $Z = 4.89$, $p < 0.0001$ from 1995 to 1999 and Wilcoxon $Z = 7.28$, $p < 0.0001$ from 1997 to 1999). An opposite trend was observed for Brown's Park, where percent fruit set increased significantly from critically low levels to an encouraging 27.7 percent in 1999 (Wilcoxon $Z = 5.35$, $p < 0.0001$ from 1995 to 1999 and Wilcoxon $Z = -3.56$, $p < 0.001$ for 1997 to 1999).

Male reproductive success, measured by percent pollinia removed per inflorescence, had patterns of variation between years and populations that were similar to fruit set (Table 3). As with fruit set, the percent pollinia removed was significantly greater in the Diamond Fork population than in Brown's Park in all years measured (Table 3, Figure 3; Sipes and Tepedino 1995, Kuta et al. 1997). Within Diamond Fork, the percent of pollinia removed varied between years (Table 3) with a significant decrease in 1999 (Wilcoxon $Z = 4.73$, $p < 0.0001$ from 1995 to 1999 and Wilcoxon $Z = 5.06$, $p < 0.0001$ from 1997 to 1999). In contrast, the percent of pollinia removed within Brown's Park did not significantly differ between years (Table 3).

Across-year trends similar to those found for the Diamond Fork population were also found within the individual colonies between years. Significant declines in percent fruit set were found for all colonies ($p < 0.01$ for all) except colony 21.

Table 3. Comparisons of reproductive and morphological data for *Spiranthes diluvialis* collected in 1995, 1997, and 1999 at the Diamond Fork and Brown's Park populations (Sipes and Tepedino 1995, Kuta et al. 1997). Data in bold indicate a significant difference among years (Kruskal-Wallis non-parametric comparison). Means with the same superscript are not significantly different (Wilcoxon $p > 0.10$). Means with different superscripts are significantly different ($p < 0.01$).

Character	Brown's Park				Diamond Fork			
	1995	1997	1999	p	1995	1997	1999	p
Flowers per inflorescence	12.9 (5.2) ^a	14.2 (5.0) ^a	15.1 (5.8) ^a	0.10	19.1 (7.1) ^a	18.6 (7.3) ^a	19.6 (5.8) ^a	0.30
Height of inflorescence	22.9 (6.3) ^a	21.5 (5.42) ^a	29.5 (7.8) ^b	< 0.001	25.8 (7.5) ^a	30.1 (7.3) ^b	33.3 (9.2) ^c	< 0.001
Percent fruit set	0.03 (0.10) ^a	0.07 (0.12) ^b	0.27 (0.23) ^c	< 0.001	0.75 (0.20) ^a	0.83 (0.21) ^b	0.61 (0.22) ^c	< 0.001
Percent pollinia removed	0.17 (0.16) ^a	0.16 (0.18) ^a	0.18 (0.14) ^a	0.40	0.55 (0.24) ^a	0.58 (0.27) ^a	0.40 (0.21) ^b	< 0.001
Avg distance to 3 nearest neighbors (cm)	102.7 (71.3) ^a	79.7 (40.6) ^a	128.9 (156) ^a	0.60	167 (183) ^a	94.8 (67.03) ^b	93.2 (87.9) ^b	< 0.001

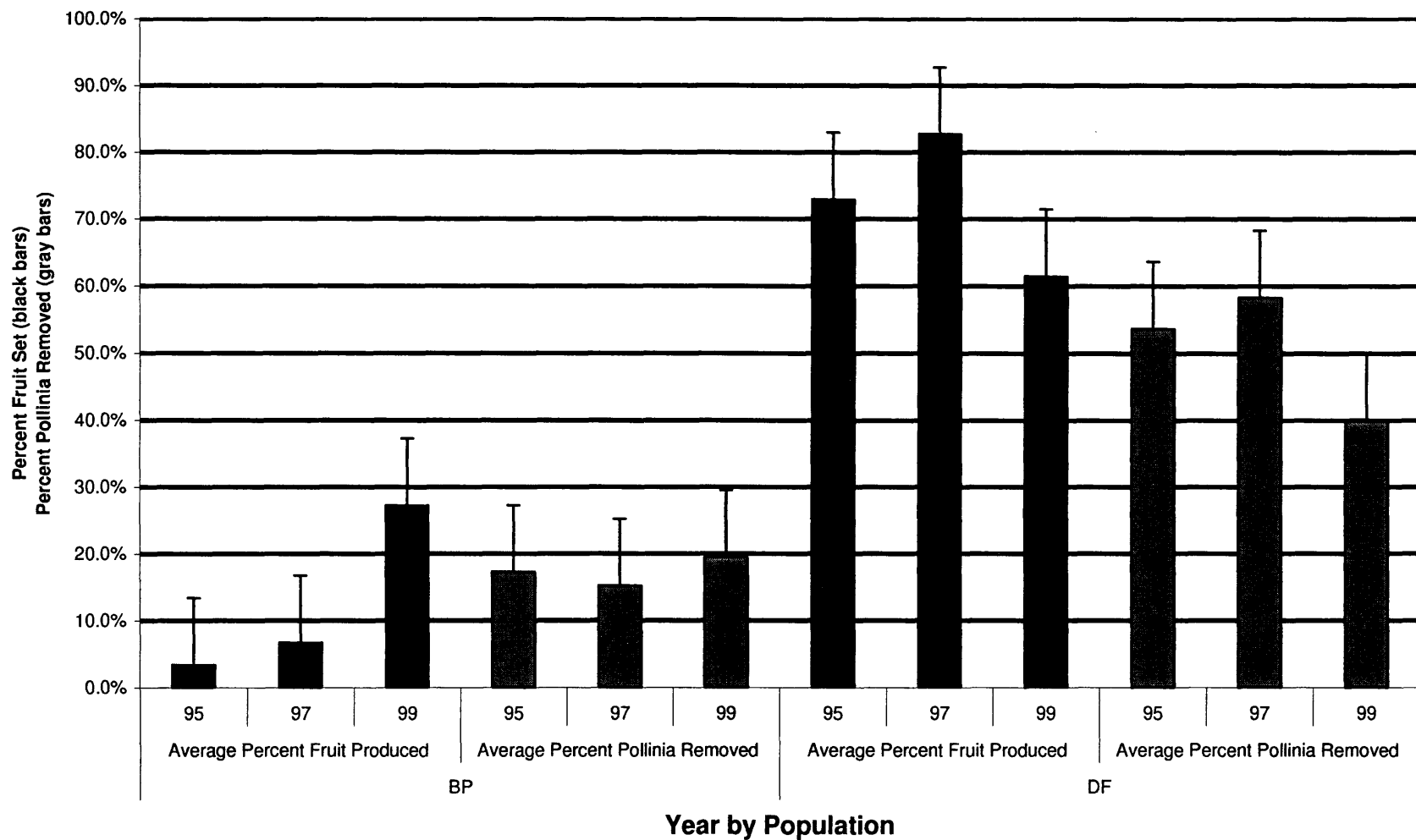


Figure 3. Comparison of percent fruit set and pollinia removal for *Spiranthes diluvialis* individuals in 1995, 1997, and 1999 in Diamond Fork and Brown's Park populations.

Percent of pollinia removal per inflorescence was also significantly lower at individual colonies in 1999 (Pierson and Tepedino 2000).

As observed in previous years (Sipes and Tepedino 1995, Kuta et al. 1997), we found a positive relationship between the number of fruits per plant and the number of flowers produced for both populations in 1999. The relationship at Diamond Fork was strong ($R^2 = 0.54$, $p < 0.0001$). It was weaker at Brown's Park ($R^2 = 0.33$, $p < 0.001$), but was still much higher than that recorded in 1995 ($R^2 = 0.09$; Sipes and Tepedino 1995). Significant, positive relationships between the number of fruits and the number of flowers were found for colonies 15, 21, 25, and 35 ($p < 0.01$ for all), but not for colonies 1, 4, 14, and 15 ($p > 0.10$).

A positive relationship between number of pollinia removed and number of flowers per inflorescence was found for the entire Diamond Fork population ($R^2 = 0.32$, $p < 0.001$) and for colony 25 in Diamond Fork ($R^2 = 0.61$, $p < 0.0001$), but not for any of the others. This relationship was not significant for Brown's Park ($p = 0.23$).

Perhaps more interesting, pollinia removal appeared to be at least partially decoupled from fruit set. At both Brown's Park and Diamond Fork populations, mean percent fruit set was significantly higher than mean percent pollinia removal per inflorescence (Brown's Park $t = 2.78$, $df = 23$, $p < 0.01$; Diamond Fork $t = 12.87$, $df = 203$, $p < 0.0001$). Comparisons were statistically significant ($p < 0.001$) for all colonies within Diamond Fork as well. These findings are important because they suggest that each pollinia fertilizes, on average, more than one fruit. This is possible because whole pollinia are not inserted onto a *S. diluvialis* stigmata; rather, grains of pollen are brushed off the pollinia and deposited individually.

We compared the density of populations at Diamond Fork and Brown's Park by measuring distances from focal plants to the three nearest neighbors. No significant difference was found between Diamond Fork and Brown's Park populations (Table 3; Wilcoxon $Z = 1.25$, $df = 1$, $p = 0.20$). However, the average distance to the three nearest conspecific neighbors did differ significantly among the colonies of Diamond Fork (ranging from 56.20 cm to 158.35 cm, Kruskal-Wallis $df = 7$, $p < 0.0001$). We found no evidence that mean distance to the nearest neighbor influenced the mean percent of fruit produced. Comparisons between Brown's Park and Diamond Fork populations, as well as among the Diamond Fork

colonies, yielded no statistically significant relationships ($p < 0.10$ for all).

Insect visitation and reproductive success are often thought to be positively related to the size and density of the floral display (Heinrich 1979, Schemske 1980, Rodriguez-Robles et al. 1992). Thus, factors such as inflorescence height, floral density (as measured by distance to nearest neighbor), and number of flowers per inflorescence may increase plant attractiveness. These variables were measured in 1999 from Brown's Park and Diamond Fork populations to determine if they were significantly correlated with the percent fruit produced or pollinia removed per inflorescence. No significant correlations between percent pollinia removed per inflorescence with height, distance to nearest neighbor, or number of flowers per inflorescence were found for Diamond Fork or Brown's Park (all comparisons $p > 0.10$). It is therefore unlikely that the size of the inflorescence and the close proximity of conspecific neighbors influenced the number of pollinator visits.

Discussion

Pollinator Visitation

The diversity and abundance of pollinators of *S. diluvialis* appears to fluctuate between populations and years. Bumblebees (*Bombus* spp.) were found to be the most important pollinators for both the Diamond Fork and Brown's Park populations in 1991 and 1992 (Sipes 1995, Sipes and Tepedino 1995), but in 1995 *A. terminalis* was most abundant and likely the most efficient pollinator for the Diamond Fork population (Sipes et al. 1996).

In 1999, only two *Anthophora* individuals (both *A. terminalis*) were seen visiting the flowers of *S. diluvialis*, whereas a variety of other pollinator taxa, such as bumblebees (*Bombus* spp.), honeybees (*Apis mellifera*), solitary bees, syrphid flies (Syrphidae), and wasps (*Vespula* spp.), were observed visiting these flowers (Table 1). Of these, only bumblebees and honeybees appeared to serve as pollen vectors. The occurrence of such large fluctuations in native bee populations is well known (Bohart and Knowlton 1953, Tepedino and Stanton 1980, 1981), though the reasons for such changes are not well understood.

Bumblebees have been shown to be among the most important pollinators of *Spiranthes* in several other studies (Ames 1921, Luer 1975, Catling 1983, Larsen and Larson 1990, Sipes and Tepedino 1995).

Of the several species observed visiting *S. diluvialis* in 1999, *B. griseocollis* (Degeer) appeared to be the most efficient pollinator at Diamond Fork. Although *B. griseocollis* carried no more pollinia or pollen on their bodies than did other species, individuals consecutively visited significantly more *S. diluvialis* spikes than did honeybees, and therefore were likely responsible for a greater number of pollinations. In contrast, honeybees may waste pollinia in deposition on non-orchid hosts because they more commonly switch from *S. diluvialis* to other flower species. Thus, honeybees are unreliable as primary pollinators of *S. diluvialis*, although they could be valuable as supplementary pollinators when native pollinators are scarce.

The two visits to the Brown's Park population provided insufficient time to identify pollinators in 1999. The significant increase in fruit production in 1999 may have been the result of many factors. Among those, an increase in pollinator abundance or diversity is the most intriguing possibility. Efforts to transplant individuals of *A. terminalis* into Brown's Park should continue and should be augmented by attempts to build up bumblebee populations by providing nest boxes.

Nesting Requirements

Anthophora terminalis used few of the artificial nesting materials provided at Diamond Fork, undoubtedly because of its low abundance. Low numbers may have been due to excessive overwinter mortality or perhaps to a previously unsuspected life cycle, common to some species, that requires 2 years to complete (Torchio and Tepedino 1982). The poor success observed in 1999 should not hinder future attempts at pollinator augmentation or transplantation into this isolated population. Given a better year, nest recruitment of *A. terminalis* could be much higher: such nesting material has been heavily used by *A. terminalis* in the past in Grand Teton National Park (Parker and Tepedino, unpublished report). Research efforts should detail the success of *A. terminalis* within this population and also attempt to confirm its role in fruit set. These findings could then be utilized to design transplantation efforts in larger populations such as at Diamond Fork. Future efforts should also attempt to attract a wider range of native pollinators, especially bumblebees.

Reproductive Success and Trends

The reproductive success of *S. diluvialis* fluctuates greatly between populations, between demes

within populations, and between years (Table 3; Pierson and Tepedino 2000). A significant overall decline in percent fruit set was observed for Diamond Fork in 1999 (Table 3, Figure 3) compared with 1995 and 1997 (Sipes and Tepedino 1995, Kuta et al. 1997). The percent of pollinia removed at Diamond Fork also declined significantly (Table 3, $p < 0.001$) in 1999 compared with previous years (Figure 3; Sipes and Tepedino 1995, Kuta et al. 1997). As in previous years, percent fruit set significantly exceeded percent pollinia removed at Diamond Fork (Figure 3), indicating that pollen from each pollinia is reaching more than one flower (Sipes and Tepedino 1995, Kuta et al. 1997).

Previously, Sipes and Tepedino (1995) hypothesized that the abundance of *A. terminalis* was responsible for the high fruit set observed in Diamond Fork in 1995. If *A. terminalis* does play such an important role in the pollination of *S. diluvialis*, then its virtual absence in 1999 might help explain why fruit set was significantly lower that year than in 1995 and 1997. The overall reduction in male reproductive success (lower percent pollinia removal) could further suggest that the pollinators observed in 1999 may either prefer the flowers of other plants more than those of *S. diluvialis*, or be less efficient at pollinating *S. diluvialis* flowers than those observed in 1995. At this point, we know only that *A. terminalis* shares its role as an important pollinator with bumblebees.

An opposite and encouraging trend for reproductive success was observed for Brown's Park, where a three-fold increase in fruit set was recorded from 1997 to 1999 (Kuta et al. 1997; Figure 3). Fruit set in 1999 was also dramatically greater than the dismal 3.3 percent recorded in 1995 ($df = 1$, $p < 0.0001$; Sipes and Tepedino 1995), although it fell short of the 37 percent recorded for this population in 1991 (Sipes 1995).

Surprisingly, the increase in percent fruit set was not accompanied by an increase in the percent pollinia removed, which did not differ between the years investigated ($df = 2$, $p = 0.40$, Table 3, Figure 3). Again, percent pollinia removed was significantly lower than percent fruit set ($p < 0.0001$), solidifying the finding that pollinia commonly pollinate more than one flower if moved by effective pollinators. The program to monitor reproductive success at Brown's Park should be continued so that we may judge our progress toward augmenting this population.

Not unlike previous years, the factor that may be limiting reproduction by *Spiranthes diluvialis* at

Diamond Fork is herbivory by voles (Sipes and Tepedino 1995, Sipes et al. 1996, Kuta et al. 1997, Black personal communication, Pierson and Bowlin personal observation). Herbivory by voles has also been reported in the *S. diluvialis* population in Boulder, Colorado, where *Microtis* sp. were primarily responsible for inflorescence destruction. In 1999, voles damaged inflorescences in most colonies and their runways were obvious. Voles tunneled under wire and mesh exclosures to attack protected inflorescences. Future studies should estimate vole population size and the impact of vole herbivory on the reproductive and vegetative success of *S. diluvialis*.

Implications for Conservation

To successfully conserve the existing populations of *S. diluvialis*, a community-based management plan is essential. Given the dependence of *S. diluvialis* on pollinators for sexual reproduction (Sipes and Tepedino 1995), an appropriate management plan must provide for the overall preservation of pollinating species and must incorporate their nutritional, provisional, and nesting needs. Understanding the biology of these species is vital to their preservation as well as to the continued conservation of *S. diluvialis*. Many efforts can be made to encourage the establishment of such bees within the ecosystem.

As outlined in Sipes and Tepedino (1995), the long activity period of bumblebees (spring to early autumn), renders their populations vulnerable to management decisions throughout the growing season. Populations of bumblebees are annual with only the young mated queens produced in late summer–early fall capable of overwintering or hibernation. The production of entire new colonies the following year is dependent upon the survival of these queens over winter. In spring, these queens will forage until the first progeny reach workerhood (Michener 1974, Alford 1975). The application of insecticides during these foraging and mating periods could prevent formation of entire colonies and could also jeopardize the health of a colony. Workers can become vectors of contaminated nectar or pollen. Because bumblebees can forage great distances (up to 3 miles), insecticide applications must be prohibited over distances much greater than the mandated 500 foot buffer around water courses.

Conservation of native pollinators not only requires minimizing the potential detrimental effects of management but also requires promoting and preserving the factors critical to their survival.

By providing for the nesting requirements and nutritional needs of these bee species, maintenance of native pollinators is possible.

The nesting and foraging requirements of the pollinators of *S. diluvialis* are varied. *Bombus griseocollis* prefers to nest in dry aboveground locations such as downed logs, abandoned sheds, or farm buildings (Eshelman and Plowright 1972), whereas other species (*B. appositus*, *B. rufocinctus*, and *B. bifarius*) typically nest in belowground burrows or in abandoned rodent nests (Medler and Carney 1963, Hobbs 1965, 1966). *Bombus occidentalis* prefers to nest in open west-southwest slopes bordered by poplar trees and sites connected to the surface with downward-sloping tunnels (Hobbs 1968). These species are often attracted to legumes such as *Melilotus*, *Medicago*, and *Trifolium*, and members of the Asteraceae including *Solidago* and *Aster* (Plath 1927, Medler and Carney 1963, Hobbs 1965, 1966).

Because the pollinators of *S. diluvialis* appear to fluctuate from year to year and site to site, a larger scale conservation plan to preserve and encourage the establishment of all native pollinators would be extremely beneficial. Efforts to encourage the establishment of *A. terminalis* should be continued. Nesting sites such as logs, snags, shrubs, and downed wood should be encouraged rather than disturbed. Augmenting populations with such nesting material may also prove beneficial to the populations of *A. terminalis*. Bumblebees should be encouraged to establish or colonize areas surrounding the *S. diluvialis* populations. Subterranean nesting boxes that have been designed to promote ground-nesting bumblebees (Alford 1975) could be employed in the more mesic areas surrounding *Spiranthes* colonies. Additional nest-building material such as cotton batting, dried moss, or old bird nests could be provided to encourage colonization by such species. Bumblebees have also been observed nesting at ground level when large accumulations of dried moss or fine grass are available (Alford 1975).

Although *B. morrisoni* and *B. fervidus* were not observed visiting the flowers of *S. diluvialis* in 1999, their importance as pollinators has been noted previously (Sipes 1995, Sipes and Tepedino 1995). Conservation efforts should incorporate the needs of these pollinators as well. Recommendations for increasing successful nesting and preservation of these species are outlined in Sipes and Tepedino (1995).

In addition to providing for the nesting requirements of these native pollinators, manage-

ment plans must consider the importance of co-occurring vegetation. Pollinators cannot use *S. diluvialis* pollen and must therefore seek their pollen elsewhere. We have provided a list of specific pollen resources (Table 2) used by *S. diluvialis* visitors. Many of the pollinators of *S. diluvialis* currently appear to depend upon the pollen of non-native vegetation for survival, and managers should not seek to eradicate these species without parallel efforts to return native vegetation to the area.

A detailed list of native entomophilous vegetation usable in revegetation efforts and to support populations of native pollinators is provided in Table 4. Of these, members of the mint family (Lamiaceae) and figwort family (Scrophulariaceae) are attractive to bees (*A. terminalis* and many *Bombus* spp.) and are also valuable for revegetation. Plants should be spaced to promote wide pollinator movement and foraging throughout the entire flowering region. Finally, plants selected for revegetation efforts should flower not only when *S. diluvialis* does, but throughout the growing season to promote long-lived populations of native pollinators.

Table 4. Native entomophilus plant species useful for revegetation efforts.

Species	Family	Flowering time
<i>Allium</i> sp.	Liliaceae	Early spring
<i>Aster</i> spp.	Asteraceae	Late sum–fall
<i>Linum perenne</i> L.	Linaceae	Spring
<i>Gutierrezia sarothrae</i> Pursh	Asteraceae	Late sum–fall
<i>Helianthella uniflora</i>	Asteraceae	Mid-late sum
<i>Helianthus annuus</i> L.	Asteraceae	Late sum–fall
<i>Heterotheca villosa</i> (Pursh) Shinn	Asteraceae	Mid sum
<i>Mentha arvensis</i> L.	Lamiaceae	Mid-late sum
<i>Mertensia ciliata</i> (James) G. Don	Boraginaceae	Spring
<i>Monarda</i> spp.	Lamiaceae	Spring–sum
<i>Penstemon</i> spp.	Scrophulariaceae	Variable
<i>Penstemon palmeri</i> Gray	Scrophulariaceae	Late spring
<i>Polemonium</i> spp.	Polemoniaceae	Variable
<i>Polemonium viscosum</i> Nutt.	Polemoniaceae	Mid summer
<i>Prunella vulgaris</i> L.	Lamiaceae	Mid summer
<i>Rosa woodsii</i> Lindl.	Rosaceae	Mid late sum
<i>Salvia reflexa</i> Hornem.	Lamiaceae	Mid summer
<i>Scutellaria</i> spp.	Lamiaceae	Mid-late sum
<i>Senecio</i> spp.	Asteraceae	Variable
<i>Solidago</i> spp.	Asteraceae	Variable
<i>Stachys plaustris</i> L.	Lamiaceae	Mid-summer

We hope that the foregoing has shown that conservation efforts for *S. diluvialis* must be designed and implemented at a community or ecosystem level to be successful. Healthy populations of *S. diluvialis* will be realized not simply by protecting riparian habitat; in addition, managers must be cognizant of the natural nesting habitat and floral needs of the bee pollinators that make reproduction and continued existence of this rare plant possible.

Acknowledgments

We thank the USDA Forest Service (Uinta National Forest) and the Central Utah Water Conservancy District for funding this study and the Uinta National Forest for camping accommodations and technical support. Also, we would like to thank the U.S. Fish and Wildlife Service for their cooperation and support. Assistance in the field was provided by S. Enright, K. Konen, and B. Bowlin. E. Klomps and J. L. Harris provided assistance in the lab. T. L. Griswold, O. Messinger, and W. Hansen identified insect specimens.

Literature Cited

- Alford, D. V. 1975. Bumblebees. Ebnezer Baylis and Sons, London.
- Ames, O. 1921. Notes on New England orchids. I. *Spiranthes*. *Rhodora* 23:3–85.
- Beattie, A. J. 1971. A technique for the study of insect-borne pollen. *Pan-Pacific Entomologist* 47:82.
- Bohart, G. E., and G. F. Knowlton. 1953. Yearly population fluctuation of *Bombus morrisoni* at Fredonia, Arizona. *J. Econ. Entomol.* 45:890.
- Catling, P. M. 1983. Pollination of northeastern North American *Spiranthes* (Orchidaceae). *Canadian Journal of Botany* 61:1080–1093.
- Eshelman, C. W., and R. C. Plowright. 1972. Factors influencing recognition of the nest entrance by returning bumble bee foragers (Hymenoptera: Apidae). *Canadian Entomologist* 104:389–398.
- Heinrich, B. 1979. Bumblebee economics. Harvard University Press, Cambridge MA.
- Hobbs, G. A. 1965. Ecology of species of *Bombus* Latr. (Hymenoptera: Apidae) in southern Alberta: III. Subgenus *Cullumanobombus* Vogt. *Canadian Entomologist* 97:1293–1302.
- Hobbs, G. A. 1966. Ecology of species of *Bombus* Latr. (Hymenoptera: Apidae) in southern Alberta: IV. Subgenus *Subterraneobombus* Vogt. *Canadian Entomologist* 98:33–39.
- Hobbs, G. A. 1968. Ecology of species of *Bombus* Latr. (Hymenoptera: Apidae) in southern Alberta: VII. Subgenus *Cullumanobombus*. *Canadian Entomologist* 100:156–164.
- Kruckeberg, A. R., and D. Rabinowitz. 1985. Biological aspects of endemism in higher plants. *Annual Review of Ecology and Systematics* 16:447–479.
- Kuta, K. A., D. G. Alston, and V. J. Tepedino. 1997. The reproduction of *Spiranthes diluvialis* from two locations in central Utah. Unpublished report prepared for USDI Bureau of Land Management, Salt Lake City, Utah.

- Larsen, K. S., and R. J. Larson. 1990. Lure of the locks, showiest ladies-tresses orchids, *Spiranthes roman-zoffiana*, affect bumblebee, *Bombus* spp. foraging behavior. *Canadian Field Naturalist* 104:519–523.
- Luer, C. A. 1975. The native orchids of the United States and Canada excluding Florida. New York Botanical Garden.
- Medler, J. T., and D. W. Carney. 1963. Bumblebees of Wisconsin (Hymenoptera: Apidae). University of Wisconsin Research Bulletin 240. 47 pp.
- Michener, C. D. 1974. The social behavior of the bees. A comparative study. Belknap Press of Harvard University, Cambridge, MA.
- Pierson, K. and V. J. Tepedino. 2000. The pollination ecology of a rare orchid, *Spiranthes diluvialis*: Implications for conservation. Unpublished report prepared for Uinta National Forest, Provo, Utah.
- Plath, O. E. 1927. Notes on the nesting habits of some of the less common New England bumblebees. *Psyche* 34:122–128.
- Rodriguez-Robles, J. A., E. J. Melendez, and J. D. Ackerman. 1992. Effects of display size, flowering phenology, and nectar availability on effective visitation frequency in *Comparettia falcata* (Orchidaceae). *American Journal of Botany* 79:1009–1017.
- SAS Institute, Inc. 1999. SAS/STAR® software: Version 7.0. SAS Institute, Inc., Cary, North Carolina.
- Schemske, D. W. 1980. Evolution of floral display in the orchid *Brassavola nodosa*. *Evolution* 34:489–493.
- Sheviak, C. 1984. *Spiranthes diluvialis* (Orchidaceae). A new species from the western United States. *Brittonia* 36:8–14.
- Sipes, S. D. 1995. Reproductive biology of the rare orchid, *Spiranthes diluvialis*: Pollination ecology, variations in reproductive success, and implications for conservation. Master's thesis, Utah State University.
- Sipes, S. D., and V. J. Tepedino. 1995. Reproductive biology of the rare orchid, *Spiranthes diluvialis*: Breeding system, pollination, and implications for conservation. *Conservation Biology* 9:929–938.
- Sipes, S. D., P. G. Wolf, and V. J. Tepedino. 1996. The pollination and reproduction of *S. diluvialis*: Implications for conservation of four populations. Unpublished report prepared for USDI Bureau of Land Management, Salt Lake City, Utah.
- Tepedino, V. J. 1979. The importance of bees and other insect pollinators in maintaining floral species composition. *Great Basin Naturalist Memoirs* 3:139–150.
- Tepedino, V. J. 2001. The reproductive biology of rare rangeland plants and their vulnerability to insecticides. In G. L. Cunningham and M. W. Simpson (technical coordinators). Grasshopper integrated pest management user handbook. Tech. Bull. 1809. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Washington, D.C.
- Tepedino, V. J., and N. L. Stanton. 1980. Spatiotemporal variation in phenology and abundance of floral resources on shortgrass prairie. *Great Basin Naturalist* 40:197–215.
- Tepedino, V. J., and N. L. Stanton. 1981. Diversity and competition in bee-plant communities on short-grass prairie. *Okios* 36:35–44.
- Torchio, P. F., and V. J. Tepedino. 1982. Parsivoltinism in three species of *Osmia* bees. *Psyche* 89:221–238.