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The Genetics, Demography, and Conservation Management of the Rare Orchid Spiranthes diluvialis

Anna Maria Arft
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Chapter 1: Introduction

The rare orchid, Spiranthes diluvialis Sheviak (Orchidaceae), gained federally threatened status under the U.S. Endangered Species Act on January 17, 1992 (Federal Registry, 1992). At the time of its listing, little was known of the genetic, ecological, and demographic processes affecting the life history and long-term survival of this threatened orchid. Knowledge of the biology of any species, including its recent and evolutionary history, genetics, and demographic patterns, is vital to understanding the causes and consequences of rarity and the implementation of recovery actions (Fiedler, 1986). This work examines the phylogenetic history, population genetics, demography, and conservation management of the rare orchid S. diluvialis.

Many nineteenth century naturalists recognized rarity as a discernible plant characteristic (Fiedler, 1986; Fiedler and Ahouse, 1992). In the final edition of *On the Origin of Species*, Darwin concluded that rarity is a preliminary step in the process of extinction (Darwin, 1872). During the early twentieth century biologists argued for and against singular causes of rarity, debating whether rarity was a result of relictual species in the process of extinction or new species in the process of divergence (Willis, 1922; Ridley, 1916). More recently Stebbins (1980) proposed a synthetic approach to understanding rarity as a result of three interacting forces: a specific evolutionary history, a unique and localized environment, and a specific genetic structure. Rabinowitz (1981) based her seven forms of rarity on the distribution, abundance, and habitat specificity of species. Recently, Fiedler et al. (1992) constructed a detailed list of factors which may cause a species' rarity including age of taxon,

coevolution, earth history, ecology, evolutionary history, land-use history, life history strategy, population dynamics, human uses, reproductive biology, stochasticity, genetics, and taxonomic history.

In the field of conservation biology, the multitude of causes and consequences of rarity have often been reduced to a debate over the relative importance of genetic versus demographic factors in the conservation of rare species (Lande, 1988; Schemske et al., 1994). Numerous biologists have proposed that knowledge of genetic processes is an integral component of the conservation of rare species (Falk, 1992). The importance of population size to the genetics and evolution of a species was first recognized by Wright (1931, 1938, 1946). Classical population genetic theory predicts severe inbreeding depression in small populations of normally outcrossing plants, particularly when gene flow from outside the population is small (Wright, 1977). Recent studies using isozyme markers suggest endemic and/or geographically-restricted species are often genetically depauperate. However, the ecological and evolutionary significance of the lack of genetic variation is unclear. Proponents of the genetic approach to the conservation of rare species stress that understanding the organization of genetic diversity is critical to the long-term survival of species since the loss of genetic variation reduces a species' ability to adapt to changing environments (Hamrick et al., 1991; Lande and Barrowclough, 1987; Schemske et al., 1994). Thus, one goal of many conservation programs is to assess and maintain existing levels of genetic variation within rare species (Frankel and Soulé, 1981; Simberloff, 1988).

Proponents of the demographic approach to the conservation of rare species assert that characterizing the life history, biotic interactions,

and habitat requirements are the most critical aspects of conservation and recovery efforts (Brussard, 1991; Schemske et al., 1994; Simberloff, 1988). Populations large enough to reduce ecological threats to species survival may reduce genetic threats as well (Holsinger and Gottlieb, 1991). In addition, the investment of time, money, and expertise to analyze and manage the genetic structure of endangered plant species is large (Holsinger, and Gottlieb, 1991; Schemske et al., 1994). Schemske et al. (1994) suggest a demographic framework for endangered plant recovery efforts focusing on knowledge of the biological status of the species, the life history stages that have the greatest effect of population growth and species persistence, and the biological causes of variation of those life history stages that have a major demographic impact. Only after the demographic framework is articulated can the need for more extensive ecological or genetic research be ascertained (Schemske et al., 1994).

The present work examined both population genetics and demography of S. diluvialis, explored the evolutionary history of this species, and assessed the effects of potential management strategies on the continued survival of this rare orchid. Spiranthes diluvialis is a terrestrial orchid species known from scattered populations in Colorado, Nevada, Utah, and Wyoming (U.S. Fish and Wildlife Service, 1994). Each of the existing populations is limited in area to under 50 acres and in the number of individual plants to under 7000 (U.S. Fish and Wildlife Service, 1994; Coyner, 1990). Some of the existing sites where populations of S. diluvialis occur have supported agriculture historically. The habitat alteration resulting from agricultural use (such as mowing, grazing, and burning) may be neutral, beneficial, or detrimental to S. diluvialis (McClaren and Sundt, 1992; Gori, personal communication). The apparent

tendency for populations of S. diluvialis to fluctuate drastically from one year to the next makes populational and distributional assessments of orchid occurrences difficult.

The four specific questions addressed are central to understanding the evolution of S. diluvialis and ensuring the future existence of the species based on knowledge of its genetics and demography: 1) What is the phylogenetic history of S. diluvialis? 2) What is the genetic structure and level of variation within and among populations of S. diluvialis? 3) What is the effect of management (grazing, mowing, burning) on the life history and ecology of S. diluvialis? and, 4) What is the population structure, demographic processes, and extinction probability of populations of S. diluvialis?

Chapter 2 explores the phylogenetic history of S. diluvialis. In his original description of S. diluvialis, Sheviak (1984) described S. diluvialis (2n=74) as a distinct species and suggested it may possibly be an allopolyploid hybrid between S. romanzoffiana (2n=44) and S. magnicamporum (2n=30) based on morphological and cytological evidence. Several recent studies have employed enzyme electrophoresis and DNA markers to elucidate phylogenetic relationships and hybridization events leading to allopolyploidy when questions of hybridity were not answered with traditional morphological studies (Ranker et al., 1989; Rieseberg, 1991; Soltis and Soltis, 1989, 1991). Chapter 2 presents the results of an electrophoretic study used to clarify the allopolyploid origin of S. diluvialis by determining its relationship with its proposed parents (S. magnicamporum (2n=30) and S. romanzoffiana (2n=44)), other appropriate congeneric species (S. cernua (2n=30), S. lucida (2n=44), S.

porrifolia (2n=44), S. ochroleuca, S. odorata, S. vernalis (2n=30)), and a morphologically similar tetraploid species (S. delitescens (2n=74)).

Central to understanding the genetics of a species is determining the amount and distribution of genetic variation within and among populations. Enzyme electrophoresis has been the predominant technique employed to survey populational genetic variation in recent years (Schaal et al., 1991) and allows relatively fast, inexpensive analysis of large numbers of individuals. The third chapter examines the genetic variation within and among populations of S. diluvialis using enzyme electrophoresis. Estimates of genetic variation are used to examine processes maintaining genetic variation in the small, fragmented populations of this species, to estimate levels of gene flow between populations, heterozygosity, the presence of population-unique alleles, and to provide insight into the historical biogeography of populations (Lacy, 1988; Ranker, 1992).

The fourth chapter summarizes the status of S. diluvialis and the effect of various management strategies on life history characteristics and environmental parameters. Demographic plots were established to collect data on the life history of S. diluvialis, evaluate the effect of management techniques, and examine environmental factors which may be critical to the persistence of S. diluvialis.

The nature of conservation biology often results in the implementation of management strategies without the benefit of basic research on the rare species (Waite and Hutchings, 1991). One strategy to developing more objectively-based management regimes is through the analysis of population dynamics and structure under particular management techniques (Waite and Hutchings, 1991). In the fifth chapter,

I analyze the population structure and extinction probabilities of populations of S. diluvialis using matrix population transition modeling: Demographic studies provide data on life history stages and form the basis for estimating the current and future probabilities of extinction for plant populations (Menges, 1992). Population dynamics and fate may be predicted through the use of matrix-projection models. A population with a given age or stage structure may be projected through time, using the observed proportion of individual transitions among age or stage classes. The goal of Chapter 5 is to characterize population structure and to use population modeling based on demographic parameters and their variation over a three year period to compare different management strategies and sites for populations of S. diluvialis. An understanding of the life history stages critical for persistence is essential to effectively manage populations and to control the risk of extinction (Menges, 1992; Shaffer, 1981).

This work provides information critical for the formulation of conservation management plans for S. diluvialis as well as data on the basic biology and evolution of this threatened species. This study is the first to explore the origin of a polyploid species in the Orchidaceae through the application of molecular techniques. The research provides information regarding the allopolyploid origin of S. diluvialis and its distinctness as a species. Knowledge of the evolutionary origin of S. diluvialis provides a meaningful framework for and insight into both genetic and ecological studies critical to the continued existence of this rare orchid. A detailed ecological study of S. diluvialis, including analyses of demographic dynamics and environmental requirements, elucidates both intrinsic and extrinsic factors constraining the continued persistence of

this species. Understanding the partitioning of genetic variation within and among populations of S. diluvialis will provide critical data for agency decisions affecting management and maximize the likelihood of its continued evolutionary adaptation and change. Knowledge of biological characteristics, such as have resulted from this project, must be the starting point for conservation and recovery efforts of rare taxa.

Chapter 2: Phylogenetic History

Introduction

The delimitation of species in Spiranthes has often been difficult due to the lack of distinctive morphological characters preserved on herbarium specimens and the occurrence of interspecific hybridization within the genus (Luer, 1975). Specimens ultimately identified as S. diluvialis Sheviak had been previously misidentified as S. romanzoffiana Chamisso, S. magnicamporum Sheviak, S. porrifolia Lindley, or S. cernua (Linnaeus) L.C. Richard. It was not until 1984 that Sheviak described S. diluvialis (2n=74) as a distinct species and suggested it may possibly be an allopolyploid hybrid between S. romanzoffiana (2n=44) and S. magnicamporum (2n=30) based on morphological and cytological evidence (Sheviak, 1984).

Allopolyploidy (i.e., the formation of a polyploid following hybridization between two genetically distinct diploid species) is an important mechanism of speciation in flowering plants (Harlan and deWet, 1975; Stebbins, 1950; Winge, 1917). Grant (1981) estimated 47-52% of angiosperm species are the result of hybrid/polyploid origin, although this may be an overestimate because speciation at the polyploid level was not taken into account. The duplication of chromosomes giving rise to a polyploid confers "instant" speciation on the new fertile polyploid due to complete reproductive isolation from the parental taxa. Generally, if the two parental species are sufficiently different, the resulting fertile tetraploid will form 2n sets of bivalents instead of n sets of irregularly segregating quadrivalents (Futuyma, 1986). In the case of the fertile

tetraploid, S. diluvialis, meiosis is generally regular with the formation of 37 bivalents (Sheviak, 1984).

Spiranthes romanzoffiana is a montane plant of moist areas along streams and near lakes, rarely found below 8000 feet in Colorado and is distributed widely from Alaska to Newfoundland and south across the Rocky Mountains to Arizona (Figure 1; Luer, 1975). Spiranthes romanzoffiana has a tight helix of inflated, ascending flowers around the spike, lateral appressed sepals and a pandurate (violin-shaped) lip. The lip is membranaceous with prominent laterally diverging veins below the constriction. Spiranthes magnicamporum is a plains plant of moist areas which has nodding, tubular flowers with free and ascending lateral sepals, and an ovate to lanceolate lip. The lip is thick and fleshy with largely parallel veins; if present, diverging veins are restricted to the very base. The center of distribution of S. magnicamporum is in the Midwest ranging from Texas to North Dakota with one disjunct population in New Mexico (Figure 1) (Luer, 1975). Although the center of distribution for S. magnicamporum is the Midwest plains, the disjunct population in New Mexico possibly indicates a once larger distribution for this species. Morphologically, S. diluvialis is intermediate between its putative progenitors S. romanzoffiana and S. magnicamporum. For example, S. diluvialis has flowers facing directly away from the stalk, neither nodding nor ascending, appressed or free lateral sepals, and a lip intermediate in shape, fleshiness, and venation between its putative parental species.

Present geographic ranges of the three taxa generally do not overlap. In his original description, Sheviak (1984) proposed that S. magnicamporum and S. romanzoffiana were sympatric or parapatric during the Pleistocene pluvial period and hybridized to form the fertile

allopolyploid S. diluvialis. As the climate became progressively drier, the putative parental species became restricted to their present ranges and S. diluvialis persisted only in scattered, permanently moist areas (Figure 2). Subsequent urbanization of the Colorado and Wasatch Front Ranges has further reduced populations of S. diluvialis and resulted in its current status as a threatened species.

Cytological data support the allopolyploid origin of S. diluvialis and the parental roles of S. romanzoffiana and S. magnicamporum. Chromosome counts for S. diluvialis from three populations in Colorado and Utah uniformly have been 2n=74 with 37 bivalents (Sheviak, 1984). The chromosome number 2n=44 generally has been found for S. romanzoffiana across widespread areas of its range. This number is uncommon in the genus and is known to occur only in two other species, S. lucida (H. H. Eaton) Ames, a northeastern U.S. species, and S. porrifolia, a western U.S. species primarily known from California. Counts for all other species within the genus are based on x=15. Throughout its range, counts for S. magnicamporum have been 2n=30. Other morphologically similar congeneric species with a sporophytic chromosome number of 30 include S. cernua, S. ochroleuca (Rydberg) Rydberg, S. odorata (Nuttall) Lindley, and S. vernalis Engelmann & Gray. Spiranthes cernua is a common and variable species found in the eastern half of the United States and Canada in wet meadows and along streams and lakes (Luer, 1975). Morphologically similar to S. cernua in appearance is S. ochroleuca which is restricted to the northeast United States and southeastern region of Ontario (Luer, 1975). Spiranthes odorata is restricted to the southeast United States where it may grow in shaded wet woods or in full sun in grassy marshes and wet meadows (Luer, 1975). Finally, S. vernalis is more

widely distributed from southern New England to Texas.with disjunct populations occurring in Mexico (Luer, 1975).

Spiranthes delitescens, a recently described tetraploid species from Arizona, has the same chromosome number (2n=74) and similar morphology as S. diluvialis, suggesting a phylogenetic relationship between the two tetraploids (Sheviak, 1990). The morphological differences between the taxa may be a result of different parental taxa hybridizing to form the allopolyploids or to isolation and genetic drift following a single or multiple hybridization event(s) involving the same parental taxa.

Several recent studies have employed enzyme electrophoresis to elucidate phylogenetic relationships and hybridization events leading to allopolyploidy when questions of hybridity were not answered with traditional morphological studies (Ranker et al., 1989; Rieseberg, 1991; Soltis and Soltis, 1991; Soltis and Soltis, 1989, 1992). The evolution of four new allopolyploid species have been documented by isozymic analysis (Thompson and Lumeret, 1992 and references therein). This chapter presents the results of an electrophoretic study used to clarify the allopolyploid origin of S. diluvialis by determining its relationship with its proposed parents (S. magnicamporum (2n=30) and S. romanzoffiana (2n=44)), other appropriate congeneric species (S. cernua (2n=30), S. lucida (2n=44), S. porrifolia (2n=44), S. ochroleuca, S. odorata, S. vernalis (2n=30)), and the morphologically similar tetraploid species (S. delitescens (2n=74)). Generally, allozymes are useful for detecting allopolyploid hybridization because the allozymes present in each of the putative parental species will be combined and detectable in the tetraploid hybrid species (Ranker et. al., 1989; Rieseberg, 1991). Allozymic profiles of allopolyploids typically

exhibit additivity of the divergent genotypes of the parental diploids (Roose and Gottlieb, 1976; Soltis and Soltis, 1991; Werth, 1989). Since congeneric diploid species often have unique alleles, isozyme markers can be used to distinguish taxa.

The specific questions addressed in this chapter concerning the phylogenetic history of S. diluvialis are:

1.) Is S. diluvialis the result of an allopolyploid speciation event between the two diploid species S. magnicamporum and S. romanzoffiana?

2.) Is S. delitescens also the result of an allopolyploid speciation event between S. magnicamporum and S. romanzoffiana and subsequent divergence from S. diluvialis? or, does S. delitescens result from an allopolyploid speciation event between two different diploid species?

2.) Is the establishment of S. diluvialis the result of multiple hybridization events or a unique origin with subsequent dispersal?

Materials and Methods

Leaf samples from populations of S. cernua, S. delitescens, S. diluvialis, S. lucida, S. magnicamporum, S. porrifolia, S. romanzoffiana, and S. vernalis were collected for enzyme electrophoresis and DNA analysis. When leaves persist past anthesis, collection was timed to

coincide with flowering since the plants were difficult to locate in their vegetative stage. Only one individual per vegetative clump was sampled to minimize collecting vegetatively-produced clones. Sample size and population localities of S. diluvialis and those of the congeneric species are listed in Table 1. Voucher specimens are deposited at the University of Colorado Herbarium.

Leaves were stored in plastic bags on ice for transportation to the laboratory. Leaves were ground in an extraction buffer: 0.1M PO_4 buffer adjusted to pH 7.5, 0.03 M Borax, 0.02 M Diethyldithiocarbamic acid (DIECA), 0.2 M ascorbic acid, 6% Polyvinyl pyrrolidone (PVP) (40,000), with 2.4% (vol/vol.) 2-mercaptoethanol and 10% Dimethylsulfoxide (DMSO) added just before grinding (Ranker et al., 1989). Leaf grindate was absorbed with filter-paper wicks and inserted into 12.5% starch gels for electrophoresis.

Three gel buffer systems were used to resolve nine enzyme systems. A discontinuous lithium borate buffer system (gel buffer, 0.004 M LiOH, 0.005 M boric acid, 0.03 M Tris[hydroxymethyl] amino methane (Tris), and 0.05 M citrate pH 7.6; electrode buffer, 0.04 M LiOH and 0.04 M boric acid, pH7.6) was used to resolve diaphorase (DIA), fluorescent esterase (FE), leucine aminopeptidase (LAP), menadione reductase (MNR), phosphoglucose isomerase (PGI), and triose phosphate isomerase (TPI). A discontinuous Tris, ethylenediamine tetraacetic acid (EDTA), borate buffer system (gel buffer, 0.046 M Tris, 0.0009 M EDTA, 0.004 M boric acid; electrode buffer, 0.15 M Tris, 0.003 M EDTA, and 0.013 M boric acid) was used to resolve alcohol dehydrogenase (ADH) and colorimetric esterase (EST). A continuous morpholine citrate buffer system (0.04 M citrate

titrated to pH 7.5 with N-(3-aminopropyl)-morpholine) was used to resolve malate dehydrogenase (MDH).

Genetic variation was assessed by estimating three commonly used parameters: the percent of loci polymorphic (P), the mean number of alleles per locus (A), and the observed heterozygosity (H_O) assuming Hardy-Weinberg conditions. The resulting estimates were used to compare genetic variation within and among species. Genetic identity values (Nei, 1978) were calculated between all pairs of populations. Genetic identity values range from 0.0 to 1.0 with zero indicating no genetic similarity between two taxa and one indicating genetically identical taxa. The genetic data were analyzed using BIOSYS-1 (Swofford and Selander, 1989).

Results

A total of nine enzyme systems were surveyed, resulting in the resolution of 14 putative loci. Allelic frequencies are tabulated in Table 2. Several additional loci, which could not be consistently resolved, were not included in the analysis (Est-3, Est-4, Fe-2). Missing loci in populations were scored as null alleles and not included in estimates of heterozygosity. A total of 82 alleles were observed with a minimum of three alleles found in Mdh-2 and Mdh-3 and a maximum of eight alleles at the Adh and Fe-1 loci. For the diploids, the number of species for which a given locus was polymorphic ranged from two taxa at Mdh-2 to seven taxa at Fe-1. For the tetraploids, the number of species for which a given locus was polymorphic ranged from zero taxa at Mdh-2 and Mdh-3 to two taxa at Adh, Dia, Est-2, Fe-1, Lap-1, Mdh-1, Mnr, Pgi, and Tpi-2.

The genetic variability found in species of Spiranthes is summarized in Table 3. The total number of alleles per taxon ranged from 14, found in S. lucida, to 36 found in S. cernua. The mean number of alleles per locus ranged from 1.1, in S. lucida, to 2.7 in S. cernua. The percentage of polymorphic loci ranged from 16.7%, in S. lucida, to 78.56%, in S. ochroleuca. Observed heterozygosity for the diploids ranged from 0.007 for S. lucida to 0.347 for S. porrifolia with higher values observed for the tetraploids, 0.460 for S. diluvialis and 0.398 for S. delitescens. Spiranthes odorata and S. lucida contained the least amount of genetic variability and were monomorphic at ten of the fourteen putative loci surveyed.

At all fourteen loci S. diluvialis possessed a combination of alleles found within the putative parental species, S. magnicamporum and S. romanzoffiana (Table 4). At nine loci, Adh, Fe-1, Lap, Mdh-1, Mdh-2, Mdh-3, Mnr, Pgi, and Tpi-2, at least in part, S. diluvialis combined alternate alleles from the putative parental species. Although many of the alleles present in S. magnicamporum and S. romanzoffiana were also present in other congeneric species within the 2n=30 and 2n=44 series, respectively, some differences were observed. At the Est-2 locus, S. vernalis and S. odorata were monomorphic for allele nine, which was not found in S. diluvialis. At the same locus, S. ochroleuca harbored three different alleles (5, 7, and 8) that were also not found in the tetraploid species. At the Pgi locus, S. odorata was homozygous for allele three which was not found in populations of S. diluvialis. At Dia, S. porrifolia possesses alleles 2 and 3, which were found in S. diluvialis, however, alleles 5 and 6 were missing in S. porrifolia, but present in both S. romanzoffiana and S. diluvialis. At Lap, S. porrifolia was monomorphic

for allele two, whereas alleles 1, 2, and 4 were present in both S. romanzoffiana and S. diluvialis. At the Pgi locus, only S. romanzoffiana and S. diluvialis possessed the low frequency allele 4.

The tetraploid S. delitescens differed from S. diluvialis in the banding patterns observed at Mdh. The two species consistently exhibited different alleles at Mdh-1, Mdh-2, and Mdh-3. The alleles found in S. delitescens were not found in either S. magnicamporum or S. romanzoffiana. Spiranthes ochroleuca and Spiranthes odorata also lacked alleles found in S. delitescens at Adh, Est-1, and Est-2. At Est-2, S. vernalis was monomorphic for allele nine which was not found in S. delitescens. At Fe-1, S. porrifolia was homozygous for allele two which was not found in S. delitescens.

Genetic identity values (Nei, 1978) were calculated between all pairs of populations and taxa. Mean genetic identities are summarized in Table 5. The mean genetic identity among populations of S. diluvialis was 0.962 (range 0.876 to 1.000) and that for four populations of S. romanzoffiana was 0.928 (range 0.852-0.995). The two populations of S. magnicamporum had a genetic identity of 0.781. Genetic identifies among populations within the remaining species ranged from 0.756 in S. porrifolia to 0.901 in S. cernua. The mean genetic identity between the two cytological series of diploid species (2n=30 and 2n=44) ranged from 0.066 for S. vernalis and S. lucida to 0.335 between S. romanzoffiana and S. cernua with a grand mean of 0.273. Higher genetic identities between diploid species within the same chromosomal series were found. For the 2n=30 group, the grand mean was 0.591, ranging from 0.453 between S. magnicamporum and S. odorata, up to 0.782 between S. cernua and S. ochroleuca. For the 2n=44 group, the grand mean was 0.510, ranging from 0.301 between S. porrifolia and S.

lucida to 0.795 between S. porrifolia and S. romanzoffiana. The genetic identity between the two tetraploid species was 0.455. In comparing the diploid genomes with S. diluvialis, the highest genetic identities were found for S. romanzoffiana (0.727) and S. magnicamporum (0.619). For S. delitescens, the highest genetic identities were found for the diploids S. porrifolia (0.580) and S. vernalis (0.595).

Discussion

The origin of *S. diluvialis*. Isozyme results favored S. magnicamporum and S. romanzoffiana as the diploid progenitors of S. diluvialis since the alleles found in S. diluvialis are a combination of those found in the two diploid species. Allozyme results do not support S. lucida, S. ochroleuca, S. odorata, or S. vernalis, as possible diploid progenitors of S. diluvialis. At several enzyme loci, those species did not contain the alleles found in S. diluvialis. However, allozyme markers did not distinguish the putative parental species as clearly from S. cernua and S. porrifolia which also contained a combination of alleles found in the tetraploid.

A comparison of allelic frequencies supports S. magnicamporum rather than S. cernua (both with 2n=30 chromosomes) as the most likely progenitor of S. diluvialis. At two loci (Adh and Est-1), S. diluvialis was typically heterozygous for the most common alleles found in S. romanzoffiana and S. magnicamporum. In S. cernua (Adh) these alleles were observed only at low frequencies (0.063 and 0.141), whereas the most common allele in S. cernua was found in S. diluvialis at low frequency

(0.062). Similarly, at the Est-1 locus, S. cernua was homozygous for an allele which was present in only one population of S. diluvialis at low frequency (0.047). Spiranthes romanzoffiana harbors the same allele and is the more likely donor given the allelic frequencies found in the tetraploid.

Differentiation of S. romanzoffiana and S. porrifolia as progenitor species is difficult due to their high genetic similarity (0.795). At eight loci, both S. romanzoffiana and S. porrifolia possessed the alleles found in S. diluvialis. Although four additional loci (Dia, Lap-1, Mdh-1, and Pgi) support S. romanzoffiana as the diploid progenitor of S. diluvialis, two loci (Adh and Est-1) favor S. porrifolia. For three of these loci (Dia, Lap, and Pgi), S. porrifolia was missing alleles found in both S. romanzoffiana and S. diluvialis. The Mdh banding patterns were complex, but also supported S. romanzoffiana as the parental species. In S. romanzoffiana, the Mdh-1 locus was missing, whereas S. porrifolia contained alleles 3 and 6 which were found in S. diluvialis. However, the predominant banding pattern found in S. diluvialis was homozygous for allele 1, an allele not found in S. porrifolia but present in S. magnicamporum. Two loci support S. porrifolia rather than S. romanzoffiana as the diploid progenitor. At the Adh locus, the presence of a rare allele (allele 3) was detected in both S. porrifolia and S. diluvialis. This allele was not found in either S. magnicamporum or S. romanzoffiana. However, due to its low frequency and presence in only one population of S. diluvialis it could be a new mutation since the original hybridization event(s) and could coincidentally have the same electrophoretic mobility as the allele found in S. porrifolia. At the Est-1 locus, allele 5 was found in both S. porrifolia and S. diluvialis but not S. romanzoffiana, however, allele 5 is also found in S. magnicamporum.

Overall, these data support S. magnicamporum and S. romanzoffiana as the diploid progenitors of the allopolyploid S. diluvialis. However, alleles were observed in S. diluvialis which could not be attributed to either S. magnicamporum or S. romanzoffiana (Adh, allele 3; and Tpi-1, allele 5). The presence of these alleles may be explained by incomplete sampling of the variability present in the diploids, loss of the alleles in the extant diploid populations (as sampled), or they may have arisen through mutation following hybridization.

Genetic identity values further support S. magnicamporum and S. romanzoffiana as the diploid progenitors of S. diluvialis. Genetic identity values indicate S. diluvialis is more similar to S. magnicamporum (0.619) and S. romanzoffiana (0.727) than to any of the other congeneric species assayed. Although previous studies of other taxa have indicated greater similarity between putative parental species (e.g. Ranker et al., 1989), in the present work relatively low genetic similarity (I=0.334) was found between S. magnicamporum and S. romanzoffiana indicating these two taxa are not close relatives. The dissimilarity between the parental species may be an advantage in the formation of bivalents rather than multivalents in the resulting tetraploid.

Several recent molecular studies (Soltis, et al., 1992 and references therein; Soltis and Soltis, 1993) have indicated multiple hybridization events are more common than traditionally suggested. Recurrent polyploidization produces substantial genetic variation within polyploids from different parental populations. The genetic variation within S. diluvialis supports the occurrence of multiple hybridization events. A minimum number of hybridization events may be estimated from the maximum total number of alleles derived from the diploid parental

species at any given locus. Five alleles were observed at Dia and Fe-1 which were present in the diploid parental species, thus, supporting a minimum of two separate hybridization events giving rise to the allotetraploid species. The genetic consequences of multiple hybridization events are high levels of genetic variation present within populations of this rare orchid which may help buffer these populations from environmental stochasticities.

Isozyme data indicate S. delitescens and S. diluvialis are not of the same origin and, therefore, that S. delitescens is a distinct, tetraploid species. The genetic identity value between the tetraploids is relatively low for comparisons within a genus (0.455). Similarly, the genetic identity between S. delitescens and each of the diploid species, S. magnicamporum and S. romanzoffiana, is low (0.453 and 0.362, respectively). For S. delitescens, the highest genetic similarities were found between S. porrifolia (0.580) and S. vernalis (0.595) which were suggested by Sheviak (1990) as the putative parental species. However, these genetic identities are lower than those found between S. diluvialis and its diploid progenitors. At eight loci, S. delitescens possesses a combination of alleles found in S. porrifolia and S. vernalis, however, at five loci the tetraploid does not combine the alleles commonly found in the two putative parental species. These data suggest the diploid parental species giving rise to S. delitescens were not sampled in the present study, significant divergence between the progenitor and derivative species has occurred since hybridization and polyploidization, or the limited number of populations sampled did not include the parental genotypes.

Genetic variability and geographic range. The wider range in distribution of polyploids relative to their diploid progenitors has been reported previously (Roose and Gottlieb, 1976; Lewis, 1980; Soltis and Soltis, 1991). The robustness of polyploid dispersal and establishment has been attributed to their possession of two divergent diploid genomes which may produce a heterozygous advantage. Multiple copies of enzyme-producing genes may extend the range of the polyploid beyond that of the diploid parental taxa and account for their frequent widespread distribution (Roose and Gottlieb, 1976 and references therein). In addition, novel heteromeric enzymes with distinctive properties may result if the duplicated genes represent different subunits of a multimeric enzyme (Roose and Gottlieb, 1976). However, in the case of S. diluvialis, the polyploid is geographically restricted whereas the diploid progenitors are both widespread common species. The relatively restricted range for S. diluvialis may have resulted from the localization of the initial hybridization event(s). The geographic ranges of the parental taxa, S. magnicamporum and S. romanzoffiana, do not presently overlap, thus, the hypothesized overlap (Sheviak, 1984) during the Pleistocene pluvial period may have been minimal. Adaptation of the resulting allotetraploid S. diluvialis to a new habitat and its subsequent dispersal may have resulted in its current distribution within riparian zones. The widespread distribution of the recent allotetraploid, Tragopogon miscellus, during the last century provides evidence for the rapid establishment of allopolyploids (Soltis and Soltis, 1991). However, the widespread destruction of wetlands within the western United States may have reduced a once-larger distribution of S. diluvialis to its current state.

The taxonomic status of *S. diluvialis.* Although S. diluvialis is accepted as a distinct species by the U.S. Fish and Wildlife Service and Colorado Natural Areas Program, in the past some controversy has surrounded its status (Naumann and England, personal communications). Although allopolyploidy has long been recognized as a mechanism of speciation in plants (Harlan & deWet, 1975; Jackson & Hauber, 1983; Stebbins, 1950; Winge, 1917), recent controversy over the treatment of primary homoploid hybrids under the Endangered Species Act (ESA) with respect to some animal populations has confused the issue (Dowling et al., 1992a, 1992b; Fergus, 1991; Nowak, 1992; O'Brien and Mayr, 1991; Wayne and Jenks, 1991; Whitham et al, 1991). A clear distinction must be made between primary, homoploid hybrid individuals and allopolyploid species (Ranker and Arft, 1994). Primary homoploid hybrids result from the union of haploid gametes from two genetically distinct diploid individuals, each from a different species. The resulting hybrid individual typically possesses lower levels of fertility or complete sterility. In contrast, an allopolyploid often results from an increase in chromosome number via nondisjunction; either separately in individuals of two diploid species that subsequently hybridize at the polyploid level (Type I polyploidization; Harlan and deWet, 1975) or after interspecific hybridization in a primary diploid hybrid (Type II polyploidization; Harlan and deWet, 1975). This duplication of chromosomes confers "instant" speciation on the allopolyploid since it is reproductively competent and isolated from the parental species. Thus, S. diluvialis is protected under the ESA as a distinct species based on any species definition, i.e., from the perspective of biological, evolutionary, or ecological species concepts

(Camp and Gilly, 1943; Clausen et al. 1941; Donoghue, 1985; Mayr, 1982; Simpson 1961; Stebbins, 1950; Wiley, 1981).

This is the first study to explore the origin of a polyploid species in the Orchidaceae through the application of molecular techniques. The research provides information regarding the allopolyploid origin of S. diluvialis and its distinctness as a species. Allozymic data allow identification of the putative parental taxa and provide insight into the genetic variability present within the genus. Knowledge of the evolutionary origin of S. diluvialis provides a meaningful framework for and insight into both genetic and ecological studies critical to the continued existence of this rare orchid.

Chapter 3: Population Genetics

Introduction

Spiranthes diluvialis Sheviak (Orchidaceae) is a terrestrial orchid species which occurs primarily along the east slope of the Colorado Front Range and in the Uintah Basin along the south slope of the Uintah Mountains in northeastern Utah. Smaller, scattered populations occur in Colorado, Nevada, Utah, and Wyoming (Figure 2; U.S. Fish and Wildlife, 1995). Populations occur at elevations between 1300 and 2100 meters in alluvial substrates along riparian edges, gravel bars, old oxbows, and moist to wet meadows in the floodplains of perennial streams where the soil is saturated during the spring and summer growing season. Some populations of the eastern Great Basin occur in similar habitats near freshwater lakes or springs. The orchid usually occurs in small scattered groups which occupy a relatively small area within the riparian system (Franklin, 1993).

Individual orchids stand 12-45 cm tall and bloom from July through September (Jennings, 1990; personal observation). Small, inconspicuous leaf rosettes may emerge at the end of the growing season and persist through the winter months. Many species of Spiranthes are initially saprophytic underground plants that persist for many years before leaves emerge above ground. These individuals rarely flower in consecutive years or under unfavorable conditions, and may survive due to specific symbiotic relationships with mycorrhizal fungi (Wells, 1981).

Reproduction appears to be strictly sexual with bumble bees (Bombus spp.) as the primary pollinators (Dressler, 1981; Sheviak, 1984; Sipes et al., 1993). The inflorescence always begins blooming with the bottom flower and proceeds sequentially up the spike. Flowers are protandrous (function first as male flowers and then female flowers) due to a change in the position of the column. During the male stage, the stigmatic surface is positioned close to the inner surface of the lip so that incoming pollen is unable to reach the stigmatic surface. After several days, the angle of the column changes, exposing the stigmatic surface. These features tend to maximize outcrossing, due to the tendency of bees to visit the bottom-most flower first and then proceed vertically up the spike. So, pollinia are removed from the top "male" flowers of one plant and transported to the bottom "female" flowers of the next plant.

Spiranthes diluvialis gained federally threatened status under the U.S. Endangered Species Act on January 17, 1992 (Federal Registry, 1992) due to habitat destruction, large fluctuations in monitored population size, the unknown impacts of grazing, and potential exotic species invasion. At the time, little was known of the genetic, ecological, and demographic processes affecting the life history and long-term survival of this threatened orchid.

Maintenance of genetic diversity has been considered crucial for long-term survival and the evolutionary response of populations to changes in the environment (Antonovics, 1984; Franklin 1980; Huenneke, 1991). Loss of genetic variation may reduce a population's ability to adapt to changing environmental conditions and result in inbreeding

depression. Population genetic analyses of rare plants, such as S. diluvialis, are particularly important for designing management programs which will allow for the conservation of maximum levels of natural genetic variation and local adaptation.

Central to understanding the genetics of a species is determining the amount and distribution of genetic variation within and among populations. A number of species traits, both biological and historical, may effect the structure of genetic diversity within and among populations. For example, geographically-restricted species generally exhibit less genetic variation than widespread congeners (Karron, 1991). Factors that may account for the reduced variability are stochastic events (genetic drift and founder effect), strong directional selection toward genetic uniformity, and/or high levels of inbreeding followed by selection against homozygous individuals with rare deleterious alleles. However, the range of genetic variation present in rare species varies considerably and in several cases, geographical range is a poor indicator of genetic diversity (Karron, 1991). Although geographic range may account for the largest proportion of genetic variation, Hamrick et al. (1989, 1991) found that seven other species traits are correlated with genetic diversity including taxonomic status, regional distribution, life form, mode of reproduction, breeding system, seed dispersal mechanism, and successional status.

In his original description, Sheviak suggested S. diluvialis ($2n=74$) may possibly be an allopolyploid hybrid between S. romanzoffiana ($2n=44$) and S. magnicamporum ($2n=30$) based on morphological and cytological evidence (Sheviak, 1984; see Chapter 2). Allopolyploidy may arise following hybridization between two genetically distinct diploid species

and is an important mechanism of speciation in flowering plants. The process of becoming and the attributes of being polyploid also relate to the development and maintenance of genetic variation. The number of hybridization events (i.e., single versus multiple) in addition to the variability present in the parental taxa will contribute to the extent of variation in the resulting allopolyploid. A single hybridization event limits the amount of genetic variability found in the polyploid to that of the combination of genomes of the parental gametes, whereas multiple or recurring hybridization events could potentially encompass all of the variability found in both parental taxa. Allopolyploid speciation often results in more individual genetic variation than in a diploid species because different parental alleles combine to form heterozygous isozymic patterns in the allopolyploid (Ranker et al., 1989). For example, Roose and Gottlieb (1976) found approximately 30-40% "fixed" heterozygosity across loci within the allopolyploids of Tragopogon. Increased heterozygosity, the generation of novel heteromeric enzymes, and the formation of new gene combinations may be critical in the successful establishment of the polyploid (Thompson and Lumaret, 1992). Additional genetic variation after the initial hybridization event(s) may be provided by mutation and recombination. The expression of mutations is also affected by ploidy level such that tetraploids should change at half the rate of their diploid progenitors because four copies of each gene are present (Haufler, personal communication). Since polyploids possess more than two copies of each gene, differential gene silencing among allopatric populations may occur, resulting in genetic divergence between populations. For example, mutations within regulatory genes could lead to significant interpopulational differences (Werth and Windham, 1991).

Genetic information also can provide insight into the historical biogeography of populations, revealing past geographic structure and distribution (Lacy, 1988; Ranker, 1992). Nothing is known of the origins of the isolated populations of S. diluvialis. By elucidating patterns of genetic variation, however, this study provides information on whether S. diluvialis was once a larger contiguous population spanning its present range or if present populations were established through long-distance dispersal. Relatively more genetic variation would be expected in relictual populations than in those established via long-distance dispersal where all the genes stem from the few carried by the original founders and from subsequent mutation or immigration.

Enzyme electrophoresis has been the predominant technique employed to survey populational genetic variation in recent years (Schaal et al., 1991) and allows relatively fast, inexpensive analysis of large numbers of individuals. This study employs isozyme analyses to assess genetic variation and structure within and among populations of S. diluvialis. The following questions were addressed concerning the genetic processes within and among populations of S. diluvialis:

1. How is genetic variability partitioned within and among populations of S. diluvialis?
2. Are the genetic differences the result of multiple hybridization events, differential selective pressures acting on isolated disjunct populations, and/or the result of genetic drift? In contrast, are the populations similar as a result of one hybridization event and dispersal with little subsequent genetic divergence?

3. What genetic processes (e.g., gene flow, sexual outcrossing, polyploidy) maintain population genetic variability in S. diluvialis?

Methods

Leaf samples from a total of twelve populations were collected for isozyme analysis. Only one individual per vegetative clump was sampled to minimize collecting vegetatively-produced clones more than once. Collections were timed to coincide with flowering because the plants were difficult to locate in their vegetative stage. Sample sizes and population localities are listed in Table 6. Six of the nine watersheds where populations of S. diluvialis occur were collected (Figure 2). Small population size at the remaining three watersheds prohibited collection of those populations.

Leaves were stored in plastic bags on ice for transportation to the laboratory. Leaves were ground in an extraction buffer: 0.1M PO_4 buffer adjusted to pH 7.5, 0.03 M Borax, 0.02 M Diethyldithiocarbamic acid (DIECA), 0.2 M ascorbic acid, 6% Polyvinyl pyrrolidone (PVP) (40,000), with 1.1% (vol/vol) 2-mercaptoethanol and 10% Dimethylsulfoxide (DMSO) added just before grinding (Ranker et al., 1989). Leaf grindate was absorbed with chromatography paper wicks and inserted into 12.5% starch gels for electrophoresis.

Three gel buffer systems were used to resolve nine enzyme systems. A discontinuous lithium-borate buffer system (gel buffer, 0.004 M LiOH, 0.005 M boric acid, 0.03 M Tris[hydroxymethyl] amino methane (Tris), and 0.05 M citrate pH7.6; electrode buffer, 0.04 M LiOH and 0.04 M boric acid,

pH7.6) was used to resolve diaphorase (DIA), fluorescent esterase (FE), leucine aminopeptidase (LAP), menadione reductase (MNR), phosphoglucose isomerase (PGI), and triose phosphate isomerase (TPI). A discontinuous Tris, ethylenediamine tetraacetic acid (EDTA), borate buffer system (gel buffer, 0.046 M Tris, 0.0009 M EDTA, 0.004 M boric acid; electrode buffer, 0.15 M Tris, 0.003 M EDTA, and 0.013 M boric acid) was used to resolve alcohol dehydrogenase (ADH) and colorimetric esterase (EST). A continuous morpholine citrate buffer system (0.04 M citrate titrated to pH 7.5 with N-(3-aminopropyl)-morpholine) was used to resolve malate dehydrogenase (MDH). Loci were estimated by comparing bands to those observed in the putative parental taxa, S. magnicamporum and S. romanzoffiana.

Genetic variation was assessed by estimating three commonly used parameters: the percent of loci polymorphic (P), the mean number of alleles per locus (A), and the observed heterozygosity (H_O) assuming Hardy-Weinberg conditions. The resulting estimates were used to compare genetic variation within and among populations. Estimates of genetic variation were also used to infer processes maintaining genetic variation in the small, fragmented populations of this species.

Genetic divergence of populations was estimated from the standardized variance in allele frequencies, F_{st}, for all pairs of populations (Wright 1931, 1943, 1969). The weighted average of F_{st} for multiple loci is equivalent to Nei's G_{st} (Nei, 1973, 1977). These values range from 0.0 (no genetic divergence between populations, i.e., all genetic diversity is within populations) to 1.0 (complete genetic divergence between populations, i.e., all genetic diversity is between populations).

Gene flow, the gain or loss of alleles from a population via immigration or emigration of fertile individuals, were estimated among pairs of tetraploid populations from the equation:

$$F_{st} \sim 1/(8Nm + 1)$$

In this formula, N is the effective breeding size of a population and m is the proportion of a population replaced each generation by immigrants (Dobzhansky and Wright, 1941; Wright, 1931, 1943, 1951). Theoretically, gene flow may range from zero (no gene flow) to infinity (complete random mating among "populations"). An Nm greater than 1.0 indicates gene flow is large enough to repress population divergence via genetic drift in the absence of diversifying selection.

Nei's genetic identity values (Nei, 1978) were calculated between all pairs of populations. Genetic identity values may range from 0.0 to 1.0 with zero indicating no genetic similarity between two taxa and one indicating genetically-identical taxa.

Results

The isozyme data consisted of putative genotypes for each individual for all loci sampled. Because S. diluvialis is a tetraploid species, differential banding intensities were recorded and interpreted as corresponding to the presence of different numbers of alleles at a given locus. For example, for two alleles, 1 and 2 at a specific locus, the genotype of a heterozygous individual might be 1112, 1122, or 1222. Allelic

frequencies were calculated from genotypic data and genetic variability statistics including number of alleles per locus, percentage of polymorphic loci, expected heterozygosity, and F statistics were determined using BIOSYS-1 (Swofford and Selander, 1989).

Fourteen putative loci provided consistently interpretable results for all populations. Allelic frequencies are tabulated in Table 7. Several additional loci were not included in the analysis since they could not be consistently resolved in all populations (Est-3, Est-4, Fe-2). For all enzymes, the number of isozymes observed was typical of diploid plants (Soltis and Soltis, 1989; Weeden and Wendel, 1989), except Mdh (four instead of three). The typical banding pattern for Mdh is shown in Figure 3. Based on the alleles found in the parental taxa (see Chapter 2), four loci were scored with the remaining bands representing interlocus heterodimers. Eleven of the fourteen loci were polymorphic in one or more population. Only Mdh-2, Mdh-3, and Mdh-4 were monomorphic across all populations. A total of 34 alleles were observed with the number of alleles per polymorphic locus ranging from a minimum of 2 alleles (Tpi-2) up to 5 alleles for Adh, Dia, and Fe-1. The number of populations for which a given locus was polymorphic ranged from one population for Tpi-1 to all twelve populations for Adh, Est-1, Lap, Mnr, Pgi, and Tpi-2.

The genetic variability found in populations of S. diluvialis is summarized in Table 8. The total number of alleles per population ranged from 23 (American Fork, Ashley Creek, Big Brush Creek, Brown's Park, Clear Creek, Diamond Fork) to 30 (Deer Creek). The mean number of alleles per locus in S. diluvialis ranged from 1.6 for the Ashley, Big Brush Creek, Brown's Park, Clear Creek, and Diamond Fork populations to 2.1 at

Deer Creek and Duchesne River. The mean number of alleles per polymorphic locus ranged from 2.6 at American Fork, Ashley Creek, and Diamond Fork to 3.3 at Deer Creek. The percent of polymorphic loci ranged from 57.1% for the Big Brush Creek, Brown's Park, Clear Creek, Cherryvale, and Powell Slough populations to 71.43% for the Deer Creek, Uintah River, and Van Vleet populations. Sample size ranged from 20.0 for Big Brush Creek to 134.9 at Van Vleet.

A summary of the alleles present within all twelve populations of S. diluvialis is shown in Table 9. Different combinations of alleles were found across the polymorphic loci in all twelve populations (Figure 4). For example, although Deer Creek, Powell Slough, and Van Vleet all possessed alleles 1, 2, and 3 at Est-1, they each possessed different alleles at Dia (Deer Creek, alleles 2, 3, and 6; Powell Slough, alleles 1 and 2; and Van Vleet, alleles 1, 2, and 3). These results were not due to sampling artifacts since number of alleles and sample size were not significantly correlated (correlation coefficient = 0.585, $p>0.05$)

The mean observed heterozygosity (Ho) across all populations and all loci ranged from 0.365 at the Deer Creek population to 0.582 at the Diamond Fork population (Table 10). In contrast, the mean expected heterozygosity under Hardy-Weinberg equilibrium for diploid populations with the same allele frequencies as those observed for populations of S. diluvialis ranged from 0.252 at Powell Slough to 0.318 at Diamond Fork with a mean of 0.282.

Pairwise genetic identity values between populations of S. diluvialis are summarized in Table 11. Mean genetic identity between pairs of populations ranged from 0.876 between American Fork and Deer Creek to 1.000 between all pairs of populations American Fork, Big Brush Creek,

Duchesne River, and Uintah River with a mean of 0.962 across all pairwise combinations.

The standardized variance in allele frequencies ($F_{st} \sim G_{st}$) and interpopulational gene flow (Nm) for each pair of populations are summarized in Table 12. The standardized variance in allele frequencies ranged from 0.000 for the pairs Ashley Creek/Big Brush Creek and American Fork/Big Brush Creek to 0.149 for Clear Creek/Deer Creek with a mean across all populations and loci of 0.083. Interpopulational gene flow ranged from 0.71 for the Clear Creek/Deer Creek pair to infinity for the Ashley Creek/Big Brush Creek and American Fork/Big Brush Creek pairs with a mean across all populations of 1.38.

Discussion

The high levels of genetic diversity found within populations of S. diluvialis are due primarily to their polyploid condition. Similar to most polyploids (Crawford, 1985, 1989; Gottlieb, 1982; Roose and Gottlieb, 1976; Thompson and Lumaret, 1992), S. diluvialis shows high levels of fixed, or nearly-fixed, heterozygosity at several loci (Adh, Lap, Est-1, Mnr, Pgi, Tpi-2). Distinct allozyme markers in the parental taxa may combine in the allopolyploid to produce "fixed" heterozygosity. Thus, polyploidy may also account, in part, for the high percentage of polymorphic loci found in S. diluvialis (57.1% to 71.4%) relative to seed plant species on average (34%) (Hamrick et al., 1991). However, the mean number of alleles per polymorphic locus in populations of S. diluvialis (2.57-3.00) was relatively low compared to both widespread and endemic diploid, seed plant species (3.19 and 3.00, respectively) (Hamrick et al., 1991). The low mean number

of alleles per polymorphic locus may also result from an allopolyploid origin. One to a few initial hybridization events would limit the number of alleles found in the tetraploid derivative species to a subset of those found in the diploid parental taxa.

Genetic variation among populations (G_{st} = 0.083) and for pairs of populations (range = 0.000 to 0.149) is low relative to other animal-pollinated, outcrossed species (G_{st} = 0.21) (Hamrick et al., 1991). Conversely, estimates of gene flow (mean = 2.800; range = 1.430 to infinity) are relatively high when compared to other animal-pollinated outcrossed species (0.940; estimated from Hamrick et al., 1991). Estimates of gene flow above 1.0 indicate there is enough migration between populations to eliminate genetic drift as an evolutionary force leading to the divergence of populations (Dobzhansky and Wright, 1941). The high level of apparent gene flow among populations of S. diluvialis, however, may result from the confounding effects of recent common ancestry and not gene flow *per se*.

Genetic identity values (average, 0.962) also indicate a high degree of similarity among populations of S. diluvialis. Thus, each population harbors most of the genetic variability found within the species. However, these estimates do not take into account the presence of population-unique alleles. In addition, each of the twelve populations of S. diluvialis contains unique combinations of alleles across the polymorphic loci.

These unique combinations of alleles indicate differentiation among populations which may have resulted from multiple hybridization events occurring between different pairs of parental diploids, sexual recombination and/or the incorporation of new mutations into the populations since hybridization. Closer examination of the allelic

combinations across loci indicates the presence of two unique alleles not found in either of the putative parental species, allele 3 at Adh and allele 5 at the Tpi-1 locus (see Chapter 2 for a discussion of the parental taxa). These alleles may represent new mutations that have arisen since hybridization or unsampled variability within the parental diploid species. A minimum number of hybridization events may be estimated from the maximum total number of alleles at any given locus. Five alleles were observed at Dia and Fe-1 which support a minimum of two separate hybridization events giving rise to the allotetraploid species.

These data shed some light on the biogeographical history of S. diluvialis (Figure 4). If present day populations are relictual populations of once larger, more widespread occurrences, then similar genetic variability should be seen across existing populations. The data do not clearly show this type of pattern. The data may support a limited number of hybridization events and subsequent long distance dispersal. If populations result from a minimum of two separate hybridization events, then the remaining populations should contain a subset of alleles found in the original populations. For example, the Uintah River population contains alleles 1, 2, and 5 at Dia with nearby Big Brush Creek containing alleles 2 and 5 and Duchesne River and Ashley Creek containing only allele 2. At Fe, the Uintah River population contains alleles 1, 2, 5, and 6 with Ashley Creek and Big Brush Creek containing only alleles 1 and 2, however, the Duchesne River population contains alleles 1, 2, and a different allele (3) which is also found within one of the diploid parental species. Multiple hybridization events and gene flow within a local area may account for these inconsistencies and contribute to the obscure patterns of allelic variation observed.

Traditionally, polyploids have been considered genetically impoverished based on the assumption that polyploidization was a rare event and each polyploid species had a unique origin (Soltis and Soltis, 1992, 1993). Recent molecular data indicate that multiple origins of polyploids are the rule rather than the exception (for a review see Soltis and Soltis, 1992, 1993). In addition, recurrent hybridization events have been documented occurring over relatively short time spans and geographic distances (Soltis and Soltis, 1992). Thus, significant genetic variability may be incorporated into the allopolyploid from genetically distinct parental populations. Isozyme data from the present study indicate that S. diluvialis follows this general trend with a minimum of two hybridization events introducing genetic variability into the polyploid derivative.

This research indicates allopolyploidy may be a primary factor responsible for the generation and maintenance of genetic variation within and among populations of S. diluvialis. Numerous recent studies using molecular techniques have supported the occurrence of multiple hybridization events over relatively short time spans and geographic distances (Ashton and Abbott, 1992; Haufler et al., 1990; Soltis and Soltis, 1993). Recurrent polyploidization produces substantial genetic variation within polyploids from different parental populations. The genetic consequences of multiple hybridization events are high levels of genetic variation present within populations of this rare orchid which may help buffer these populations from environmental stochasticities. Maintenance of genetic diversity is important in reducing the deleterious effects of inbreeding and stochastic genetic processes (such as genetic drift), and in allowing for continued evolutionary adaptation and change.

Genetic variation has increasingly been recognized as crucial to success in the long-term management of rare and endangered species and should be a central concern for the long-term conservation of populations of S. diluvialis (Franklin 1980; Antonovics, 1984; Holsinger and Gottlieb, 1991; Huenneke, 1991). Population genetic analyses of rare orchids such as S. diluvialis are particularly important in light of conserving alleles or combinations of alleles unique to these small populations. Allelic differences between populations add weight to the distinctness of populations and argue for the preservation of each population as a unique genetic entity. Genetic diversity such as this may be critical in buffering populations of S. diluvialis against the genetic problems associated with small isolated populations and allow for its continued evolutionary change and adaptation.

Chapter 4: Demographics

Introduction

The short-term persistence of rare and endangered species is often dominated by ecological and demographic considerations (Holsinger and Gottlieb, 1991; Lande, 1988; Schemske et al., 1994). The recovery plan for S. diluvialis identifies the life history, demographics, and habitat requirements as important recovery goals (U.S. Fish and Wildlife Service, 1994). This work comprises a detailed ecological study of S. diluvialis, including analyses of demographic dynamics and environmental requirements, which may help elucidate both intrinsic and extrinsic factors constraining the continued persistence of this species.

Spiranthes diluvialis is a terrestrial orchid species known from scattered populations in Colorado, Nevada, Utah, and Wyoming (Figure 2; U.S. Fish and Wildlife Service, 1994). Populations occur in wetland habitats such as subirrigated meadows, alluvial terraces, and abandoned stream channels where the water table is near the surface throughout the growing season and into the late summer or early autumn. The orchid occurs at elevations between 1300 and 2100 meters, stands 12-45 cm tall, and blooms from July through September (Jennings, 1990; personal observations). Small, inconspicuous leaf rosettes may emerge at the end of the growing season and persist through the winter months.

Each of the existing populations is limited in area to under 50 acres and in the number of individual plants to under 7000 (U.S. Fish and Wildlife Service, 1994; Coyner, 1990). Many of the historically documented populations of S. diluvialis have been extirpated as a result of urban development (U.S. Fish and Wildlife Service). The remaining populations

occur primarily in areas where vegetation is relatively open and not overly dense, overgrown, or overgrazed (Coyner 1990; Jennings 1989, 1990). A few populations in eastern Utah and Colorado are found in the shade of riparian woodland communities, but the species generally seems to prefer open, forb-dominated sites. The orchid usually occurs in small scattered groups which occupy a relatively small area within the riparian system (Franklin, 1993).

Some of the existing sites where populations of S. diluvialis occur have supported agriculture historically. The habitat alteration resulting from agricultural use (such as mowing, grazing, and burning) may be neutral, beneficial, or detrimental to S. diluvialis (McClaren and Sundt, 1992; Gori, personal communication). In Colorado, the largest population of S. diluvialis is on City of Boulder Open Space at the Van Vleet Ranch which has been used agriculturally for the past 50-75 years. This site is still grazed each year in the winter from February through May, mown in the summer around the beginning of July, and irrigated in the spring and early summer. When exclosures were placed around patches of S. diluvialis at the Van Vleet site, to exclude mowing and grazing, the orchids disappeared (Tamara Naumann, personal communication). Since the initial disturbance of the creek's flood plain at the Van Vleet site, the population may have persisted as a result of agricultural use because grazing may control competing weedy species such as Canada thistle (Cirsium arvense), a problem at the Van Vleet site, which may outcompete the orchid for light and nutrients.

Since its discovery in 1986, the population at Van Vleet has been monitored by the City of Boulder Open Space Department. Large fluctuations in population size have been reported from 1986 to 1992, excluding 1991 since no data were collected that year (Figure 5; City of Boulder Open Space, personal communication). The apparent tendency for

populations of Spiranthes diluvialis to fluctuate drastically from one year to the next makes populational and distributional assessments of orchid occurrences difficult. The monitoring counts were consistently conducted during the first week of August; however, different individuals as well as different techniques were used each year. Thus, some of the variation in population size may be due to methodology. Due to the difficulty in finding vegetative individuals, the monitoring counts were based on flowering individuals and did not include vegetative plants. Previous work done by Wells (1981) in England on S. spiralis indicated that population size did not fluctuate when both flowering and vegetative plants were surveyed.

Spiranthes diluvialis became federally listed as threatened January 17, 1992. The reasons for listing were numerous. Foremost is the current or threatened destruction of habitat. Urbanization of the Colorado Front Range and Utah's Wasatch Front has directly impacted wetland habitat and changed natural stream dynamics. Second, monitoring counts of some populations have indicated dramatic fluctuations in population size. Third, many of the populations occur on public lands which are impacted by grazing, the effects of which are unknown. Finally, the invasion of exotic, weedy species has threatened some populations.

The shape of this work was formed, in part, by the reasons for listing. Demographic plots were established at Van Vleet, along Clear Creek, and at Deer Creek to collect data on the life history of S. diluvialis, evaluate the effect of management techniques, and examine environmental factors which may be critical to the persistence of S. diluvialis. The specific questions addressed by this phase of research included:

1.) Do populations fluctuate in size annually when both vegetative and flowering plants are monitored?

2.) What is the life history of S. diluvialis? What proportion of individuals produce an inflorescence and set fruit each year? Can plants remain subterranean during unfavorable periods and reemerge when conditions are more favorable?

3.) How does phenology compare across different populations? What factors may be important?

4.) What effect does management have on the Van Vleet population? Which management strategy provides the best conditions for the continued persistence of S. diluvialis?

5.) Because this species occurs in semi-aquatic habitats, is soil moisture the most critical factor affecting the continued survival of S. diluvialis? What other environmental factors are important?

Materials and Methods

Detailed information on the survival, growth, and fecundity of S. diluvialis was collected in a manner similar to studies on other species of Spiranthes (Wells, 1981; McClaren and Sundt, 1992; see below). These data were collected primarily on the City of Boulder Open Space population (Van Vleet site), where 29 experimental plots were established and monitored during the 1992 field season, five additional plots were added during 1993, and three more plots were added in 1994.

In addition to the Van Vleet haymeadow, demographic data were also collected at three other nonagricultural riparian sites: at the mouth of Clear

Creek in Jefferson County, Colorado (Clear Creek site); along Clear Creek at Prospect Park in Jefferson County, Colorado (Prospect Park site); and along Deer Creek in Garfield County in southern Utah (Deer Creek site) (Figure 2)

Orchid patches at Van Vleet were initially located from monitoring maps provided by the City of Boulder Open Space Department. A systematic "hands and knees" search was conducted and individual plants were flagged. A power analysis based on the growth and reproduction of S. diluvialis at Dinosaur National Monument and Brown's Park, Utah (Kraemer and Thiemann, 1987; data provided by Lynn Riedel and Sedonia Sipes, respectively) suggested 30 plants per plot would provide sufficient resolution of differences in means of growth and reproduction among treatments (α=0.05 to detect a 10% difference in means). Three-meter by three-meter plots were established such that each plot contained an average of 30 plants. Individual plants were permanently marked with six-inch plastic tent stakes, each engraved with the plant number and located 10 cm to the magnetic north of the plant. Plot corners were marked with either rebar or plastic PVC pipe. Mapping coordinates were measured from the northwest and southwest corners of each plot. Monitoring plots at Clear Creek, Deer Creek, Prospect Park and the "grazed only" plots at Van Vleet were established in a similar manner in 1993.

To provide information on potential management techniques, four plots of each of six treatments were established within cattle exclosures at the Van Vleet site: "no treatment", control plots with no external treatment; "early clip", simulating winter grazing; "late clip", simulating summer mowing; "clipping twice", which simulates the combination of winter grazing and summer mowing; "early burn"; and "late burn". In addition, two types of plots were set up outside the exclosures: one in an area which

had been and continued to be grazed in the winter and mown in the summer and another which had been grazed only, with no summer mowing. These plots were located from one meter to over 100 meters from the exclosures.

Individual plants were monitored during the growing season on a monthly basis from May to September to characterize the life cycle of S. diluvialis and the impact of the management strategies on that cycle. Measurements were recorded for each individual's growth and reproduction including longest leaf, widest leaf, number of leaves, height of inflorescence, number of flowers, number of fruits, and seed set, as well as recording any damage to individuals from herbivory, grazing, or mowing. Each plant was categorized in one of four phenological stages including absent (no above-ground foliage was observed), remained vegetative (no inflorescence was produced), produced an inflorescence (an inflorescence formed but had not set fruit), and set fruit (an inflorescence formed and fruit set was successful). Inflorescence damage was categorized into one of four types including: no damage (individuals set fruit), mowing, vole herbivory, and other damage (the above-ground parts died-back or were completely absent). Since seed set is dependent on the position of the fruit on the inflorescence spike (Sipes et al., 1993), the second, fifth, and eighth fruits from the bottom of the spike were collected. Seed set was estimated by uniformly spreading the seeds from one fruit into a petri plate lined with 1cm x 1cm squared graph paper, counting viable seeds within 12 randomly chosen squares, and estimating total numbers.

Environmental factors possibly associated with the persistance of S. diluvialis were also monitored including associated vegetation, vegetation cover, light intensity, soil moisture, soil nutrients, as well as other soil

characteristics. Associated vegetation and cover were assessed monthly using stratified random sampling with one random sample per quadrat of the plot (Eberhardt and Thomas, 1991). For each random sample, the percentage of all species and bare cover within a 30 cm by 30 cm square quadrat was estimated and recorded. Photosynthetically active radiation was measured using a quantum meter positioned four inches above ground level at four randomly chosen plants per plot. The organic and nutrient contents of soil were determined from soil cores taken from each plot and analyzed by the Soil Testing Laboratory at Colorado State University and the Long-Term Ecological Research Group at the University of Colorado, Boulder. Six ground water wells were drilled at the Van Vleet site and monitored by Open Space personnel beginning in 1991. Gravimetric soil moisture measurements were obtained from soil cores taken from each plot on a monthly basis during the field season (Jason Jaeger, personal communication). Monthly measurements of hydrology at the scale of individual plants was ascertained using the time domain reflectometry method (TDR; Herkelrath et al., 1991; Roth et al., 1992) and a stratified random sampling design with one random sample per quadrat of each plot (Eberhardt and Thomas, 1991). TDR calibrations based on gravimetric measurements were conducted on soil excavated from the Van Vleet site (Dasberg and Hopmans, 1992; Dirksen and Dasberg, 1993).

Results

Estimated Population Fluctuations

Figure 6 shows an estimate of population fluctuations at Van Vleet based on the number of individuals within each treatment during the three

year study period. To facilitate treatment comparisons, the graph is standardized to begin with 100 individuals for each treatment in 1992 and treatment size calculated for the subsequent years. A maximum change of 25% was found for any given year or treatment. A comparison of estimated population size for the three riparian areas is shown in Figure 7. Although these populations were only monitored from 1993-1994, less than a 25% difference was found between years for each site.

Phenological State

Van Vleet Site

Since orchid plots were established in May, 1992, traditional grazing occurred on all treatment plots and simulated grazing was not carried out. Early burning and late burning occurred in May, 1993, and October, 1992, respectively. A summary of the phenology within each treatment group for the 1992 field season is presented in Figure 8. Phenological state varied by treatment with 38-75% of the individuals remaining vegetative, 26-62% producing an inflorescence (including individuals which later set fruit), and 1-19% setting fruit. The number of orchids producing an inflorescence stalk and setting fruit was statistically different among treatments when analyzed using multivariate analysis of variance (Wilks' Lambda test of significance, $df=6, 22$; $p< 0.05$), however, at the univariate level neither variable differed significantly among treatments ($df=6, 22$ $p>0.05$). Fruit set was lowest in plots in which mowing or simulated mowing occurred. Simulated mowing was not significantly different than traditional mowing at the univariate level ($df=2, 10$; $p>0.05$).

A summary of the phenological state by treatment for the 1993 field season is shown in Figure 9. Phenological state differed statistically among

treatments at the multivariate level (Wilks' Lambda, df=7, 26; $p<0.05$). Less than 2% of the individuals recorded in 1992 were absent in 1993. The traditionally "grazed and mown" and "grazed only" plots contained the largest proportion of individuals producing inflorescences (79% and 77%, respectively) with the "no treatment" control plots containing the smallest number of individuals producing an inflorescence (32%; df=7, 26; $p<0.05$). Despite the high proportion of flowering plants in 1993, particularly in the "grazed and mown" and "grazed only" plots, few individuals were able to set fruit (3% for the "grazed only" plots, 0.7% for the "clipped twice" plots, and 0.5% for the "late burn" plots). No other individuals monitored in the other treatment groups were able to successfully set fruit. Neither absence or fruit set differed significantly among treatments at the univariate level.

Phenological state for the 1994 field season is summarized in Figure 10. Statistical analysis indicates all phenological variables at both the univariate and multivariate levels are significantly different among treatments (df=7, 26; $p<0,05$; Wilks' Lambda, df=7, 26; $p<0.05$). Absence was highest in the "no treatment", "early clip", and "early burn" plots (approximately 19%) and dramatically less in the "grazed and mown", "grazed only", and "clip twice" plots (approximately 5%). Fewer individuals produced an inflorescence within any of the treatment plots (less than 23%) compared to individuals within the traditionally managed plots (50% and 55%). The percentage of individuals producing an inflorescence in the "grazed only" plots in 1994 was not significantly different than the previous year, however, fruit set was significantly greater (16% versus 3%) (df = 7, 26; $p<0.05$). Fruit set significantly different than zero occurred only in the "grazed only" plots with no other plots successfully producing fruit (df = 4, $p<0.05$).

Additional information pertaining to the differences outlined above can be ascertained from graphs and multivariate analysis of monthly phenology. Differences in phenology among treatments were seen as early as June, 1993, and continued through August (Figure 11) (Wilks' Lambda, df = 7, 26; $p<0.05$). By June, the proportion of individuals producing an inflorescence was significantly different between plots (df = 7, 26; $p<0.05$) with the "grazed only" and "grazed and mown" plots producing the highest proportion of inflorescence stalks and the "no treatment" producing the least. This trend continued into July although the "grazed only", "late burn", and "early burn" plots produced the earliest flowers. By August, both "grazed and mown" and "grazed only" plots harbored significantly more inflorescence-producing plants than any of the treatment groups. The late burn plots suffered high orchid mortality from July to August. Although the above-ground parts of most plants had died-back by September, a large proportion of orchids had already produced a leaf rosette for the next season, ranging from 82% in the "grazed only" plots to 32% in the "no treatment" plots.

Monthly phenological state for 1994 is shown in Figure 12. As early as May, a higher proportion of individuals are absent from the "no treatment" and "early clip" plots (Wilks' Lambda, df = 7, 26; $p<0.05$; univariate df = 7, 26; $p<0.05$). Differences in absence continued throughout the field season into August with the "grazed and mown", "grazed only", and "clip twice" plots having the smallest proportion of individuals absent. Similar to the previous year, the "grazed and mown" and the "grazed only" plots produced significantly more inflorescence buds by June, 18% and 5%, respectively (Wilks' Lambda, df = 7, 26; $p<0.05$). This trend continued into July, with these plots having the highest proportion of individuals in bud (55% and

50%) and the earliest flowers. By August, the "grazed only" plots had the highest flowering rate (44%), however, individuals within the "grazed and mown" plots suffered high mortality possibly as a result of mowing in mid-July. By September (data not shown), 45.8% of individuals in the "grazed and mown" plots had produced an overwintering rosette, whereas only 12.3% of individuals within the "late clip" plots had produced rosettes.

In setting up the original 29 demographic plots, two subpopulations of orchids were used: one situated in an area of slightly higher elevation which has been traditionally grazed and mown and the other slightly lower which has been grazed only. Comparison of these plots indicated significant differences in phenology between the two patches in 1992 (Figure 13; Wilks' Lambda, df=1, 22; $p<0.05$). In the "high" patch over 55% of individuals flowered in all treatments. In contrast, in the "low" patch, only 12-50% of the individuals flowered with 88% of the plants remaining vegetative. This general trend continued into 1993 and 1994, however, phenological differences were not significant among treatments (Figure 14 and Figure 15) (Wilks' Lambda, df = 1, 26; $p>0.05$). Therefore, to simplify multivariate statistical analyses, patch location was not used as a factor effecting phenology among treatments for any other portion of this work.

Site Comparison

During the 1993 field season, phenological state differed significantly at the three riparian areas relative to the Van Vleet haymeadow (Figure 16) (Wilks' Lambda, df = 3, 16; $p<0.05$). These differences were detected for all four variables at the univariate level (df = 3, 16; $p<0.05$). Inflorescence production was higher for the traditional treatments at Van Vleet (81% and 77%) than at any of the riparian areas (37-64%). Despite the large proportion

of flowering individuals, the "grazed and mown" plots at Van Vleet produced no fruit set, in contrast to individuals observed at the three riparian sites (4-27%). Fruit set in the Van Vleet "grazed only" plots (3%) was at the low end of the range observed for the nonagricultural riparian populations (4% at Prospect Park), despite the discrepancy in overall inflorescence production. Although the highest fruit set occurred at Clear Creek (27%), several miles downstream, at the Prospect Park site, only 4% of the orchids set fruit.

Phenological state in 1994 did not differ significantly among locations (Figure 17) (Wilks' Lambda, df = 3, 16 $p>0.05$), however at the univariate level fruit set did vary (df = 3, 16; $p<0.05$). At the riparian sites absence ranged from only 5% at Clear Creek to 11% at Prospect Park. The proportion of individuals producing an inflorescence ranged from 70% at Clear Creek to only 33% downstream at Prospect Park. Whereas, inflorescence production for the traditional treatments at Van Vleet ranged from 55% for the "grazed and mown" to 50% for the "grazed only" plots. Fruit set at the riparian areas ranged from 22% at Clear Creek to 9% just downstream at Prospect Park and only 5% at Deer Creek. Fruit set in the traditional treatment plots at Van Vleet ranged from 16% ("grazed only") to 0.3% ("grazed and mown").

Damage

Van Vleet

A summary of inflorescence damage in 1992 among treatments at Van Vleet is shown in Figure 18. Mowing was easily distinguished from vole damage, since mowing produced a straight cut whereas vole herbivory produced a more jagged edge. Differences among treatments occurred at the multivariate level (Wilks' Lambda, df = 6, 22; $p<0.05$) with types of damage

(mowing and vole herbivory) differing at the univariate level (df = 6, 22; $p<0.05$). The total proportion of damaged versus undamaged individuals did not vary among treatments. In the "grazed and mown" plots, 65% of flowering individuals lost their inflorescence due to mowing. Simulated mowing (i.e., late clipping) did not produce significantly different inflorescence damage than traditional mowing (df = 2, 10; $p>0.05$). Perhaps the most surprising observation was the large number of orchids that were damaged by herbivores other than cattle (i.e., 44-73%) in the plots not mown. Field voles were the primary herbivores (Microtus pennsylvanicus and Microtus ochrogaster; Dr. David Armstrong, personal communication). Numerous tunnels or runways constructed by voles were observed and in many cases, if an orchid was on or near one of the runways, the inflorescence was chewed off and left lying a few inches away, uneaten. Other damage ranged from 6% ("late clip") to 27% ("grazed and mown").

Inflorescence damage for 1993 is summarized in Figure 19. Multivariate analysis indicates significant differences across treatments (Wilks' Lambda, df = 7, 26; $p<0.05$) with damage due to mowing and vole herbivory differing among treatments at the univariate level (df = 7, 26; $p<0.05$). No differences were observed in the proportion of damaged versus undamaged inflorescences among treatments. During 1993, inflorescence damage as a result of vole herbivory in plots not mown was higher overall (74-92%) than for 1992 (44-73%) (df = 1, 22; $p<0.05$). In the "grazed and mown" plots a large number of individuals were damaged from mowing (90%). Simulated mowing produced significantly less damage (8-13%) than did traditional agricultural mowing (df = 2, 10; $p<0.05$). Other damage was ranged from 6% in the "early burn" plots to 21% in the "late burn" plots.

During 1994, significant differences among treatments occurred for both the proportion of individuals damaged and the type of damage suffered (Figure 20) (Wilks' Lambda, df = 7, 26; $p<0.05$). The proportion of inflorescence stalks undamaged differed among treatments ranging from 32% for the "grazed only" plots to less than 1% for all other treatments (df = 7, 26; $p<0.05$). Mowing produced substantial damage in the "grazed and mown" plots (76%), whereas simulated mowing produced only 11% to 39% of the damage in the clipped plots. A large proportion of individuals sustained damage due to vole herbivory in plots not damaged by mowing, from 32% in the "late clip" plots to 80% in the "early burn" plots (df = 4, 16; $p<0.05$). Damage due to other reasons ranged from 11% in the "grazed only" plots to 58% in the "late clip" plots.

Monthly herbivory data provided additional insight into the effects of vole herbivory (Figure 21). The differences in vole herbivory among treatments were significantly different throughout the growing season. As early as May, 1993, a high percentage of individuals within the "no treatment" plots were damaged (48%). In contrast, within both "grazed and mown" and "grazed only" plots few individuals were damaged (Wilks' Lambda, df = 7, 26; $p<0.05$). Within one month, vole herbivory sharply increased, although damage to both "grazed and mown" and "grazed only" plots remained low. By August, all plots except the "grazed and mown" plots had significant vole damage (Wilks' Lambda, df = 7, 26; $p<0.05$). However, this lack of damage due to herbivory in the "grazed and mown" plots may not be due to a lack of activity on the part of voles but as a direct result of the mowing which took place in mid-July (prior to the July data collection).

In 1994, vole herbivory again produced significant differences in damage among treatments (Figure 22) (Wilks' Lambda, df = 7, 26; $p<0.05$). As

early as May, from 28% to 41% of all individuals within all treatment plots except the "grazed and mown" and "grazed only" plots were damaged. Vole herbivory continued to increase throughout the summer, producing over 30% of the damage in the "grazed only" plots by August. Insects damaged individuals within all treatment plots, ranging from 5% in the "grazed and mown" plots" to 28% in the "late clip" plot. Insect herbivory, however, affected only a small percentage of an individual's surface area (generally less than 10% approximated from casual observation).

To examine the effect of the exclosure on vole herbivory three additional plots were established at the Van Vleet site in 1994. The extent of vole herbivory outside of the exclosures was independent of distance from the exclosure ($df = 2, 12$; $p>0.05$).

Site Comparison

A site comparison of 1993 inflorescence damage indicated significant differences in the proportion of individuals damaged, vole herbivory, and other damage at both the multivariate and univariate levels (Figure 23) (Wilks' Lambda, $df = 3, 16$; $p<0.05$). Vole herbivory ranged from 90% for the Van Vleet "grazed only" plots to 14%-24% at the nonagricultural riparian sites. The "grazed and mown" plots at Van Vleet contained only minor inflorescence damage resulting from vole herbivory (4%) as a consequence of prior damage due to mowing. The proportion of undamaged individuals ranged from 0-4% at Van Vleet to 9-44% at the nonagricultural riparian sites. Other damage at the riparian areas was relatively high compared to Van Vleet ranging from 42-74%. This damage was due, in part, to stochastic events which occurred at different sites. For example, flash flooding at Deer Creek, high water levels at Prospect Park such that plots were submerged,

and trampling due to off-road vehicles at Clear Creek (Arft, personal observations).

Inflorescence damage during the 1994 field season is summarized in Figure 24. Multivariate analysis indicated differences among locations for the proportion damaged, vole herbivory, and other damage (Wilks' Lambda, df = 3, 16; $p<0.05$). The proportion of individuals undamaged at Van Vleet ranged from less than 1% at the "grazed and mown" plots to 32% for the "grazed only" plots, with the three riparian sites containing 16-31% of individuals undamaged. Vole herbivory at the riparian sites was low (2%-17%) relative to the Van Vleet haymeadow. Very few individuals within the "grazed and mown" plots suffered vole herbivory or other damage (<1%) due to the prior impact of mowing. A large number of individuals at the nonagricultural sites sustained damaged from other causes, from 64% at Clear Creek to 69% at Prospect Park.

Plant Growth

An aggregate measure of area (longest leaf times widest leaf) and the number of basal leaves was used for analysis of plant growth. Multivariate analysis was performed to examine differences in plant growth for each year. A summary of monthly average leaf area and average number of basal leaves for 1992-1994 is presented in Figures 25, 26 and 27, respectively. Average leaf area was statistically different among treatments for July, 1992, whereas basal leaf number was significantly different among treatments for both July and August (Wilks' Lambda, df = 6, 22; $p<0.05$; Figure 25). The average leaf area during 1992 ranged from 12.4 cm^2 (s.e. 0.43) for the "late clip" plots in August to 20.8 cm^2 (s.e. 0.61) for the "grazed and mown" plots in July. The average number of basal leaves during 1992 ranged from 2.5 (s.e. 0.90) for the "grazed

and mown" plots in August to 3.4 (s.e. 0.71) for the "grazed and mown" plots in July. Growth differences continued into 1993 and 1994, where both growth variables were significantly different among treatments throughout the growing season (Wilks' Lambda, df = 7, 26; $p<0.05$). For example, after mowing in July, 1993, leaf area ranged from 9.1 cm^2 (s.e. 0.59) in the "no treatment" control plots to 17.3 cm^2 (s.e. 0.47) in the "grazed only" plots (Figure 26). The average number of basal leaves during 1993 ranged from 1.14 (s.e. 0.091) for the "late burn" plots in August to 4.28 (s.e. 0.101) for the "early burn" plots in June (Figure 26). The difference in leaf areas during the 1994 season ranged from 16.66 cm^2 (s.e. 0.96) for the "grazed only" plots to 6.53 cm^2 (s.e. 0.298) for the "no treatment" control plots. The average number of basal leaves during 1994 ranged from 0.867 (s.e. 0.074) for the "early clip" plots in August to 4.124 (s.e. 0.090) for the "grazed only" plots in June (Figure 27).

Analysis of seed set was limited by low fruit set at the Van Vleet site. Estimated mean viable seed set per fruit of individuals able to set fruit was 6400 (s.e. 360) for second fruit from the bottom of the spike, 6000 (s.e. 330) for the fifth fruit from the bottom of the spike, and 5100 (s.e. 360) for the eighth fruit from the bottom of the spike. Seed set for the second fruit from the bottom of the spike is significantly more than seed set for the fifth and eighth fruits from the bottom of the spike (df = 2, 118; $p< 0.05$). Since only individuals within the "grazed only" plots were able to set significant fruit in 1993 and 1994 differences among treatments were not calculated.

Environmental Variables

Groundwater and soil moisture. Groundwater well data collected by the City of Boulder Open Space Department for 1993 are shown in Figure 28. Wells 2 and 6 are located in areas near known Spiranthes patches. Water levels at both of these wells were quite close to ground level (less than 1 foot) during the growing season.

Gravimetric measurements indicated a high percentage of soil moisture throughout the summer growing season (Figure 29 and 30), however, no significant differences among treatments were observed for either the 1993 or 1994 season (Wilks' Lambda, df = 7, 26; $p>0.05$). The TDR measurements were calibrated twice to gravimetric soil moisture using Van Vleet soil (Dasberg and Hopmans, 1992; Dirksen and Dasberg, 1993). Regression analysis of gravimetric moisture versus TDR moisture measurements produced an $r^2=0.90$. This curve was fitted to a third order polynomial from which the equivalent volumetric soil moisture was calculated (Herkelrath et al., 1991; Roth et al., 1991). Microsite hydrology using TDR indicated no significant differences among treatments in 1993 or 1994 (Figure 31 and 32) (Wilks' Lambda, df = 7, 26; $p>0.05$).

Ground cover. The percentage of bare cover (including litter) for 1993 was significantly different among treatments at the multivariate level with bare cover for June differing at the univariate level (Figure 33) (Wilks' Lambda, df = 7, 26; $p<0.05$). The "early burn" plots produced the highest percent of bare cover, 50% (s.e. 2.24) versus the "grazed and mown" plots which produced the lowest, 33% (s.e. 3.8). Significant differences among treatments were found for 1994 with univariate differences occurring throughout the growing season (Figure 34) (Wilks' Lambda, df = 7, 26; $p<0.05$).

Associated species. Table 13 lists species found within treatment plots during the 1993 and 1994 field seasons. Spiranthes indicator species such as Sisyrinchium montanum, Triglochin maritimum, Lobelia siphilitica, and Verbena hastata were observed within S. diluvialis patches (Coyner, 1990; Jennings, 1990; U.S. Fish and Wildlife Service, 1994). The presence of the exotic, weedy species Cirsium arvensis (Canada Thistle) was not significantly different among treatments at the 95% confidence level ($p>0.05$), however, results were different at the 90% confidence level ($p<0.05$), with more Cirsium arvense in the control "no treatment" plots during the 1994 season (Figure 35). Voucher specimens are deposited at the University of Colorado Herbarium.

Photosynthetically active radiation. Light measurements for August, 1993 are shown in Figure 36. Although average readings per treatment ranged from 650 $mEm^{-2}sec^{-1}$ (s.e. 102) in the "no treatment" plots to over 1400 $mEm^{-2}sec^{-1}$ (s.e. 115) in the "grazed and mown" plots, no significant differences were found for 1993 or 1994 using multivariate and univariate analyses (Wilks' Lambda, df = 7, 26; $p>0.05$).

Soil characteristics. Soil samples were analyzed for pH, conductivity, lime content, percent organic material, ammonium, nitrate, phosphorus, potassium, zinc iron, manganese, copper, and soil texture. Multivariate analysis indicated differences among treatments at Van Vleet for soil pH, iron content, and manganese differing at the univariate level (Figure 37) (Wilks' Lambda, df = 7, 26; $p<0.05$). Analysis of available nitrogen (ammonium and nitrate) indicated differences for ammonium in 1994 (June

and July; Wilks' Lambda, df = 7, 26; $p<0.05$) and nitrate in 1993 at the multivariate level (Wilks' Lambda, df = 7, 26; $p<0.05$; Figure 38) .

A site comparison of soil characteristics is summarized for June, 1994 in Figure 39. A number of variables differed sharply across sites (Wilks' Lambda, df = 7, 26; $p<0.05$). Gravimetric soil moisture was high across all sites ranging from 31.12% (s.e. 2.726) at Prospect Park to 54.55% (s.e. 4.383) for the "grazed only" at Van Vleet. Soil pH ranged from 6.60 (s.e. 0.153) at Prospect Park and Clear Creek to 8.15 (s.e. 0.119) at Deer Creek. Typically, agricultural soil conductivity ranges from 0-2 mmhos/cm (Soil Testing Laboratory, SLC, personal communication). The four study sites ranged from 0.37 (s.e. 0.033) mmhos/cm at Clear Creek to 1.9 (s.e. 0.30) mmhos/cm for the "grazed only" at Van Vleet. Soil organic material at the four sites was high, relative to a typical agricultural reading of 2.5% (SLC), and ranged from 7% (s.e. 2.7) along Clear Creek to 16% (s.e. 4.0) for the "grazed only" plots at Van Vleet. Soil iron concentration at the four sites ranged from 60 ppm (s.e. 23) at Prospect Park to 280 ppm (s.e. 28) for the "grazed and mown" plots at Van Vleet relative to 0-5 ppm for typical agricultural soils (SLC). Soil copper concentrations ranged from 11 ppm (s.e. 3.1) for the "grazed only" plots at Van Vleet to 56 ppm (s.e. 15.2) at Prospect Park relative to typical agricultural soils (0-0.2 ppm; SLC). Soil potassium concentrations for Van Vleet and the three riparian sites ranged from 65 ppm (s.e. 2.6) for the "grazed only" plots at Van Vleet to 160 ppm (s.e. 29) at Prospect Park relative to moderate agricultural levels of 60-120 ppm (SLC). Manganese concentrations of agricultural soils typically range from 0-0.5 ppm (SLC), whereas orchid sites ranged from 8 ppm (s.e. 1.5) for the "grazed and mown" plots at Van Vleet to 26 ppm (s.e. 16.0) at Clear Creek. Soil nitrate levels ranged from 2 ppm (s.e. 1.0) at Clear Creek to 29 ppm (s.e. 2.9) for the "grazed only" plots at Van Vleet.

Typically for agricultural soils, moderate nitrate levels range from 19-36 ppm (SLC). All sites were relatively low in soil phosphorus concentration ranging from 1.0 ppm (s.e. 0.46) at Clear Creek to 3.4 ppm (s.e. 0.63) at Prospect Park (moderate agricultural soil phosphorus levels = 4-7 ppm, SLC). Finally, soil zinc concentrations ranged from 3 ppm (0.51) at Deer Creek to 270 ppm (s.e. 112) at Prospect Park well above the 0-0.2 ppm range (SLC) for moderate zinc levels in agricultural soils. Within the Van Vleet haymeadow, soil conductivity, percent organic material, and soil iron and nitrate concentrations ranged widely between the "grazed and mown" and "grazed only" plots. Soil characteristics also varied dramatically from the Clear Creek site to the downstream Prospect Park site, particularly for soil pH, and soil concentrations of potassium, phosphorus, and zinc.

Correlation of plant growth and reproduction and environmental variables

Throughout the three year study, correlations occurred between plant growth and reproduction and environmental characteristics. Because of the large number of variables measured and the resulting high probability of Type II error, only correlation coefficients greater than 0.50 or $p<0.01$ were considered significant (Michael Grant, personal communication). Table 14 summarizes the correlations and the orientation of the association (positive or negative) for environmental and plant variables at the Van Vleet site. At the microsite level, soil moisture was positively associated with plant growth and reproduction (fruit set), however, a negative association occurred with gravimetric soil moisture and plant growth. Bare cover and light readings were negatively associated with plant growth and reproduction but positively associated with vole herbivory and mowing. Soil pH and lime content were negatively associated with plant growth and reproduction (i.e.

more basic soils produce less growth and reproduction). The essential nutrients, phosphorous, nitrate, and iron were positively associated with plant growth and reproduction, however, ammonium had a negative correlation.

Discriminant Analysis

Direct discriminate function analysis was performed using environmental variables, as well as plant phenology, as predictors of membership in treatment groups at Van Vleet and at different sites. Predictor variables used included phenology, absence, proportion remaining vegetative, inflorescence production, fruit set, soil moisture, percent bare cover, light, and soil characteristics. Groups were either the treatments at Van Vleet ("grazed and mown", "grazed only", "no treatment", "early clip", "late clip", "clip twice", "early burn", and "late burn") or the sites ("grazed and mown" and "grazed only" at Van Vleet, Deer Creek, Clear Creek, and Prospect Park). For the 44 plots at Van Vleet and the three riparian habitats, evaluation of assumptions of linearity, normality, multicollinearity or singularity, and homogeneity of variance-covariance matrices revealed no threat to multivariate analysis.

Table 15 summarizes the results of discriminant function analyses of phenological and environmental variables. Two discriminant functions or canonical axes were defined. At the Van Vleet site, environmental variables (93%) were better predictors for a posteriori classification than phenology (62%). Similarly at the riparian sites the environmental variables (100%) were much better predictors than phenology (55%).

At the Van Vleet site, phenology, absence, and the proportion of individuals remaining vegetative were the best predictors in separating the

traditional treatments (G and GM) from all other treatments along the first canonical axis (Table 15 and Figure 40). The traditional treatments had fewer individuals remaining vegetative (mean=0.364 and 0.421) and less absence (mean=0.063 and 0.065) than the other treatment groups (vegetative mean range=0.761-0.950 and absence mean range=0.050-0.210). The second canonical axis separated the two traditional treatments from each other with general phenology, proportion vegetative, and absence making the largest contributions. The remaining plots overlapped across both the first and second axes. The first and second canonical axes (discriminant functions) had a combined $X^2=88.2$, $p<0.05$. After removal of the first canonical axis, there was still a strong association between groups and predictors with $X^2=26.3$, $p<0.05$. The chi-squared value is a statistical measure of the reliability of the association between groups and predictors. The two discriminant axes accounted for 80.6% and 14.9% of the between-group variability, respectively.

The environmental variables separated the traditional treatments from each other as well as all other treatments along the first two canonical axes (Figure 41). Percent bare cover (July, 1994), soil pH (June, 1994), and soil conductivity (June 1994) were the best predictors distinguishing between treatments along the first canonical axis, whereas soil conductivity (June, 1994), and percent bare cover (June, 1993 and August, 1994) separated treatments along the second canonical axis. Percent bare cover for July, 1994, ranged from 26% (s.e. 1.8) for the "grazed and mown" plots to 41% (s.e. 4.1) for the "late clip" plots. Soil pH ranged from 7.0 (s.e. 0.17) for the "grazed and mown" plots to 8.3 (s.e. 0.07) for the "late burn" plots. Soil conductivity ranged from 0.84 mmhos/cm (s.e. 0.129) for the "grazed and mown" plots to 1.9 mmhos/cm (s.e. 0.30) for the "grazed only" plots. Percent bare cover in

June, 1993 ranged from 33% (s.e. 3.8) for the "grazed and mown" plots to 50.0% (s.e. 2.24) for the "early burn". Percent bare cover for August, 1994, ranged from 26% (s.e. 1.8) for the "grazed and mown " plots to 41% (s.e. 4.1) for the "late clip" plots. The first and second canonical axes had a combined X^2=147.8, $p<0.05$. After removal of the first axis the association between groups and predictors was still significant with X^2=48.4, $p<0.05$. The two canonical axes accounted for 97.5% and 1.6% of the between-group variability, respectively.

At the riparian sites, phenology produced the correct a posteriori classification only as well as random chance along the first two canonical axes due to the wide variability within sites (Figure 42). The group centroids separated across the first and second discriminant functions based on fruit set and inflorescence production. Fruit set was highest at Clear Creek (mean=0.225) and lowest for the "grazed and mown" plots at Van Vleet (mean=0.003). Similarly, inflorescence production is highest at Clear Creek (mean=0.705) and lowest at Prospect Park (mean=0.329). The first and second canonical axes had a combined X^2=19.9, $p<0.05$. After removal of the first axis the association between groups and predictors was not significant with X^2=7.0, $p>0.05$. The two canonical axes accounted for 69.3% and 30.7% of the between-group variability, respectively.

In contrast to phenology, the environmental predictors clearly separated sites (Figure 43). Soil pH, soil iron concentration, and soil ammonium concentration were the best predictors of site membership along the first canonical axis, whereas soil nitrate, soil conductivity, and soil copper concentrations were the best predictors along the second canonical axis. Soil pH ranged from a low at Prospect Park (mean=6.6, s.e. 0.153) to a high at Deer Creek (mean=8.15, s.e. 0.119). Soil iron concentrations ranged from a low at

Prospect Park (mean=60 ppm, s.e. 23) to a high for the "grazed and mown" plots at Van Vleet (mean=280, s.e. 28). Soil ammonium concentrations ranged from a low at Clear Creek (mean=0.23, s.e. 0.049) to a high at Deer Creek (mean 2.3, s.e. 0.32). Soil nitrate ranged from a low at Clear Creek (mean=2.0, s.e. 1.00) to a high for the "grazed only" plots at Van Vleet (mean=29, s.e. 2.8). Soil conductivity ranged from a low at Clear Creek (mean=0.37, s.e. 0.33) to a high for the "grazed only" plots at Van Vleet (mean=1.9, s.e. 0.30). Soil copper concentrations ranged from a low for the "grazed and mown" plots at Van Vleet (mean=10.8, s.e. 0.97) to a high at Prospect Park (mean=56, s.e. 15.2). The first and second canonical axes had a combined X^2=133.2, $p<0.05$. After removal of the first axis the association between groups and predictors was still significant with X^2=74.9, $p<0.05$. The two canonical axes accounted for 83.7% and 14.5% of the between-group variability, respectively.

Discussion

Population size at Van Vleet and the three riparian areas does not fluctuate on an annual basis as dramatically as suggested by previous monitoring counts. A maximum 25% difference was found for any treatment or site in any given year, relative to the 800% difference based on the previous monitoring counts. Thus, when both flowering and vegetative individuals are included populations are relatively stable.

Several other factors may have impacted the previous monitoring counts with respect to timing. For example, timing of surveys may be critical since vole herbivory can significantly reduce observable flowering stalks. In addition, mowing severed a large number of flowering stalks within "grazed

and mown" plots in all three years. Damaged plants such as these were not included in the official monitoring counts which were consistently conducted the first week of August each year. Annual monitoring counts, such as this, are not sufficient to track the health of a population. Variation in the proportion of flowering individuals, year-to-year environmental variation, herbivory, and management techniques impact the "population size" throughout the growing season. Monthly data collection within established plots provides a more complete profile of the population and factors affecting its persistance.

Monthly data collection from 1992-1994 indicates orchid growth usually begins with the production of an overwintering rosette during the previous late summer or fall. Growth primarily occurs during the growing season from May to August of the year following rosette formation. Inflorescence buds are produced as early as June, producing flowers by mid-July to mid-August. The proportion of individuals producing an inflorescence varied widely among treatments and sites. Fruits matured and dehisced from mid-August into September with both timing and success varying among locations. Similar to previous work (Sipes et al., 1993), the large number of apparently viable seed produced in each fruit suggests fruit set is not pollinator-limited. Although previous reports indicate individuals may remain vegetative or flower depending on environmental conditions (Wells, 1981), at the Van Vleet site only two individuals were absent in 1993 that returned in 1994.

Higher inflorescence production at Van Vleet within the "high" patch relative to the "low" patch occurred only in 1992. This difference may have been due to microsite differences such as hydrologic characteristics or past management practice. The "high" patch has been mown for the past 50-75

years, but the "low" site has not been mown due to topography and limitations of the mowing equipment. The lack of a significant difference between the patches in 1993 and 1994 suggests the differences seen in 1992 may have been due to traditional agricultural practices.

Both multivariate and univariate analyses of variance indicates differences among treatments and sites for both phenology and inflorescence damage. Traditional management strategies at Van Vleet produce the largest proportion of individuals producing an inflorescence. However, fruit set at Van Vleet was relatively low when compared to the three riparian populations. Significant fruit set occurred only for the "grazed only" plots at Van Vleet, whereas relatively higher fruit set occurred at all three riparian habitats.

Differences among riparian sites appear to result from variation in individual site conditions. Although Prospect Park is just downstream from Clear Creek, fewer individuals set fruit. This may be indicative of the occurrence of natural stream and population dynamics where local colonizations and extinctions take place. At any given time, some patches of orchids are doing very well, whereas others may be doing poorly as a result of changes in hydrology. These types of differences were also observed within populations. For example, at Prospect Park one plot is located on the stream bank approximately six feet above the water level. These individuals are not doing well overall and may have become established during previous hydrologic conditions. In addition, the low fruit set at Prospect Park relative to the upstream Clear Creek population may result from differences in local habitat on a larger scale. The Clear Creek plots occur in a much more open area, whereas the plots at Prospect Park occur in a more shaded understory.

Insight into what is limiting fruit set is gained from examining inflorescence damage. At Van Vleet within the mown or clipped plots, a large number of individuals are damaged due to mowing. Because inflorescence development begins in mid-June and mowing takes place in early to mid-July, depending on weather and the growth of grasses, significant inflorescence damage occurred each year during the study period. Mowing in mid-July, as occurred in 1993 and 1994, damaged a larger proportion of flower stalks relative to 1992 when mowing occurred in early-July. Inflorescence damage could be avoided with earlier mowing, before significant inflorescence development. Although mowing damages inflorescence development, it also reduces vegetation cover and may help control the vole population.

Perhaps the most surprising result is the amount of damage due to vole herbivory. Significant damage at Van Vleet occurred in all plots not mown. Vole herbivory contributed to inflorescence damage in all three study years at the Van Vleet site, however, this type of damage increased significantly during 1993. The increase may have been due, in part, to enclosing the orchids in an effort to exclude cattle. By early summer, the cattle had eliminated substantial vegetative cover throughout the field, except within the orchid exclosure; the field voles may have relocated to the more vegetated exclosures, resulting in significant inflorescence damage. However, by the end of the growing season no difference in vole herbivory was observed for the "grazed and mown" and "grazed only" plots at various distances from the exclosures. Natural fluctuations in vole population size are suggested by the annual changes in vole herbivory during the three year period at Van Vleet. Numerous studies have supported annual fluctuations, multi-annual cycles, and combinations of fluctuations and cycles in Microtus

populations (Tamarin and references therein, 1985). Potentially both intrinsic and extrinsic factors are responsible for the observed population patterns including food source, predation, spacing-behavior, phenotypic-behavior, and genotypic-behavior (Tamarin, 1985)

A site comparison indicates vole herbivory is not the critical factor limiting reproductive success in nonagricultural riparian habitats. Although a large proportion of orchids at the riparian sites suffered no inflorescence damage in 1993, many individuals suffered damage due to other causes. Other damage at the riparian areas was much greater as a result of stochastic events (e.g. high water, trampling, flash flooding). In addition to local extinctions and recolonizations along riparian corridors, the stochastic events also support a watershed approach to managing riparian populations. Thus, if one orchid patch is destroyed, other seed sources are available for recolonization.

Management should be based on the different factors impacting different populations. Traditional management practices at Van Vleet may provide the best conditions for the persistance of S. diluvialis if mowing and grazing are timed with respect to inflorescence development. Grazing and mowing reduce competing vegetation as evidenced by the higher growth rate calculated for S. diluvialis in the more open plots ("grazed and mown") and the higher proportion of absent individuals in the "no treatment" plots. Plots within traditionally "grazed and mown" areas produced the highest proportion of flowering plants, however, fruit set was significantly less. Perhaps as a result of their reduced growth, plants in denser plots, such as "no treatment" plots, produced fewer inflorescence stalks. Mowing before significant development of the inflorescence is critical for successful fruit set.

On a large scale, the population may benefit from mowing since it reduces vegetative cover and, as a result, may contribute to a reduced vole population. Vole herbivory may be the most critical threat to the long-term survival and reproductive success of S. diluvialis at the Van Vleet Ranch. Therefore, any management treatment which helps to control the vole population would be potentially beneficial to the orchid. For example, grazing, mowing, and burning significantly reduce vegetative cover available to the rodents and, thereby, may limit vole population growth. However, these treatments impact the orchids in other ways that may not be beneficial. Although mowing reduces vegetative cover for rodents, it also damages a large number of flowering stalks. Thus, timing (i.e., early mowing) or a change in mowing height is critical for successful fruit set. In addition, timing is critical for grazing, in that cattle should be removed before significant vegetative growth of the orchid and the formation of an inflorescence.

Failure to produce fruit was due to external environmental factors rather than intrinsic biological phenomena based on a comparison among sites. Fruit set within a single individual was high (over 50% of flowers per inflorescence), however, some areas may suffer from pollinator limitation (Sipes, personal communication). Bumble bees, the orchid's primary pollinator, may be affected by reducing the vole population, since some bumble bees use rodent burrows for nesting (Sipes et al, 1993).

At the riparian populations, a watershed approach should be taken to reduce the effect of stochastic events and to take natural colonizations and extinctions into account. This approach has been increasingly recognized in the recovery of rare taxa. The Bureau of Land Management and the U.S. Forest Service have both undertaken riparian area and watershed

management strategies (U.S. Fish and Wildlife, 1994). In addition, the Forest Ecosystem Management Assessment Team, an interagency group composed of representatives from the Forest Service, Environmental Protection Agency, and Fish and Wildlife Service, is responsible for using an ecosystem approach to forest management (U.S. Fish and Wildlife Service, 1994). An ecosystem approach has also been used for the conservation of individual species such as the Bull Trout (Salvelinus confluentus) and for S. diluvialis (U.S. Fish and Wildlife Service, 1994).

Environmental measurements support microsite soil moisture as one factor effecting plant growth and reproduction. However, gravimetric measurements suggest oversaturating the soil may inhibit phenological development of the orchids. The negative association of plant growth and reproduction with light and bare cover is counter-intuitive since orchids occur in more open, sunny areas. However, this correlation may be a result of the impact of vole herbivory since voles construct surface pathways through the vegetation which creates bare soil. The tendency for populations not to occur in more alkaline conditions has been reported previously (Jennings, 1990). This work supports the inhibition of orchid growth and reproduction as soils become more alkaline. The correlation of plant growth and reproduction to essential nutrients (phosphorus, nitrate, and iron) is not surprising. The strong association with soil iron concentrations may be tied to the orchid not doing well in more basic soils, because iron forms insoluble oxides at neutral or alkaline pH (Brady, 1990; Hopkins, 1995). The negative correlation of plant growth and reproduction with ammonium concentration is surprising. The microbial conversion of ammonium to nitrate which usually occurs in soils may be inhibited under

anaerobic conditions such as occur at Van Vleet. The resulting buildup of ammonium may be detrimental to the orchids (Brady, 1990; Hopkins, 1995).

A site comparison of environmental variables provides insight into the range of soil characteristics of Spiranthes habitats. Based on the four sites surveyed, S. diluvialis requires high soil moisture (31-55%), moderate soil pH (6.6-8.1), and tolerates a range of soil conditions, organic material, and soil nutrients. If conditions at Van Vleet reflect the traditional agricultural practices, these strategies have potentially depressed copper and manganese concentrations, while elevating organic material and nitrogen content. The Prospect Park site shows much higher concentrations of copper, potassium, nitrate, phosphorous, and zinc than the Clear Creek site which is located several miles upstream, potentially as a result of environmental pollution from nearby Golden, Colorado. This study suggests populations of S. diluvialis persist under a variety of environmental conditions. Discriminant analysis indicates that the structure of the environmental variables differs to a much greater extent across treatments and sites than phenology.

In conclusion, populations do not fluctuate as dramatically as previously indicated. The life history of S. diluvialis includes a large number of individuals producing an inflorescence but fruit set may be limited. Limitation of fruit set at Van Vleet is due to mowing and vole herbivory and at the three riparian habitats stochastic events and natural population colonizations and extinctions. Management should be based on the different factors impacting the different populations. At Van Vleet, traditional grazing and modified mowing should be continued to maintain orchid habitat. At the riparian sites a watershed approach should be continued to reduce the effect of stochastic events and to take natural

colonizations and extinctions into account. The general results of this work can be applied to other rare plants in terms of management strategies. However, it is important to design management strategies based on the specific demography of not only individual rare species, but also for different populations within a species.

Chapter 5: Matrix Population Modeling

Introduction

Matrix algebra is a powerful and elegant technique for understanding population dynamics and structure (Geramita and Taylor, 1990). Numerous studies have employed transition matrix analysis and modeling to examine a suite of ecological questions (Bierzychudek, 1982; Lefkovitch, 1965; Silva et al., 1991; Silvertown et al., 1993) and, more recently, to provide insight into the management of rare species (Menges, 1990; Schemske et al. 1994; Waite and Hutchings, 1991). An understanding of the life history stages critical for persistence is essential to effectively manage populations and to control the risk of extinction (Menges, 1992; Shaffer, 1981). Demographic studies provide data on life history stages and form the basis for estimating the current and future probabilities of extinction for plant populations (Menges, 1992; Shaffer, 1990).

Population dynamics and fate may be predicted through the use of matrix-projection models. Changes in a population with a given age or stage structure may be predicted through time, using the observed proportion of individuals transferred among age or stage classes across years. In predicting a population's demography, stage or size is often superior to age (Caswell, 1989; Gross, 1981; Werner and Caswell, 1977), because they reflect the important stages of a plant's life cycle (Lefkovitch, 1965; Menges, 1992). Several important variables can be calculated from a transition matrix including the equilibrium finite growth rate (lambda), equilibrium age structure, and the contribution of each element of the projection matrix to the intrinsic growth rate (elasticity) (Menges, 1992).

Deterministic population models assume constant demographic parameters through time, whereas stochastic models include temporal variation. Stochastic models provide more realistic projections of populations, particularly with rare plant populations for which stochastic processes may precipitate extinction (Caswell, 1989; Menges, 1990). Schaffer (1981) outlined four stochastic factors that may affect extinction probabilities. First, environmental stochasticity involves random variation in a population's environment such as the physical environment, herbivory, and competitive interactions. Second, natural catastrophes include random or periodic events such as floods, fires, and drought. Third, demographic stochasticity refers to chance events in survival and reproduction, not related to the environment. These problems often occur with very small populations. Finally, genetic stochasticity pertains to genetic consequences of chance events, such as founder effects or inbreeding.

In plants, no comprehensive study of the effects of stochastic factors on minimum viable population size and extinction probabilities has been completed. Menges (1991) outlined a number of plant traits relating to stochasticity, however, which are pertinent to the present study including genetic structure, gene flow, disturbance ecology, microsite specialization, spatial aggregation and metapopulations, cryptic life history stages, breeding system, and general life history. Environmental stochasticity and natural catastrophes may be the primary forces shaping the fate of plant populations, whereas genetic stochasticity is more important in small populations (Menges, 1992). Sufficient population size to buffer populations from environmental stochasticity will be large enough to protect the genetic integrity of plant

populations as well (Menges, 1992). Similarly, populations large enough to overcome problems due to environmental and genetic stochasticity will be large enough to overcome those due to demographic stochasticity.

The nature of conservation biology often results in the implementation of management strategies without the benefit of basic research on the rare species (Waite and Hutchings, 1991). One strategy for developing more objectively-based management regimes is through the analysis of population dynamics and structure under particular management techniques (Waite and Hutchings, 1991).

The goal of this project was to characterize population demographic structure and to use population modeling based on demographic parameters and their variation over a three year period to compare different management strategies and populations of S. diluvialis. The following specific questions were addressed:

1. What is the effect of management strategies on the intrinsic growth rate, stable stage distribution, mortality, reproductive value, and elasticities at the Van Vleet haymeadow population of S. diluvialis?
2. How does the intrinsic growth rate, stable stage distribution, mortality, reproductive value, and elasticities for the Van Vleet haymeadow population compare to riparian populations?
3. Do the demographic parameters vary with time at the Van Vleet haymeadow population?

Species description

Spiranthes diluvialis is a terrestrial orchid species known from scattered populations in Colorado, Utah, and Wyoming. This rare orchid inhabits moist floodplains near streams at elevations between 1300-2100 meters, stands 12-45 cm tall, and blooms from July through September (Jennings, 1990). These orchids are perennial, producing tuberous roots during the growing season as storage organs for the following summer (Wells, 1981). Small, inconspicuous leaf rosettes persist throughout the winter months (personal observation). Many congeneric species of Spiranthes initially grow underground saprophytically for many years before leaves surface above ground. Other species within the genus rarely flower in consecutive years or under unfavorable conditions and may survive due to specific symbiotic relationships with mycorrhizal fungi (Wells, 1981). Bees are the primary pollinator for Spiranthes, with Bombus being the most common genus (van der Pijl and Dodson, 1966; Dressler, 1981; Sipes et al., 1993). Recent pollination studies indicate S. diluvialis follows this trend with Bombus being the primary pollinator (Coyner, personal communication). Although plants often grow in clumps, there is no indication that vegetative reproduction occurs. Spiranthes diluvialis gained federally threatened status under the U.S. Endangered Species Act on January 17, 1992 (Federal Registry, 1992) due to habitat destruction, large fluctuations in monitored population size, the unknown impacts of grazing, and potential exotic species invasion. At the time, little was known of the genetic, ecological, and demographic processes affecting the life history and long-term survival of this threatened orchid.

Materials and Methods

Detailed information on the survival, growth, and fecundity of S. diluvialis was collected in a manner similar to studies on other species of Spiranthes (Wells, 1981; McClaren and Sundt, 1992; see below). These data were collected primarily on the City of Boulder Open Space population (Van Vleet site), where 29 experimental plots were established and monitored during the 1992 field season, five additional plots were added during 1993, and three more plots were added in 1994.

In addition to the Van Vleet haymeadow, demographic data were also collected at three other nonagricultural riparian sites: at the mouth of Clear Creek in Jefferson County, Colorado (Clear Creek site); along Clear Creek at Prospect Park in Jefferson County, Colorado (Prospect Park site); and along Deer Creek in Garfield County in southern Utah (Deer Creek site) (Figure 44)

Orchid patches at Van Vleet were initially located from monitoring maps provided by the City of Boulder Open Space Department. A systematic "hands and knees" search was conducted and individual plants were flagged. A power analysis based on the growth and reproduction of S. diluvialis at Dinosaur National Monument and Brown's Park, Utah (Kraemer and Thiemann, 1987; data provided by Lynn Riedel and Sedonia Sipes, respectively) suggested 30 plants per plot would provide resolution of differences in means of growth and reproduction among treatments (α=0.05 to detect a 10% difference in means). Three meter by three meter plots were established such that each plot contained an average of 30 plants. Individual plants were permanently marked with six inch plastic tent stakes, each engraved with the plant number and located 10 cm to the magnetic north of the plant. Plot corners were marked with either rebar

or plastic PVC pipe. Mapping coordinates were measured from the northwest and southwest corners of each plot. Monitoring plots at Clear Creek, Deer Creek, Prospect Park and the "grazed only" plots at Van Vleet were established in a similar manner in 1993.

To provide information on potential management techniques, four plots of each of six treatments were established within cattle exclosures at the Van Vleet site: "no treatment", control plots with no external treatment; "early clip", simulating winter grazing; "late clip", simulating summer mowing; "clipping twice", which simulates the combination of winter grazing and summer mowing; "early burn"; and "late burn". In addition, two types of plots were set up outside the exclosures: one in an area which has been and continues to be grazed in the winter and mown in the summer and another which has been grazed only, with no summer mowing. These plots are located from one meter to over 100 meters from the exclosures.

Individual plants were monitored during the growing season on a monthly basis from May to September to characterize the life cycle of S. diluvialis and the impact of the management strategies on that cycle. Each plant within a plot was mapped and the status of each was recorded for each year from 1992-1994 at Van Vleet and during 1993-1994 for the three riparian areas. The plants were classified into one of four categories: absent (no above-ground foliage was observed), vegetative (no inflorescence was produced), produced an inflorescence (an inflorescence formed but had not set fruit), and in fruit (an inflorescence formed and fruit set was successful).

The Model

The Leslie matrix is a special age-classified population projection matrix which can be developed using survival and fertility data. The number of individuals (n) surviving from one year (t) to the next (t+1) can be estimated from the equation:

$$n_i (t + 1) = P_{i-1} n_{i-1}(t) \qquad \text{for } i = 2, 3, \ldots$$

where P_{i-1} = survival probability of members of age class (i-1). New recruits can be calculated from the fertility coefficients, F_i (the number of age class 1 individuals at time t+1 per individuals in age class i at time t), and the number of individuals in each stage class the previous year.

$$n_1 (t+1) = F_1 n_1 (t) + F_2 n_2 (t) + \ldots$$

These two equations can be combined to find the population projection matrix at time t+1:

$$\begin{bmatrix} n_1 \\ n_2 \\ . \\ . \\ . \\ n_s \end{bmatrix} (t+1) = \begin{bmatrix} F_1 & F_2 & . & . & . & F_S \\ P_1 & 0 & & & & 0 \\ 0 & P_2 & . & . & . & 0 \\ . & . & . & . & . & 0 \\ . & . & . & . & . & 0 \\ . & . & . & . & P_{S-1} & 0 \end{bmatrix} \begin{bmatrix} n_1 \\ n_2 \\ . \\ . \\ . \\ P_S \end{bmatrix} (t)$$

or, $$n (t+1) = \mathbf{A}\, n(t)$$

where **A** is the population projection matrix and n(t) is the age distribution vector at time t. In this model, individuals moving to the next age class are represented by the survival probabilities arranged diagonally in the matrix (Caswell, 1989). Beginning with an initial distribution, n(0), the subsequent states of the population are projected by repeated matrix multiplication (each column vector resulting from matrix multiplication is multiplied by the projection matrix). This model assumes present conditions are maintained indefinitely.

Leslie matrices are often limited in that an individual's age may not accurately describe its demographic properties (Caswell, 1989). For example, age may be inadequate when individuals exhibit plastic growth, multiple modes of reproduction, or environmental heterogeneity. A Lefkovitch matrix model uses a stage-classified matrix model and has been used in a number of studies where age was found to be inappropriate (Lefkovitch, 1965). In a Lefkovitch model, an individual may theoretically move from any stage to any other stage. The model then becomes:

$$\begin{bmatrix} n_1 \\ n_2 \\ . \\ . \\ . \\ n_s \end{bmatrix}_{(t+1)} = \begin{bmatrix} P_{1,1} & P_{1,2} & . & . & P_{1,s} \\ P_{2,1} & P_{2,2} & . & . & P_{2,s} \\ . & . & . & . & . \\ . & . & . & . & . \\ . & . & . & . & . \\ P_{s,1} & P_{s,2} & . & . & P_{s,s} \end{bmatrix} \begin{bmatrix} n_1 \\ n_2 \\ . \\ . \\ . \\ n_s \end{bmatrix}_{(t)}$$

where p_{ij} is the proportion of individuals moving to stage i from stage j.

The transition model for S. diluvialis was based on a Lefkovitch model of four stages: vegetative (V), producing an inflorescence but no fruit (I), producing fruit (F), and absence of above ground leaves (A) (Figure 45). The proportion of individuals surviving and moving from a given state to any other state (or remaining in the same state) was calculated for each site and for each treatment at the Van Vleet site. At the Van Vleet site two transitions could be calculated, from 1992-1993 and from 1993-1994. At the riparian sites, however, the plots were not established until 1993, thus only a 1993-1994 transition could be calculated.

Mortality rates were averaged from all years for each stage from the number of individuals absent in a given year, taking subterranean plants into account since orchids may remain below ground in any given year and produce above-ground foliage during subsequent years. Taking into account both the observed transition probabilities and the mortality rates produced the transition matrices of each population and management regime based on life stages. The main diagonal elements of the matrix are the probabilities that individuals within a given state survive and return the following year in the same stage class. The remaining elements are the probabilities that individuals will survive and return in a different stage class the following year. The column vector is the number of individuals in each stage class at any time, t. Multiplication of the transition matrix by the stage distribution vector at time t yields a new vector with the number of individuals in each stage class at time t+1. The stage distribution of a population in any future time period (e.g., 100 years from time t) can be predicted by repeated

multiplication of the transition matrix by the appropriate stage vector (Caswell, 1989; Waite and Hutchings, 1991).

A transition matrix must also account for annual recruitment of new individuals. Recruitment may be calculated as a function of the number of plants flowering in some previous year. A reproductive value, R, was estimated from the number of recruits in year t divided by the number of flowering plants in some previous year t-n. In orchids, the situation is complicated because seeds may germinate and remain subterranean for years before above-ground leaves emerge. For example, in Spiranthes spiralis plants may remain underground on average from 8-11 years before appearing above ground (Wells, 1981). Because this study was only conducted for three years, the number of flowering plants in some previous year, t-n, was calculated as the average number of flowering plants per year during the three year study period. In a similar study on the spider orchid Ophrys sphegodes, mean values of R calculated from data using from one- to eight-year time delays between fruit set and above ground emergence resulted in little variation among R values (Waite and Hutchings, 1991). New recruits were incorporated into the model using data collected on the number of new recruits and fruiting individuals. The mean number of recruits entering each stage class was calculated for the three-year monitoring period. The number of new individuals entering each stage in any year was calculated by multiplying the mean proportion of recruits entering each stage class, the R value, and the number of fruits the previous year.

A given matrix has characteristic variables known as eigenvalues and eigenvectors which form the basis of demographic analysis (Caswell,

1989). By definition, a vector **x** is an eigenvector if matrix multiplication is equivalent to scalar multiplication, or mathematically:

$$\mathbf{A}x = \lambda x$$

where **A** is a given matrix, and lambda (λ) is the eigenvalue. The intrinsic rate of population growth is equal to the largest eigenvalue of the transition matrix. If the eigenvalue is less than 1.0 the population is decreasing in size; if the eigenvalue is greater than 1.0 the population is increasing in size; whereas an eigenvalue of 1.0 indicates a stable population. The corresponding eigenvector (the right eigenvector) represents the stage distribution of the population at equilibrium. The left eigenvector (x where $x\mathbf{A}=\lambda x$, or the transpose of the right eigenvector) represents the contribution of the different stages to the growth of the population (Caswell, 1989). Eigenvalues and eigenvectors were estimated using MATLAB (The Mathworks, 1992), a software package for numeric and matrix analysis.

Bootstrap confidence intervals for eigenvalues were calculated using MATLAB (The Mathworks, 1992). Bootstrapping randomly resamples the original demographic data with replacement (Caswell, 1989; Efron and Tibshirani, 1986). The bootstapped sample was equal in size to the original sample for each treatment, site, and transition year. A new transition matrix was constructed from the bootstrapped sample and the characteristic eigenvalue determined. Bootstrapping was repeated 1000 times and the 95% confidence interval calculated. Differences between treatments, sites, and years were considered significant if the 95% confidence intervals did not overlap.

The contribution of each element of the projection matrix to the intrinsic growth rate can be assessed from two parameters, the sensitivity and the elasticity for each element in the transition matrix where the proportional sensitivities of the matrix elements are the elasticities (de Kroon et al., 1986). The sensitivity of a matrix element is calculated from the equation:

$$s_{ij} = \frac{v_i w_j}{\langle \mathbf{w}, \mathbf{v} \rangle}$$

where v_i is the ith element of the left eigenvector, w_j is the jth element of the right eigenvector, and $\langle \mathbf{w}, \mathbf{v} \rangle$ is the scalar product of the right and left eigenvectors. These values can be used to calculate the elasticities using the equation:

$$e_{ij} = (s_{ij}) \frac{a_{ij}}{\lambda}$$

where e_{ij}, s_{ij}, and a_{ij} are the ith row and jth column of the elasticity, sensitivity and projection matrices, respectively.

Future extinction probabilities and minimum viable population sizes were estimated using POPPROJ, a computer simulated, stochastic, nonequilibrium model developed by Menges (1987, 1990). Populations and treatments were projected deterministically (using only one transition matrix) or stochastically (randomly alternating transition matrices or including environmental stochasticity which is simulated by including variance in matrix elements).

Results

Transition matrices are summarized in Table 16 for all treatments, sites, and transition years. Transition matrices are the proportion of individuals moving from one stage to another stage the following year and do not include either mortality or reproductive values. At the Van Vleet site, few if any individuals were able to set fruit in any plot except the "grazed only" plot for both transition years. In contrast, individuals at all three of the riparian sites were able to move from vegetative, producing an inflorescence, setting fruit, and absent to setting fruit the following year. For both transition years and all treatments, except the "grazed and mown" and "late burn" plots during the 1992-1993 transitions, the majority of vegetative plants returned as vegetative plants the following year. During the 1992-1993 transition, most of the plants producing an inflorescence without fruit set returned to the same stage the following year except for the "no treatment" plots which returned as vegetative plants. By contrast, for the 1993-1994 transition, the majority of inflorescence-producing plants returned as vegetative plants except for individuals within the "grazed and mown" and "grazed only" plots which returned to the same stage. Individuals that set fruit in 1992 were most likely to produce an inflorescence but not set fruit the following year, except for the "early clip" plots which returned to vegetative plants. For the 1993-1994 transition, the "grazed only" plants most individuals returned from setting fruit to producing an inflorescence without fruit set and the "late burn" plants returned to vegetative plants. Successful fruit set did not occur in the remaining

plots in 1993. Absent plants were most likely to return as vegetative plants in all treatment regimes.

At Deer Creek and Prospect Park, vegetative individuals were most likely to return as vegetative plants, whereas at Clear Creek they usually returned to produce an inflorescence. Individuals producing an inflorescence one year were most likely to return to the same stage class the following year at all three riparian sites. Individuals setting fruit one year were most likely to return to produce an inflorescence the following year at Deer Creek and Clear Creek, however, return to any stage was equally likely at Prospect Park. Deer Creek produced the lowest proportion of plants returning to set fruit.

Mortality rates averaged over the three year study period were calculated and are summarized in Table 17. Nonzero mortality rates ranged from 1.00% for individuals producing an inflorescence in the Van Vleet "grazed and mown" plots to 14.2% for vegetative individuals in the "early burn" plots. In the three riparian habitats, mortality ranged from 3.7% for vegetative individuals at Clear Creek to 17.7% for individuals producing an inflorescence at Deer Creek.

Reproductive values were estimated and are summarized in Table 18. With the exception of the 1992-1993 "grazed and mown" plots, all management regimes at the Van Vleet site produced the highest reproductive values in the vegetative stage. Deer Creek and Clear Creek produced the highest values in the inflorescence stage and all stages had equal reproductive values at Prospect Park.

Population projection matrices followed similar trends as those found in the transition matrices but with the addition of new recruits from the fruiting stage and mortality in all stages (Table 19). The

projection matrices are similar to the transition matrices but include higher probabilities of fruiting individuals producing vegetative, inflorescent, and fruiting individuals due to the addition of new recruits.

The intrinsic rates of growth (λ) and their 95% confidence intervals are summarized in Table 20. For all treatment regimes at the Van Vleet site, except the "grazed only" plots (1.006), the characteristic eigenvalue was significantly less than one. In contrast, all of the riparian plots had an intrinsic growth rate greater than one (range 1.142 to 1.657) except the Deer Creek population which was not significantly different than 1.0. For the 1992-1993 Van Vleet transition, the intrinsic growth rates for the "grazed and mown" and "clip twice" plots were significantly greater than for any other treatment. The growth rates for the "no treatment", "early clip", and "early burn" plots were not significantly different from each other but were significantly lower than any other treatment. The growth rates for the "late clip" and "late burn" plots differed from each other and were intermediate in value between the "grazed and mown" and "clip twice" plots and all other plots. For the 1993-1994 transition at the Van Vleet site, the "grazed only" and "clip twice" plots had the significantly highest growth rates; followed by the "grazed and mown", "late clip", and the "late burn" plots; the "no treatment" plots; and the significantly lowest growth rates were estimated for the "early clip" and "early burn" plots.

Right eigenvectors are presented in Table 21. The equilibrium population structure is given by the right eigenvector. The largest proportion of individuals occurred in the vegetative stage at equilibrium for the "grazed only", "no treatment", and "early clip" plots for all years. In the "grazed and mown" plots at equilibrium, most individuals

produced an inflorescence but did not set fruit for both transition years. For the "late clip", "clip twice", "early burn", and "late burn" plots, the largest proportion of individuals changed from producing an inflorescence in the 1992-1993 transition to remaining vegetative in the 1993-1994 transition. At Deer Creek and Clear Creek the largest proportion of individuals at equilibrium occurred in the inflorescence stage, whereas at Prospect Park most individuals remained vegetative.

The equilibrium stage structure was compared to the observed stage structure (Table 22). The observed stage structure in the "grazed and mown", "no treatment", and "early clip" plots followed the same trends as those predicted at equilibrium. The structure observed in the "late clip, " clip twice" and "early burn" plots differed in the higher proportion of individuals remaining vegetative than was predicted by the 1992-1993 equilibrium model. The "late burn" plots produced the highest proportion of individuals producing an inflorescence in 1993, as predicted by the 1992-1993 transition, but a higher proportion of vegetative individuals in 1992 and 1994, as predicted by the 1993-1994 transition. The population structure observed in 1994 for the "grazed only" plots followed the trends predicted by the 1993-1994 model, however in 1993 a higher proportion of individuals produced an inflorescence.

The contribution of each stage to growth in the population is given by the left eigenvector Table 21. The fruiting stage contributed most to the growth of the population for all treatment regimes for the 1992-1993 transition and for the "grazed only" and "late burn" plots in 1993-1994. The vegetative stage contributed most to the growth of the population in the 1993-1994 transitions for the "clip twice" plots. The

inflorescence stage contributed primarily in the "grazed and mown", "no treatment", "early clip", "late clip", and "early burn" plots. For all three riparian habitats, fruiting individuals contributed most to the growth of the population.

The affect of particular elements in the matrices on population growth were estimated from elasticities. Elasticity matrices are presented in Table 23. The largest elements of an elasticity matrix are those upon which population growth is most dependent. For the riparian populations at Clear Creek and Prospect Park the largest elasticities were for fruiting individuals surviving in the next year as fruiting individuals. At the Deer Creek population individuals producing an inflorescence and returning to set fruit the following year had the largest elasticity. At Van Vleet for the 1992-1993 transition, the highest elasticities were estimated for vegetative stages surviving and returning to the vegetative stage ("no treatment" and "early clip") or inflorescence stage individuals returning to the same stage (" grazed and mown", "late clip", "clip twice", "early burn", and "late burn"). For the 1993-1994 transition at Van Vleet, the highest elasticities were observed for vegetative individuals returning to the same stage, except in the "grazed and mown" plots where the highest elasticity occurred for individuals producing an inflorescence and returning in the same stage the following year.

Results of deterministic and stochastic modeling are summarized in Table 24 for all treatment regimes, sites, and years. Both deterministic and stochastic models began with an initial population size of 500 and were projected for 100 years. For deterministic modeling, only populations represented by the 1993-1994 "grazed only" and "clip twice"

plots at Van Vleet did not reach extinction. However, only the "grazed only" plots produced a larger population than the initial population size after 100 years. All three riparian site populations did not go extinct and produced a larger population size than the initial population. Similarly stochastic modeling by random alternation of transition matrices resulted in extinction for the "grazed and mown", "no treatment", "early clip", "late clip", "clip twice", "early burn", and "late burn" plots. The "grazed only" plots and riparian sites could not be projected using alternating matrices since only one transition was recorded. For treatments and sites which did not become extinct under the deterministic model, environmental stochasticity was introduced into the projections. Environmental stochasticity was simulated by increasing the variance for all elements in the matrix until the probability of extinction was greater than 5%. The required variance ranged from a weak level (0.0005 and 0.0024) for the "clip twice" and "grazed only" plots at Van Vleet to moderate levels (0.0799) for the Clear Creek population to strong levels (0.1997 and 0.4999) at the Deer Creek and Prospect Park populations.

Discussion

Matrix models based on eight different management regimes at Van Vleet and three separate riparian areas have been constructed and analyzed. Although only three years of demographic data were collected at the Van Vleet site and two at the riparian habitats, some general trends emerge from data analysis.

The much higher intrinsic growth rates found at all three riparian sites possibly reflects the unnatural Spiranthes habitat at Van Vleet. However, results clearly indicate that any change in management regime from the traditional agricultural techniques to any of the other regimes tested would be detrimental to the population and increase the probability of extinction. Intrinsic growth rates greater than 1.0 based on the 95% confidence intervals, were found only in the "grazed only" plots at Van Vleet. Although all other treatments produced growth rates of less than 1.0, both actual and simulated grazing and mowing produced plots with growth rates significantly higher than any of the other treatment regimes. The significantly higher lambdas for the simulated mowing ("late clip") plots relative to the simulated grazing ("early clip") plots suggests that mowing may have a more beneficial effect on orchid growth and reproduction. The lack of fruiting individuals in the "grazed and mown" plots is a direct result of the loss of inflorescence buds during mowing (see Chapter 2). Thus, timing of mowing (i.e., earlier mowing) such that inflorescence buds are not damaged could substantially increase successful fruit set and the intrinsic growth rate of the population.

These results are repeated in the deterministic model projecting population growth which predicts extinction for all but the "clip twice" and "grazed only" plots. However, only the "grazed only" plots resulted in an increase in population size. Similarly, when the two matrices for each treatment are randomly alternated, extinction occurs under each of the management regimes projected (the "grazed only" and riparian plots could not be projected since only one transition was recorded). Relative

to the three riparian areas the Van Vleet "grazed only" plots are over 30-fold more susceptible to extinction due to environmental stochasticity.

Critical states and fluxes can be identified from the demographic data which are important in the conservation management of S. diluvialis. At the Van Vleet site, an increasing proportion of individuals occur in the vegetative state for each equilibrium population in 1993-1994 relative to 1992-1993. In the "no treatment", "early clip", "early burn", and "late burn" plots at Van Vleet, the proportion of absent individuals increased as well for the 1993-1994 transition. This change is most likely due to the implementation of the new management regimes at Van Vleet, since a higher proportion of vegetative individuals was not observed at any of the three riparian areas for the 1993-1994 transition.

In comparison to the riparian habitats, all of the management regimes at Van Vleet, including the "grazed only" plots, are deficient in the proportion of individuals able to set fruit. The introduction of new management regimes at Van Vleet resulted in a progressively lower proportion of individuals producing an inflorescence and setting fruit at equilibrium. Similarly, the left eigenvectors at the riparian sites indicate flowering individuals contribute the most to population growth. At Van Vleet, all treatments started out the study with flowering individuals contributing the most, however, by 1994 flowering individuals contributed the most only for the "grazed only" plots.

Elasticities indicate that population growth rate is most dependent on vegetative individuals at Van Vleet with the exception of the "grazed and mown" plots for which values are greatest for the individuals producing an inflorescence. In contrast, at the three riparian areas elasticity is highest for fruiting individuals remaining in the same stage

class or individuals producing an inflorescence staying in the same stage or producing fruit the following year. These results again suggest a lack of individuals producing an inflorescence and setting fruit at the Van Vleet site.

Comparison of the 1992-1993 and 1993-1994 transition matrices at Van Vleet is insufficient to determine the range of temporal variation in demographic parameters. The intrinsic growth rates calculated for the two transition matrices were not significantly different for the "no treatment", "early clip", "late clip" "early burn", "early burn", and "late burn" plots. Significant differences were observed for the "grazed and mown" and "clip twice" plots, however, the implementation of mowing and simulated mowing may have affected individuals differently at Van Vleet during each of the three study years.

Several previous studies using a matrix modeling approach indicated that the survival of some species may depend on human intervention. For example, Silva et al. (1991) found that Andropogon semiberbis populations were dependent on fire frequency resulting from human occupation of neotropical savannas. For the rare orchid Ophrys sphegodes, the introduction of sheep grazing reversed the decline in population size (Waite and Hutchings, 1991). In the case of S. diluvialis, the population at Van Vleet may no longer be viable without human intervention. Results indicate that traditional grazing and mowing, if modified to permit earlier mowing, may provide the best conditions favoring survival of this rare orchid.

In terms of conservation biology, the results suggest S. diluvialis is not rare due to intrinsic biological phenomena. Mean average values of growth parameters indicate natural riparian populations are healthy and

growing. However, these calculations may conceal the variation found within a population. Menges (1990) found that natural disturbance and recolonization processes were essential to the survival of Pedicularis furbishiae. Similar processes may occur naturally in populations of S. diluvialis. For example, at Prospect Park calculation of the intrinsic growth rate for each plot ranged from 0.980 to 2.025. These growth rates may reflect Spiranthes habitats in various stages of succession. Thus, similar to the Furbish's lousewort, management strategies should take the entire watershed into consideration when implementing recovery actions for S. diluvialis.

Chapter 6: Conclusions

Many of the causes and consequences of rarity debated since the nineteenth century bear on the threatened status of S. diluvialis. In recent years, the focus of conservation biology has narrowed to the importance of genetic versus demographic factors. This work attempted to characterize both factors by tracking the evolutionary history, population genetics, and demography of S. diluvialis in the context of its conservation and recovery. The following is a summation of the conclusions drawn from each of these areas of research.

Evolutionary history may impact the relative abundance of species (Fiedler, 1992). Isozyme data support S. magnicamporum and S. romanzoffiana as the diploid progenitors of S. diluvialis because the alleles found in S. diluvialis are a combination of those found in the two diploid species. Although the geographic ranges of the parental taxa do not currently overlap, disjunct populations suggest a once larger distribution, possibly during the Pleistocene pluvial period when conditions were cooler and moister (Sheviak, 1984). Thus, the current limited geographic range of S. diluvialis may have resulted from the historically-overlapping ranges of the parental taxa and more recent destruction of habitat.

The genetics of S. diluvialis may also impact its relative abundance. The allopolyploid origin of S. diluvialis is reflected in the high levels of genetic diversity found within populations. Similar to most polyploids, S. diluvialis shows high levels of fixed, or nearly fixed, heterozygosity at several loci, and a high percentage of polymorphic loci. Furthermore, isozyme data indicate that multiple hybridization events

have introduced genetic variation into S. diluvialis from different parental populations. This type of genetic diversity may be important in reducing the deleterious effects of inbreeding and stochastic genetic processes (such as genetic drift), and in allowing for continued evolutionary adaptation and change. In several recent studies, it was found that allopolyploids have been more successful than their diploid progenitors in the colonization of new habitat, probably as a result of relatively higher levels of genetic diversity (Soltis and Soltis, 1991).

In contrast to the high levels of genetic variation observed within species, a high degree of similarity among populations of S. diluvialis was observed with each population harboring most of the genetic variability found within the species. These estimates, however, do not take the presence of population-unique alleles into account. In addition, all twelve populations of S. diluvialis contain unique combinations of alleles, indicating differentiation among populations, possibly as a result of multiple hybridization events occurring between different pairs of parental diploids and/or the incorporation of new mutations into the populations since hybridization. Whether these unique combinations of alleles resulted from different hybridization events or local adaptation, they may represent the beginning of unique evolutionary trajectories. The different multilocus genotypes observed between populations add weight to the distinctness of populations and argue for the preservation of each population as a unique genetic entity. Population-genetic analyses of rare orchids, such as S. diluvialis, are particularly important in light of conserving alleles or combinations of alleles unique to these small populations.

Isozyme data also shed light on the biogeographic history of S. diluvialis suggesting a minimum of two hybridization events and subsequent long distance dispersal. Multiple hybridization events produce higher levels of genetic variation within the polyploid populations since more of the genetic variation within the diploid parental taxa may be represented. The high levels of genetic divergence observed among populations of S. diluvialis may have provided populations with sufficient variability to withstand potential negative effects of significant environmental stochasticity. Because of their limited size and distribution, populations of rare species are often considered more susceptible to environmental stochasticity. The high levels of genetic diversity observed in populations of S. diluvialis may be critical in providing a buffer against the genetic problems associated with small isolated populations and allow for its continued evolutionary change and adaptation.

Although knowledge of the population genetics of a species is important, particularly if gene banking or restoration projects are initiated, in the case of S. diluvialis, demographic data provide more vital information with respect to its immediate management and recovery. The ecology, stochasticity, life history strategy, population dynamics, and reproductive biology of rare species are integral factors contributing to their relative abundance as well as their short-term persistence (Feidler, 1992; Holsinger and Gottlieb, 1991; Lande, 1988). Analyses of demographic dynamics and environmental requirements help to elucidate both intrinsic and extrinsic factors constraining the continued persistence of rare species. The impact of specific ecological factors varied widely among populations of S. diluvialis, depending on

the specific population and the year. For example, although vole herbivory played an important role in preventing successful fruit set within the Van Vleet population, particularly in 1993, voles exhibited significantly less impact on the riparian populations throughout the three-year study period. Similarly, stochastic events ranged from trampling by a four-wheel drive vehicle at Clear Creek to flooding of some orchids at Prospect Park. Thus, management strategies must be based on the specific ecology and stochastic events within each population.

The life history of Spiranthes is unusual in that, after seed germination, an individual may remain subterranean for several years nourished by associated mycorrhizal fungi (Wells, 1981). Demographic data indicate a small proportion of orchids may remain subterranean for at least one year, returning the following year to produce above ground foliage. This mechanism potentially allows individuals and populations to survive periods of environmental stress. In terms of the recovery of S. diluvialis, individuals within populations which appear to be declining or extinct may simply be subterranean and will recover given the appropriate management strategy.

Population size at the Van Vleet haymeadow does fluctuate on an annual basis, although not as dramatically as suggested by previous monitoring counts of only flowering individuals. The much higher intrinsic growth rates found at all three riparian sites reflect the unnatural Spiranthes habitat at Van Vleet. However, results clearly indicate that any change in management regime from the traditional agricultural techniques to any of the other management regimes tested would be detrimental to the population and increase the probability of

extinction. Deterministic and stochastic population projection modeling indicates a decrease in population size and subsequent extinction for all treatment regimes at Van Vleet except for the "grazed only" plots and an increase in population size at the three riparian sites. Relative to the three riparian areas the Van Vleet "grazed only" plots are over 30-fold more susceptible to extinction due to environmental stochasticity. Thus, the population at Van Vleet may no longer be viable without human intervention.

The high intrinsic growth rates estimated at each riparian area obscure the variation found within orchid patches along the riparian corridor. Natural disturbance and recolonization processes may be essential to survival of S. diluvialis. The variability observed among patches along a particular riparian corridor suggests dynamic processes of colonization and extinction, therefore, management strategies should take entire watersheds into consideration when implementing recovery actions for S. diluvialis.

Sexual reproduction is critical to the intrinsic growth rate of populations and essential to the survival of S. diluvialis. At the Van Vleet site, the transition population modeling indicated the introduction of new management regimes resulted in a progressively lower proportion of individuals producing an inflorescence and setting fruit for a population at equilibrium. In comparison to the riparian habitats, all of the management regimes at Van Vleet, including the "grazed only" plots, resulted in lower proportions of individuals able to set fruit. The lack of fruiting individuals in the traditionally "grazed and mown" plots is a direct result of the loss of inflorescence buds during mowing. In the past, timing of mowing varied from year to year based on climatic

conditions, thus, in some years mowing must have occurred early enough to permit sufficient seed set for the continued persistence of S. diluvialis. Mowing earlier in the year, so that inflorescence buds are not damaged, could substantially increase successful fruit set and the intrinsic growth rate of the population.

The biogeographic distribution of Spiranthes diluvialis results from its evolutionary history with a minimum of two allopolyploid hybridization events taking place and subsequent long distance dispersal. Since riparian populations appear to be healthy and growing, extant populations of S. diluvialis do not appear to be the result of relictual populations moving towards extinction.

The disappearance of historic populations appears to correlate with significant urban development and alterations in the natural dynamics of stream systems along both the Colorado Front Range and the Wasatch Front. With the exception of the Colorado Van Vleet population, S. diluvialis occupies a unique and localized habitat along riparian corridors in Colorado, Nevada, Utah, and Wyoming. Successful sexual reproduction is critical to the growth of populations and may be the single-most important biological factor constraining the persistence of S. diluvialis. The unique combination of all these factors has led to the current status of S. diluvialis as a threatened species, a status that is not necessarily a precursor to extinction.

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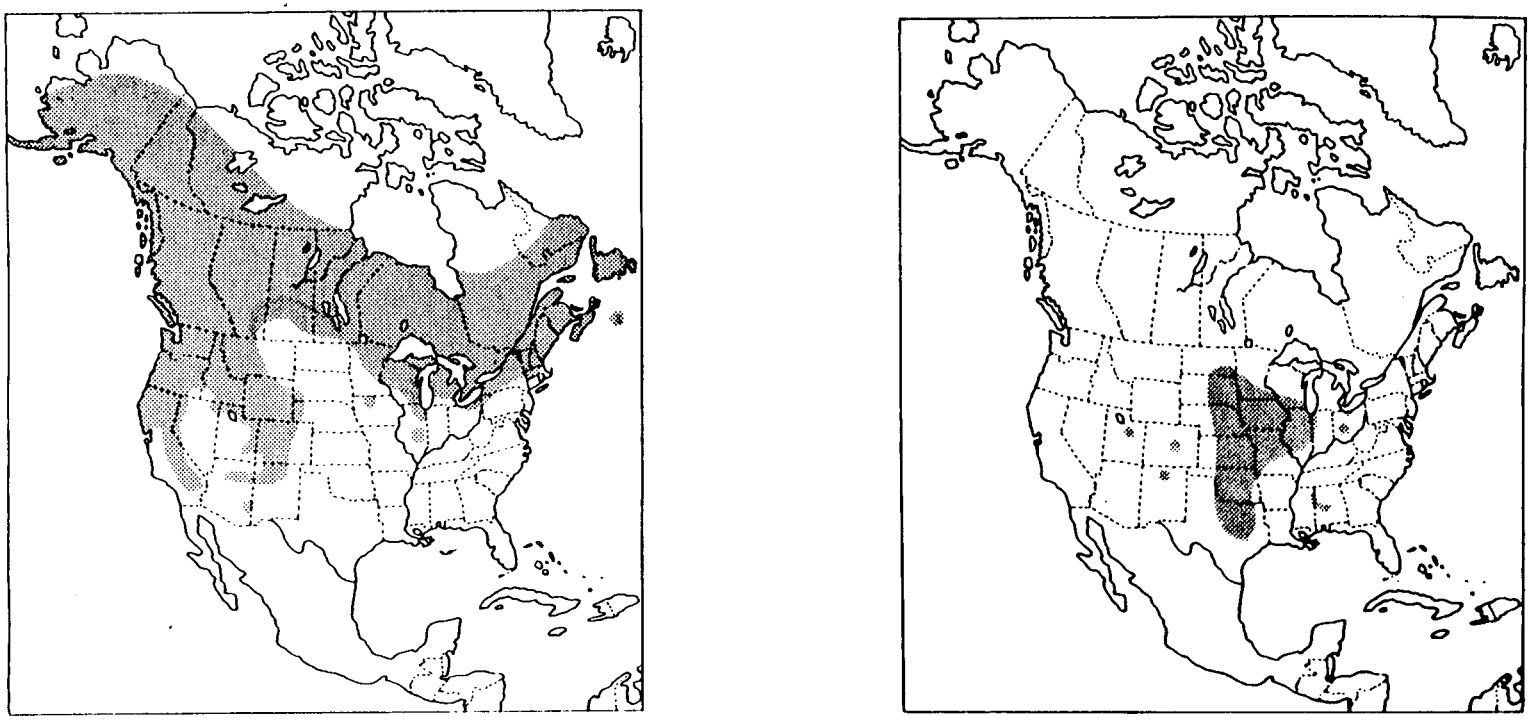


FIGURE 1
Distributions of S. romanzoffiana (on left) and S. magnicamporum (on right).
These maps are taken from Luer (1975).

Figure 2: Distribution of *S. diluvialis* in Colorado, Utah and Wyoming.

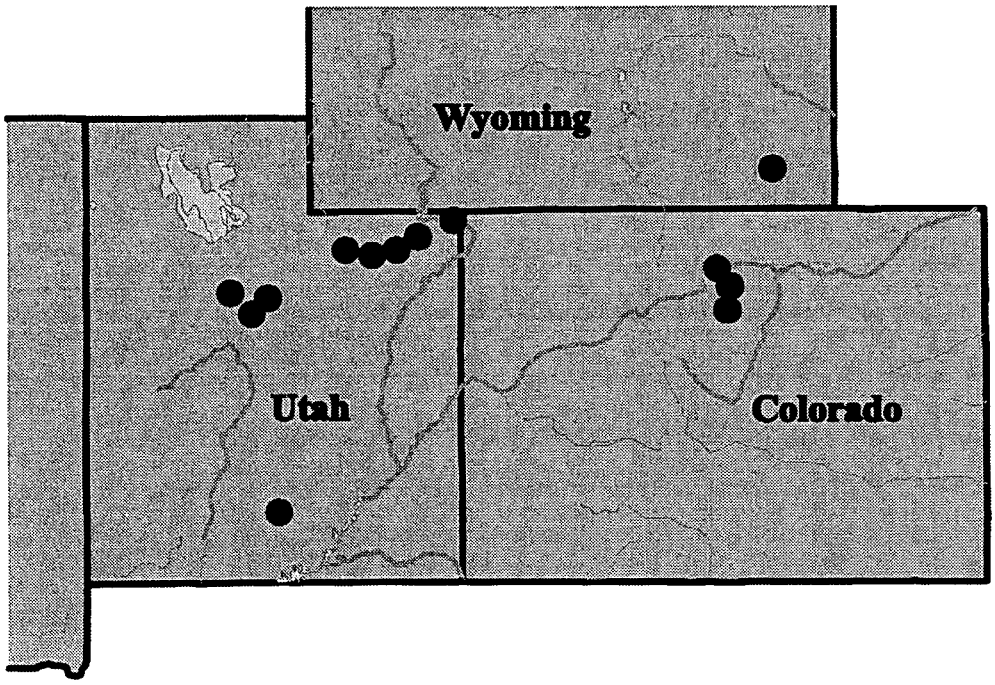


Figure 3: Schematic representation of the banding pattern present for MDH in Spiranthes diluvialis. Four loci (Mdh-1 through Mdh-4) and two interlocus heterodimers (IH) were observed.

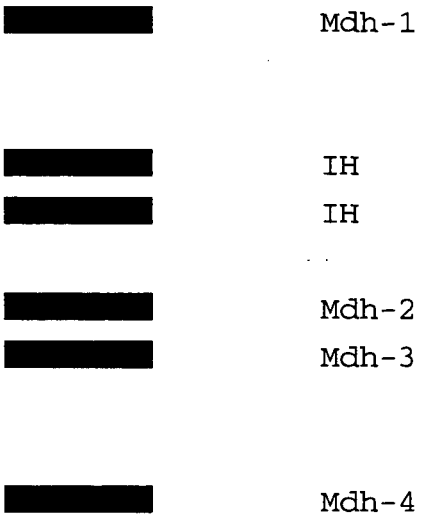


FIGURE 4: Diagramatic representation of the geographic distribution of alleles within twelve populations of Spiranthes diluvialis across ten polymorphic loci. The top left square indicates the relative location of each population. Alleles not found in the parental populations sampled are followed by an asterisk (*).

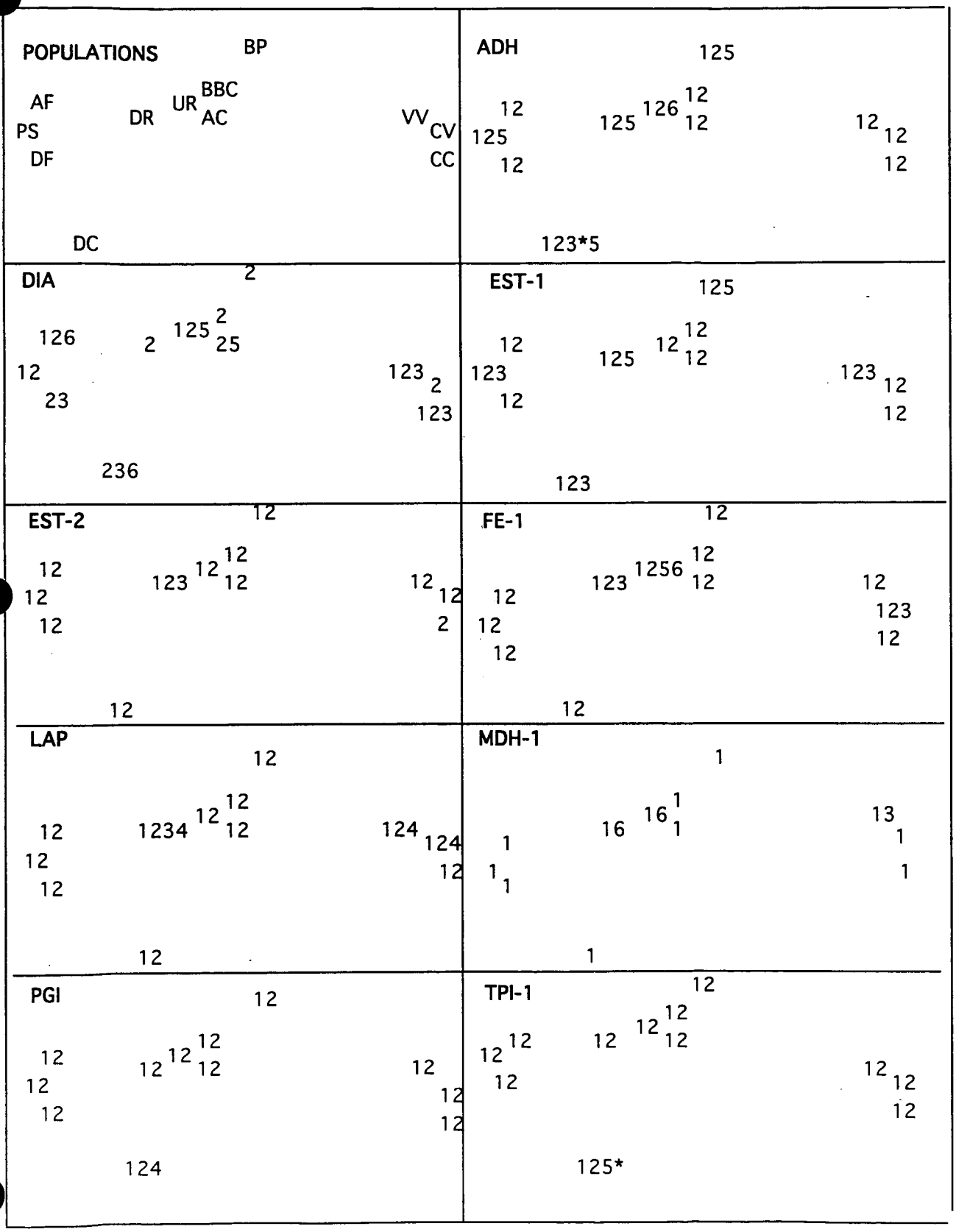


Figure 5: Monitoring counts of flowering individuals at the Van Vleet site from 1986-1992, excluding 1991.

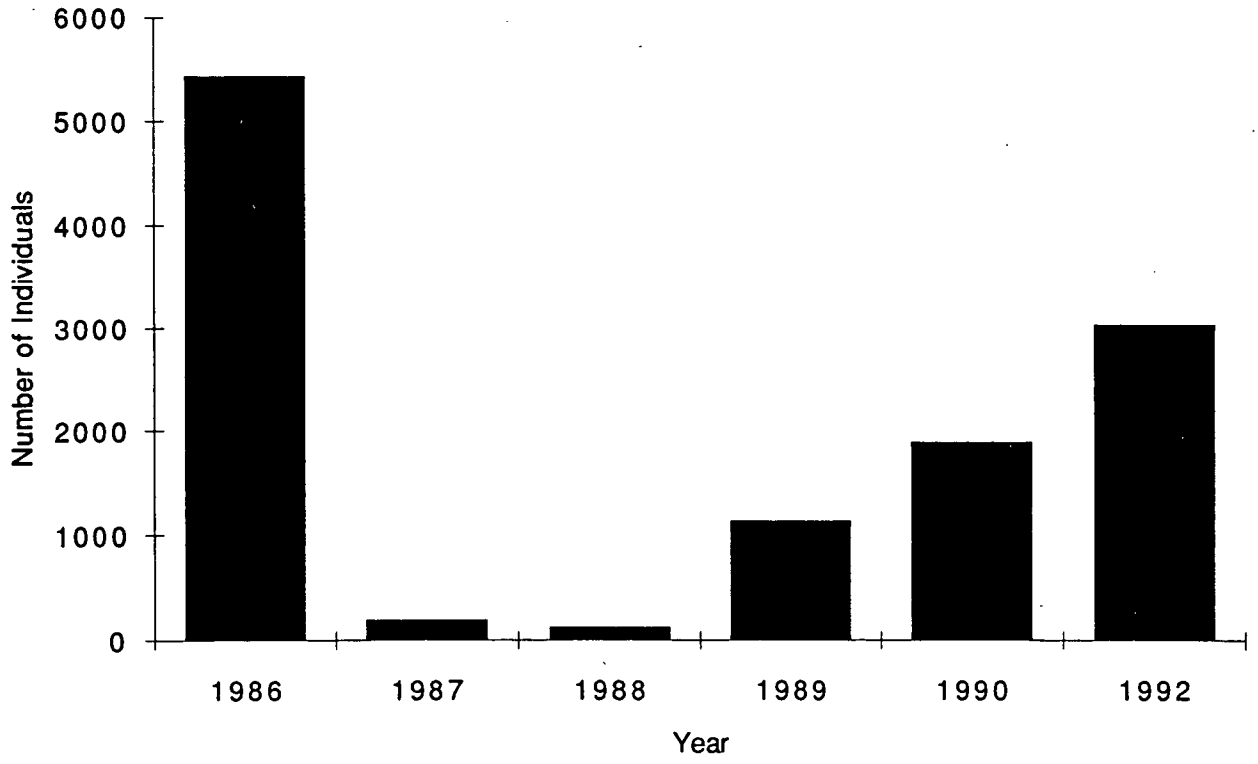


Figure 6: Estimated population fluctuations at Van Vleet standardized to 100 individuals per treatment in 1992. Treatments are grazed, G; grazed and mown, GM; none, NT; early clip, EC; late clip, LC; clip twice, CT; early burn, EB; and late burn, LB.

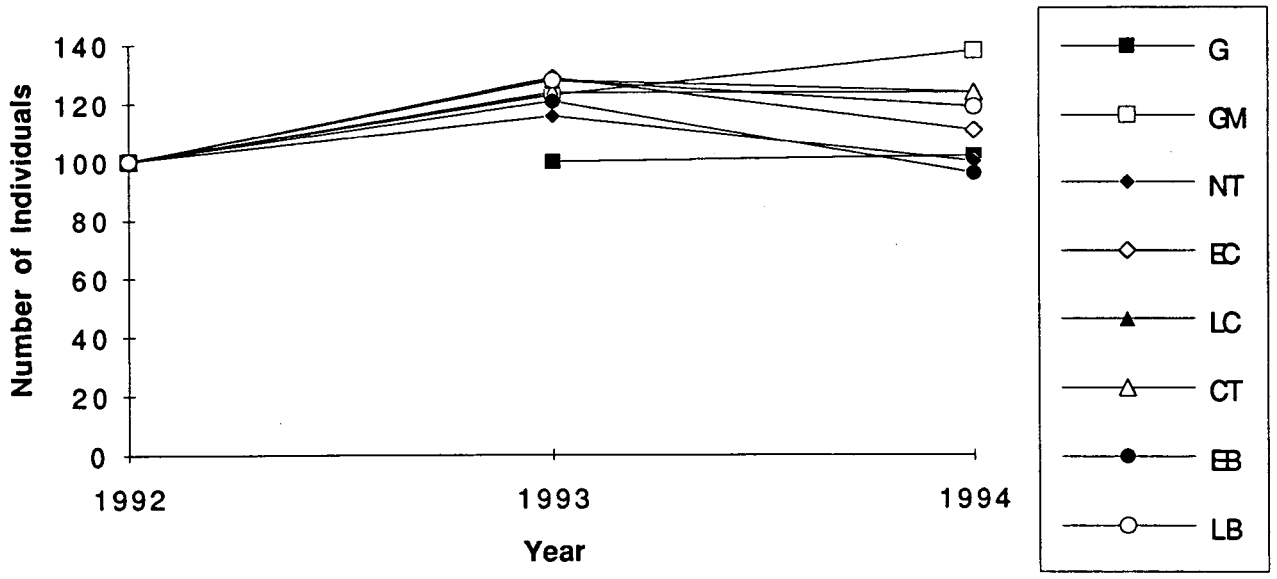


Figure 7: Estimated population fluctuations at Clear Creek (CC), Prospect Park (PP), and Deer Creek (DC) based on the number of individuals within each treatment. Numbers are standardized to 100 individuals in 1993.

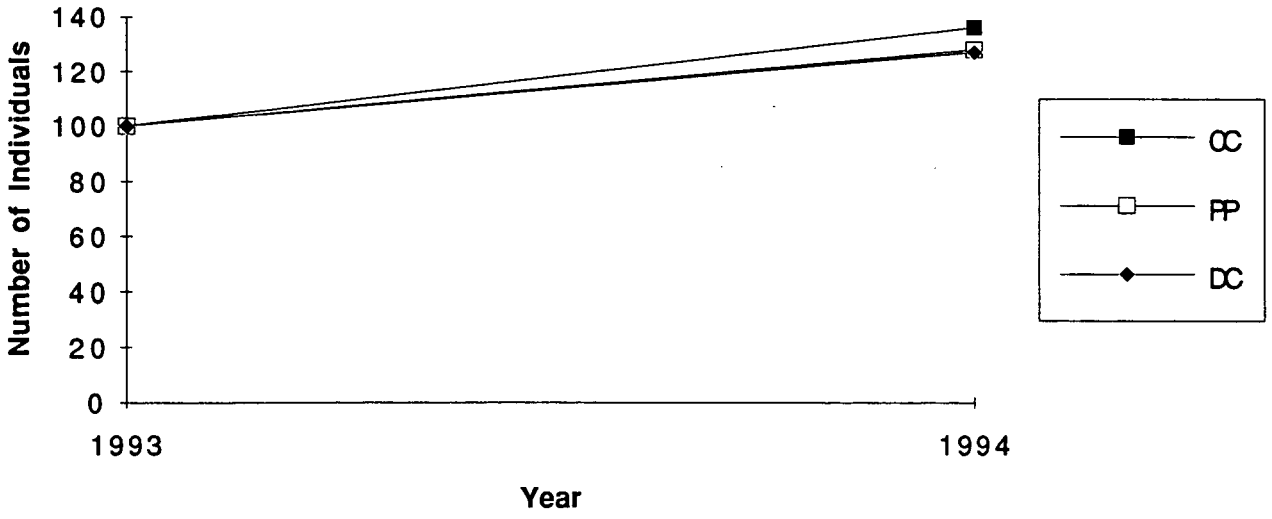


Figure 8: The percentage of individuals remaining vegetative, producing an inflorescence, and setting fruit for the 1992 field season. Abbreviations as in Figure 6.

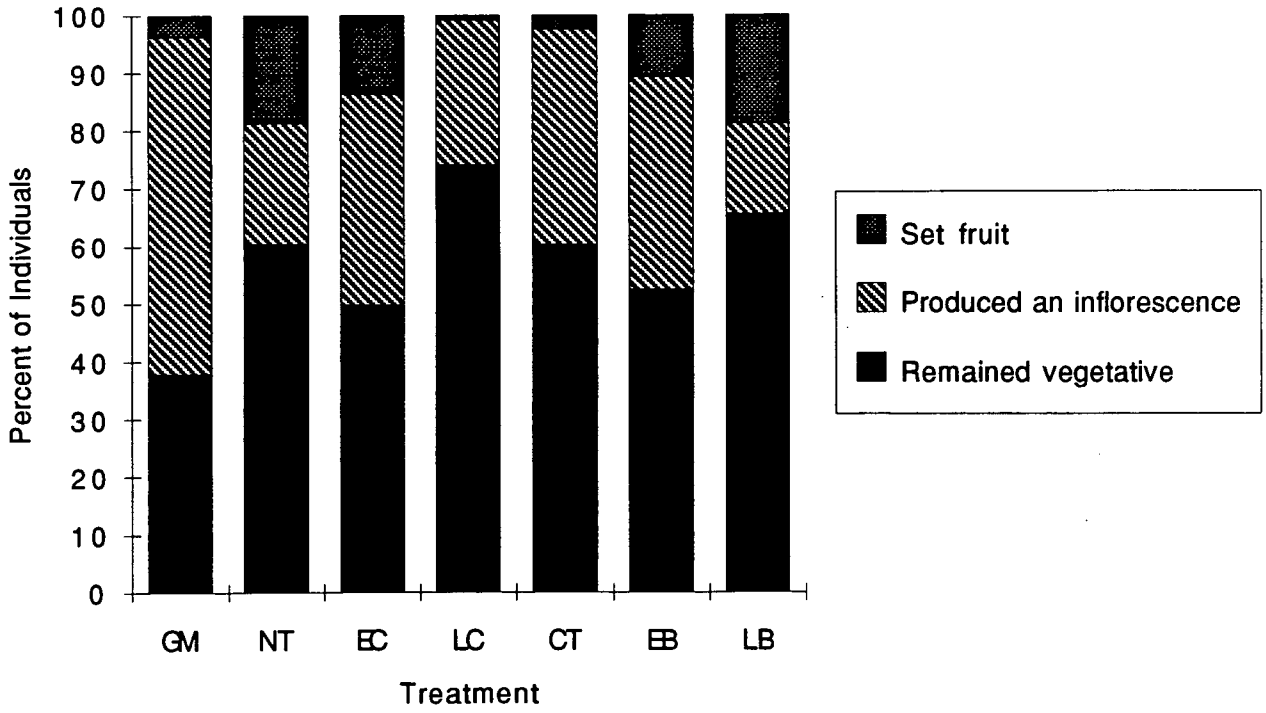


Figure 9: The percentage of individuals remaining vegetative, producing an inflorescence, and setting fruit for the 1993 field season. Abbreviations as in Figure 6.

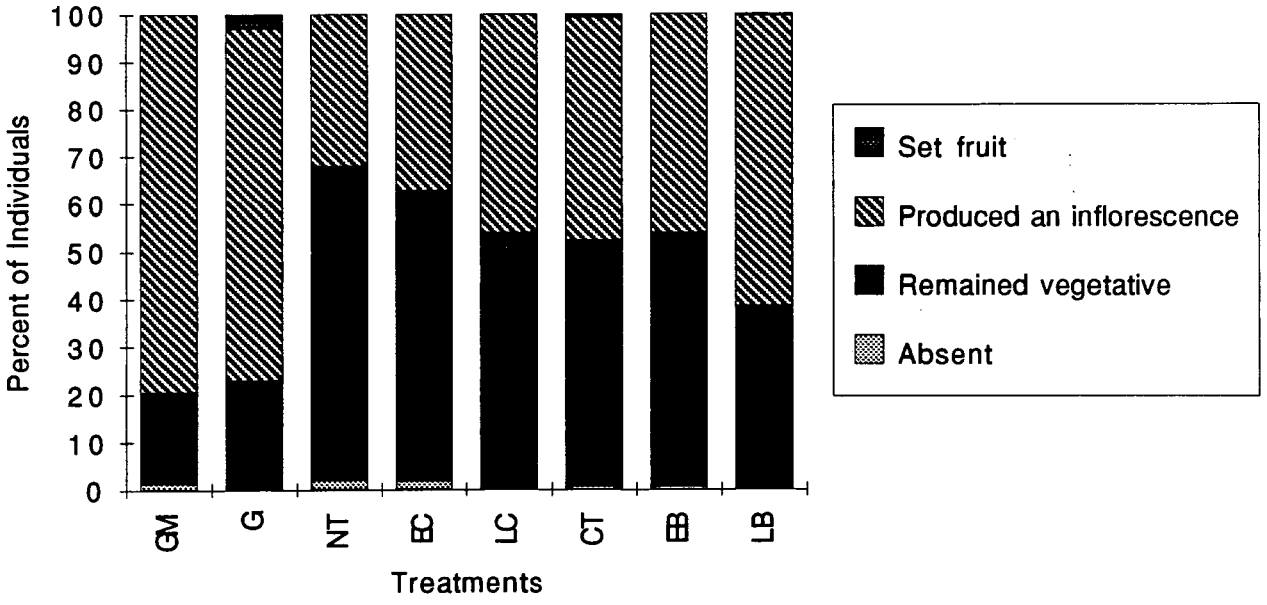


Figure 10: The percentage of individuals absent, remaining vegetative, producing an inflorescence, and setting fruit in 1994 at the Van Vleet Ranch. Abbreviations as in Figure 6.

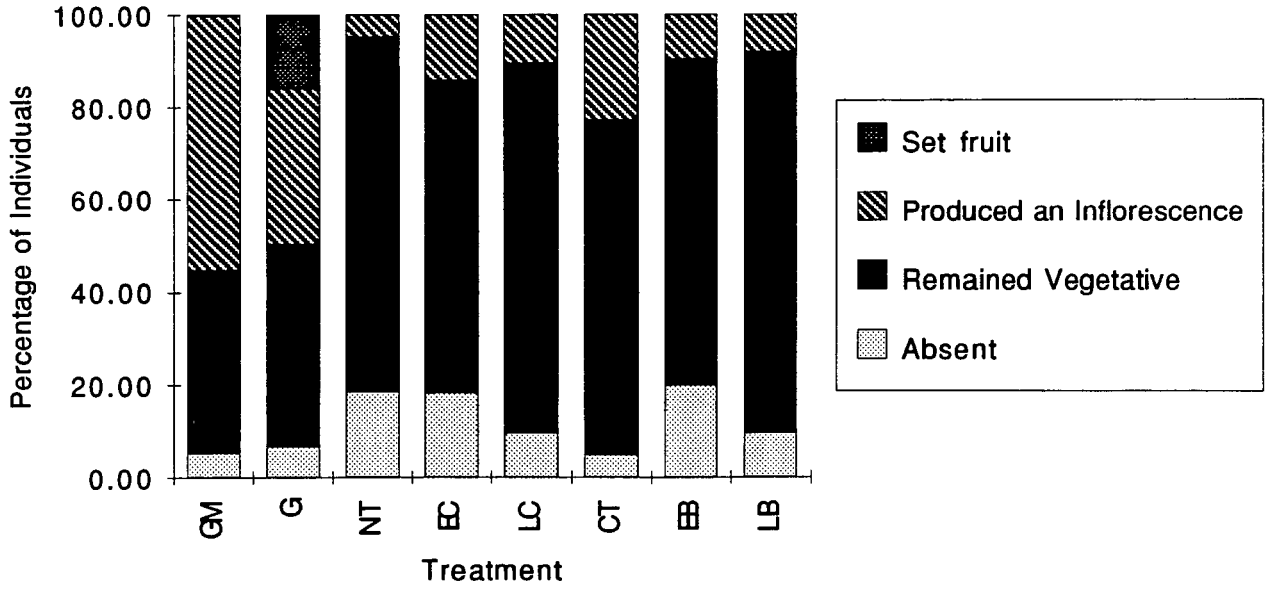


Figure 11: Monthly status of orchids at Van Vleet in June (top) through September (bottom)1993. Abbreviations: absent, A; vegetative, V; budding, B; flowering, F; fruiting, FR; died-back, D; and rosette, R.

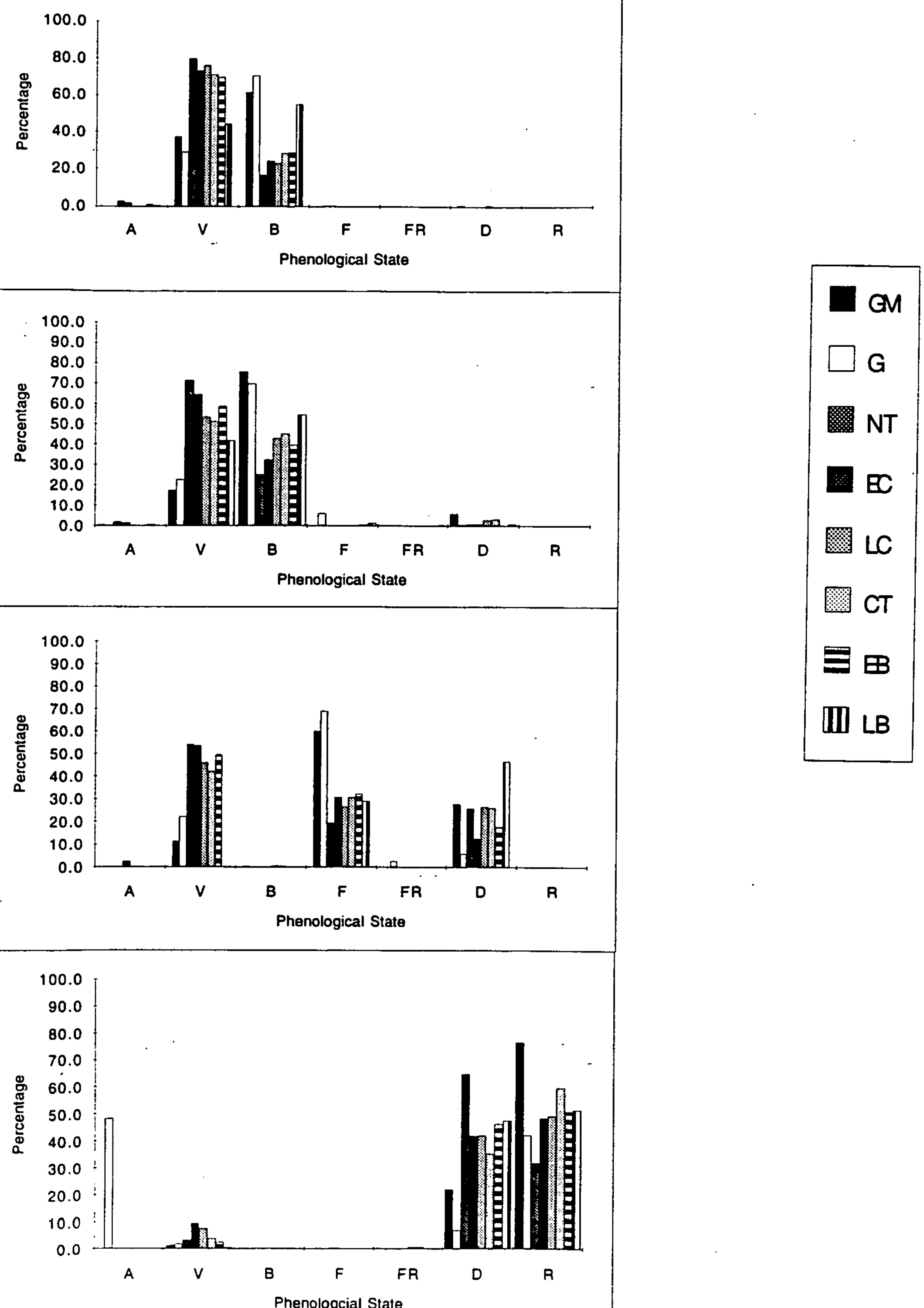


Figure 12: Monthly phenological state at Van Vleet in May (top) August (bottom), 1994. Abbreviations as in Figure 11.

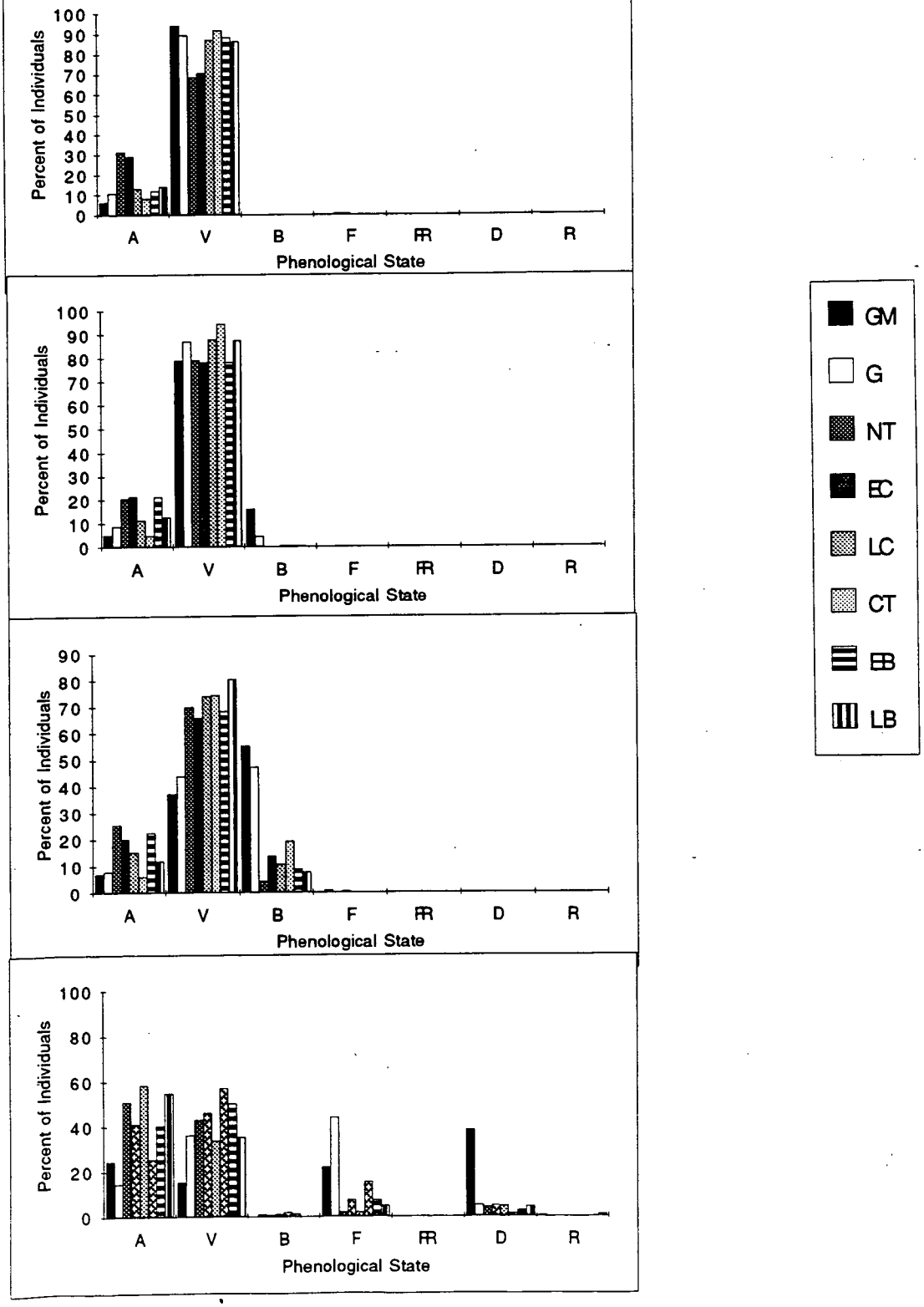


Figure 13: The percentage of individuals remaining vegetative, producing an inflorescence, and setting fruit in 1992. Treatment abbreviations as in Figure 6. Patch location indicated as follows: higher patch, "hi"; lower patch, "lo".

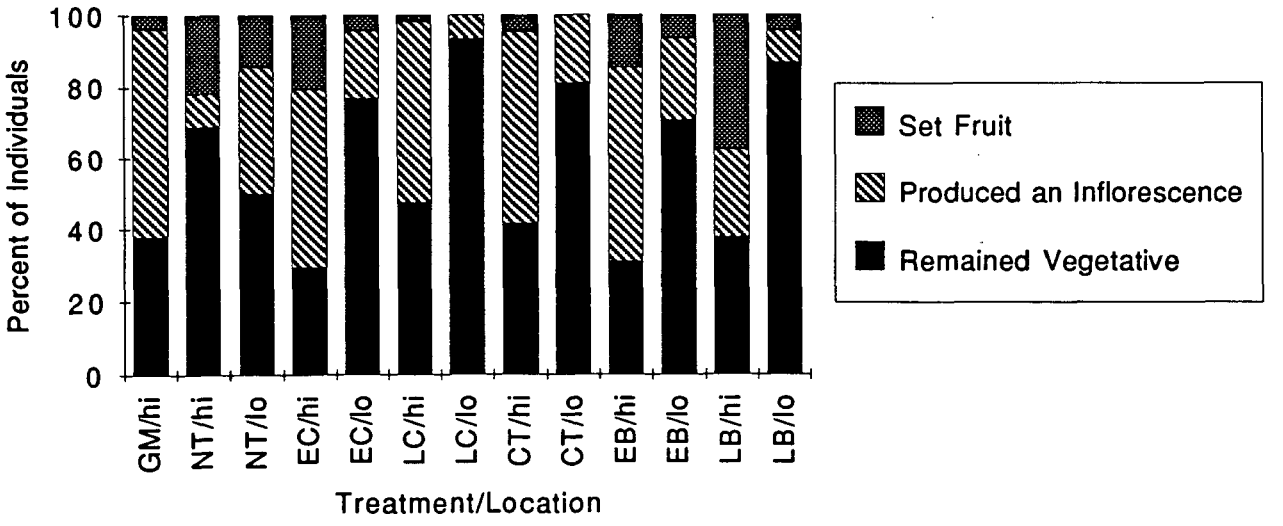


Figure 14: The percentage of individuals remaining vegetative, producing an inflorescence, and setting fruit in 1993 taking both treatment and patch location into account. Abbreviations as in Figure 13.

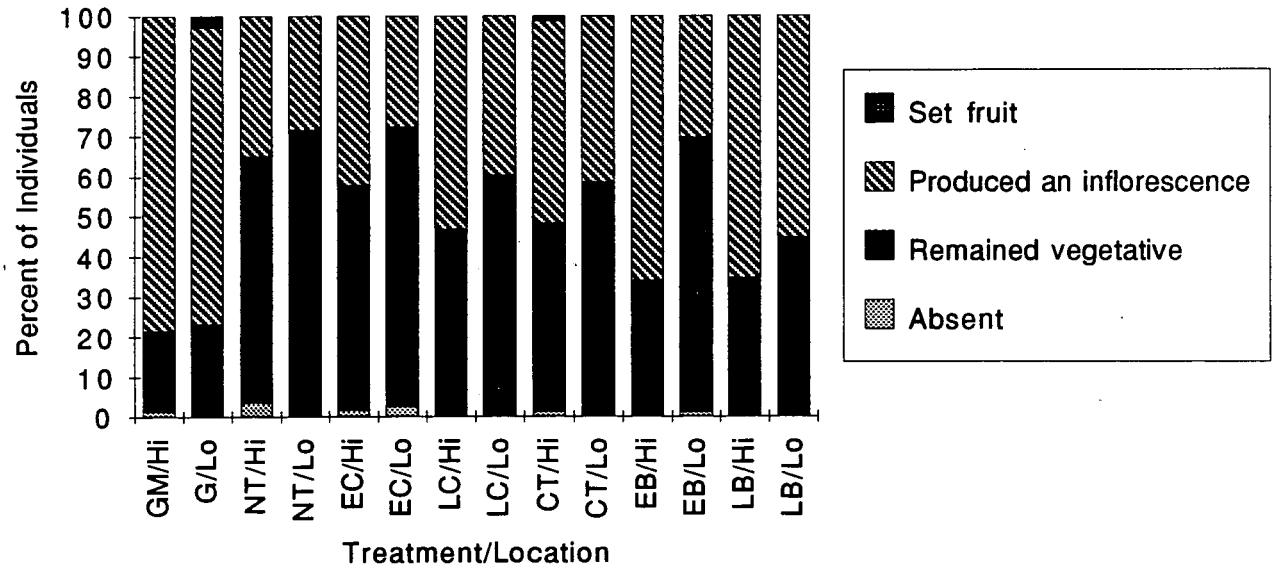


Figure 15: Summary of phenological state for the 1994 field season taking both treatment and location into account. Abbreviations as in Figure 13.

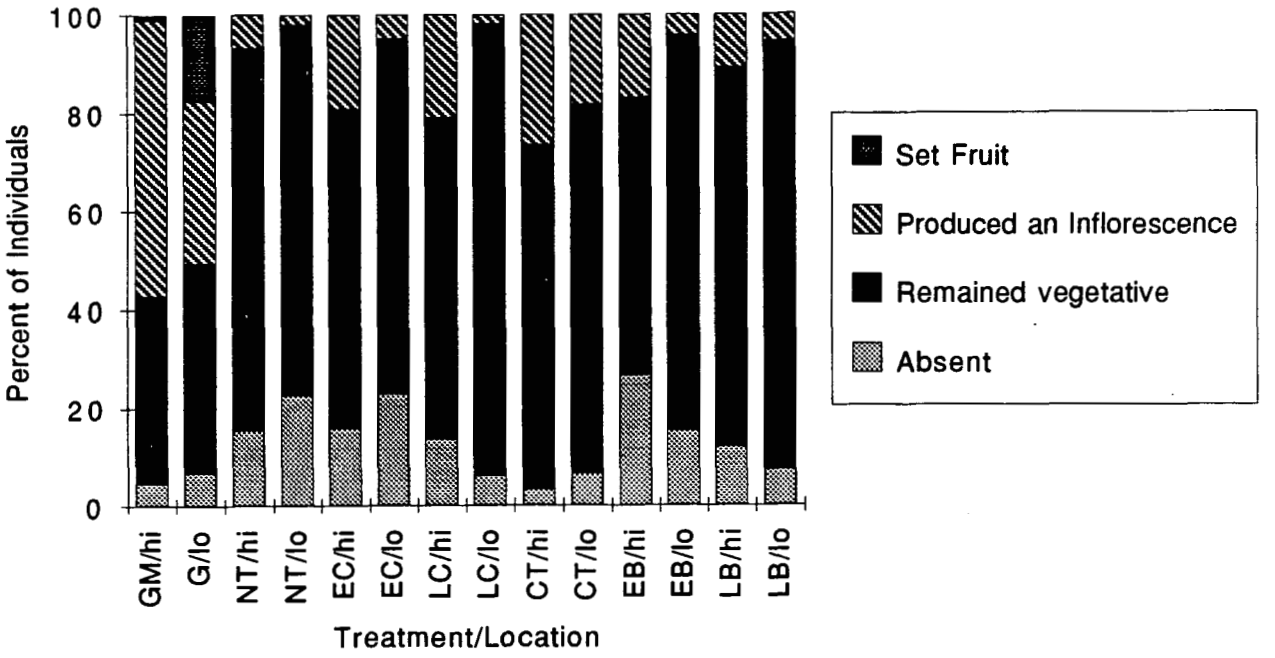


Figure 16: A comparison of 1993 phenological state of Van Vleet "grazed and mown", "grazed only", Clear Creek, Prospect Park, and Deer Creek plots. Abbreviations as in Figures 6 and 7.

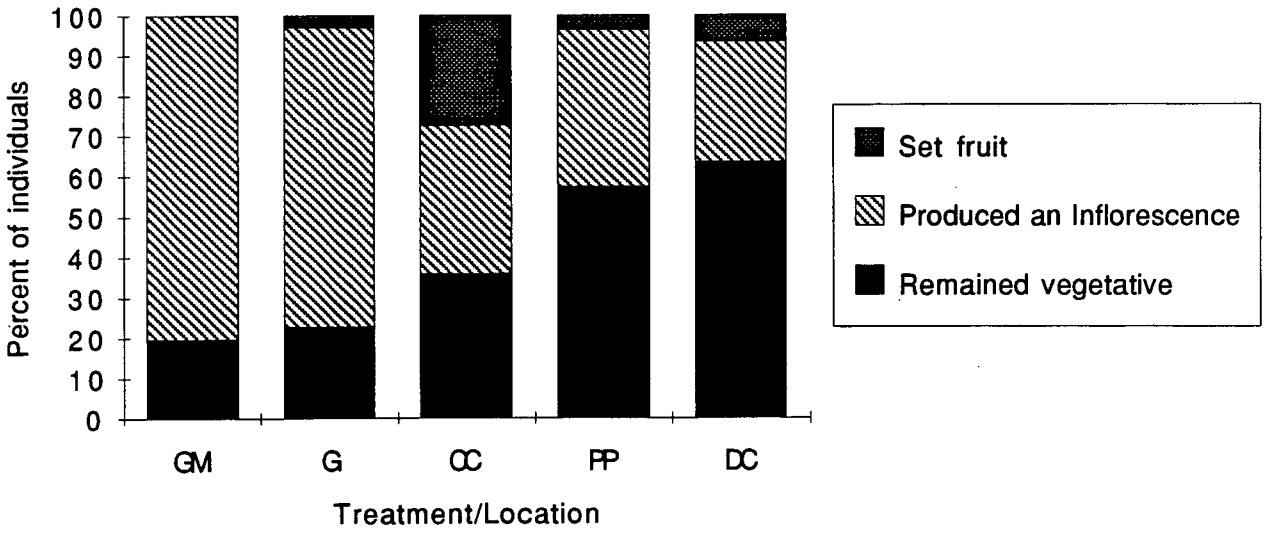


Figure 17: A comparison of 1994 phenological state of Van Vleet "grazed and mown", "grazed only", Clear Creek, Prospect Park , and Deer Creek plots. Abbreviations as in Figures 6 and 7.

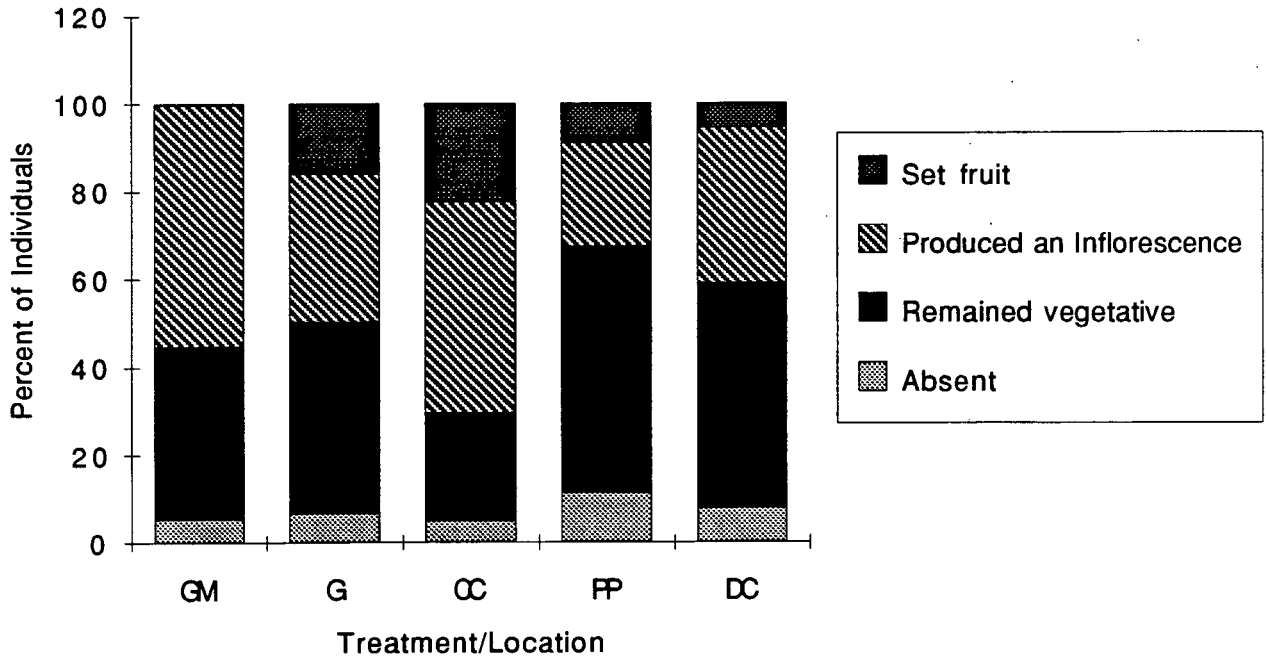


Figure 18: The percentage of inflorescences undamaged and damaged in 1992 by vole herbivory, traditional and simulated mowing, and unknown causes. Treatments as indicated in Figure 6.

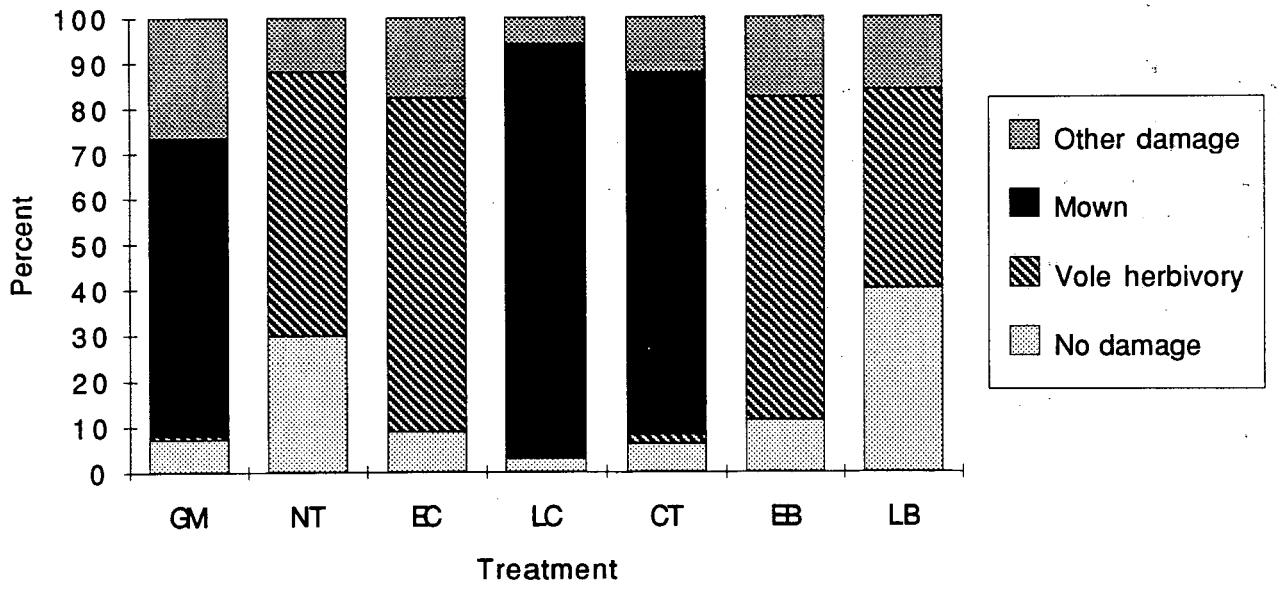


Figure 19: The percentage of inflorescences damaged by vole herbivory, mowing, clipping, and other causes during 1993. Treatments as indicated in Figure 6.

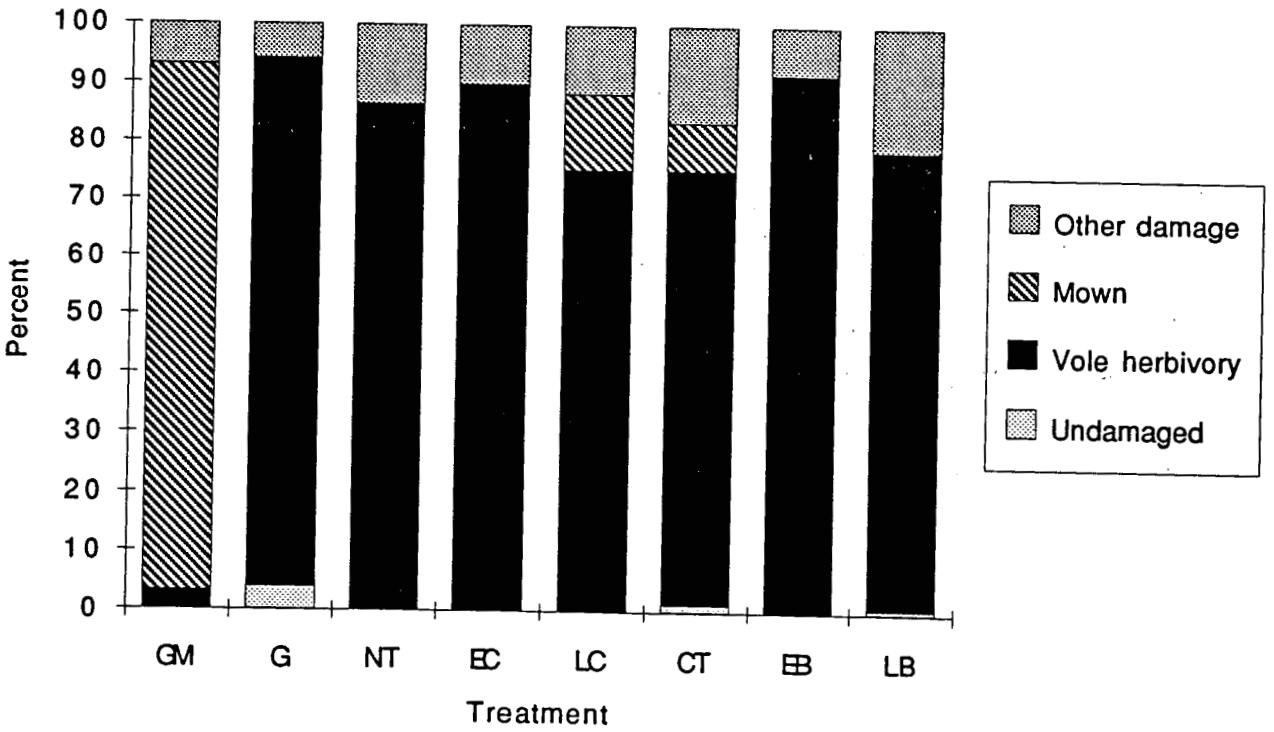


Figure 20: Summary of inflorescence damage at Van Vleet during the 1994 field season. Abbreviations as in Figure 6.

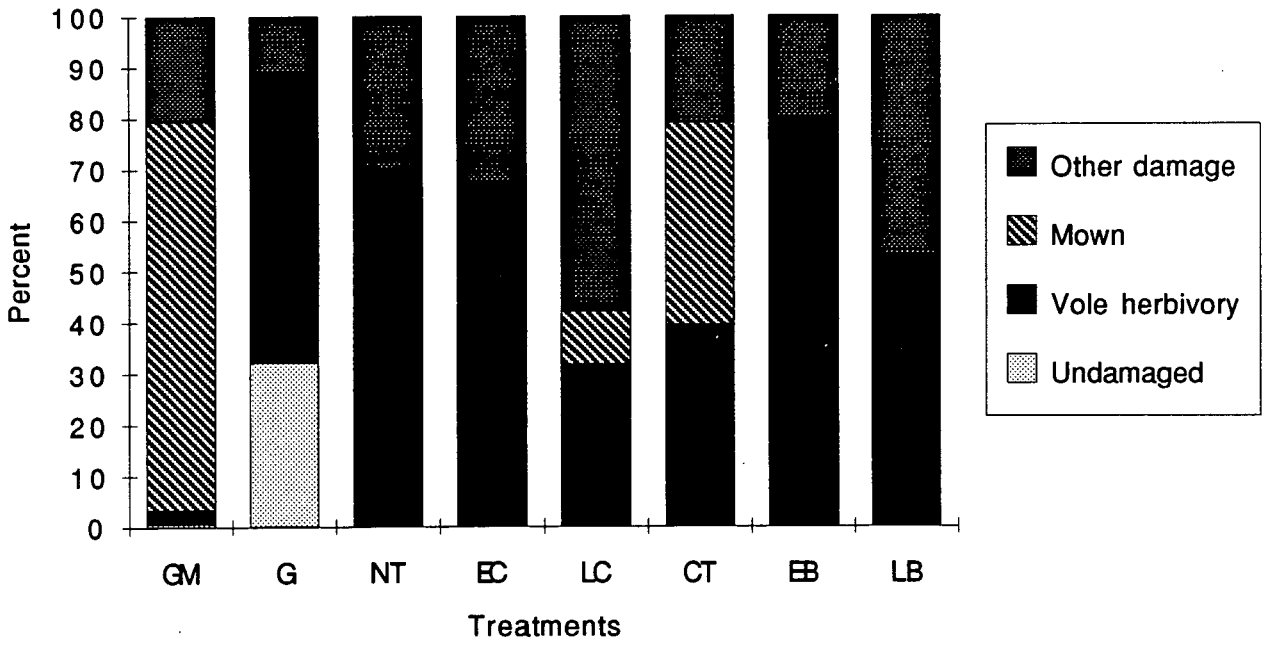
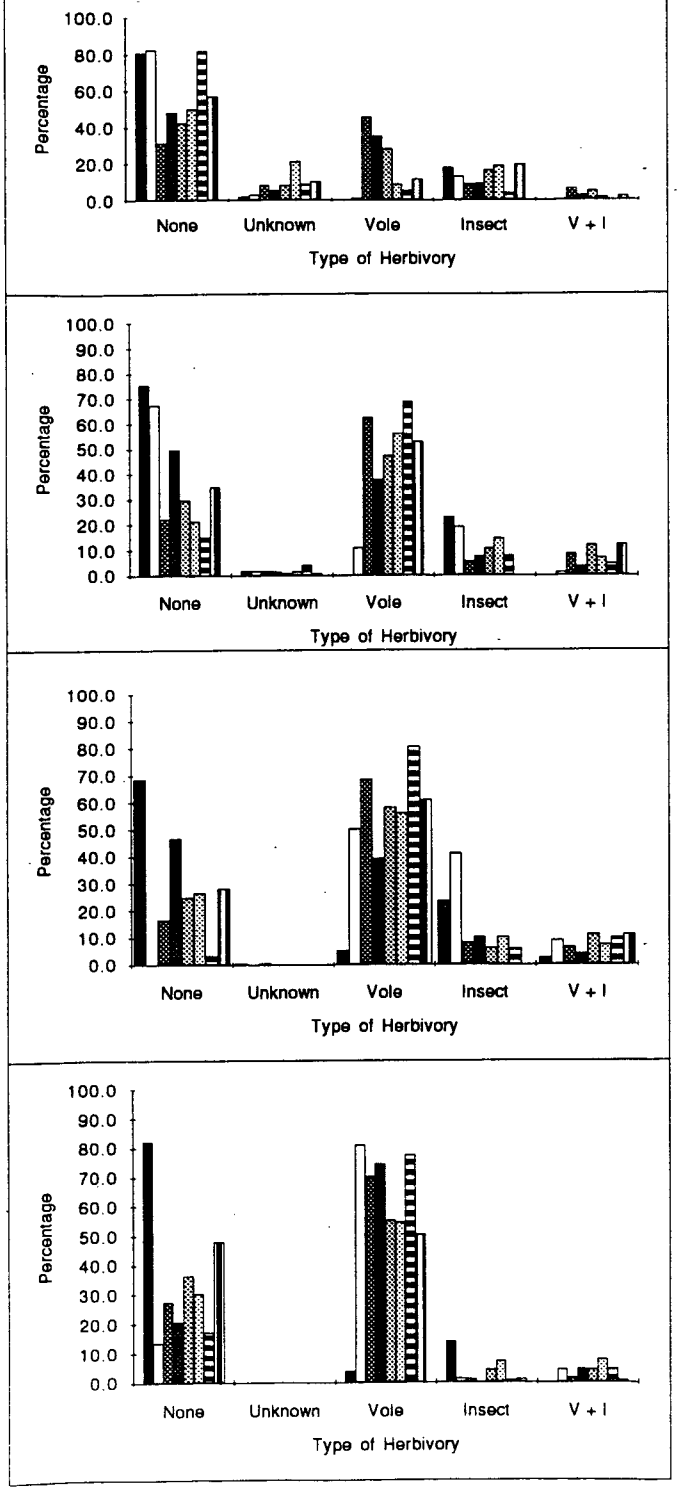


Figure 21: Monthly inflorescence damage at Van Vleet in May (top) through August (bottom),1993. Types of damage are: no damage (none), unknown, vole, insect and vole and insect (V+I). Abbreviations as in Figure 6.



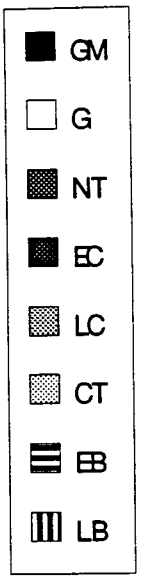


Figure 22: Monthly inflorescence damage at Van Vleet in May (top) through August (bottom),1994. Abbreviations as in Figure 21.

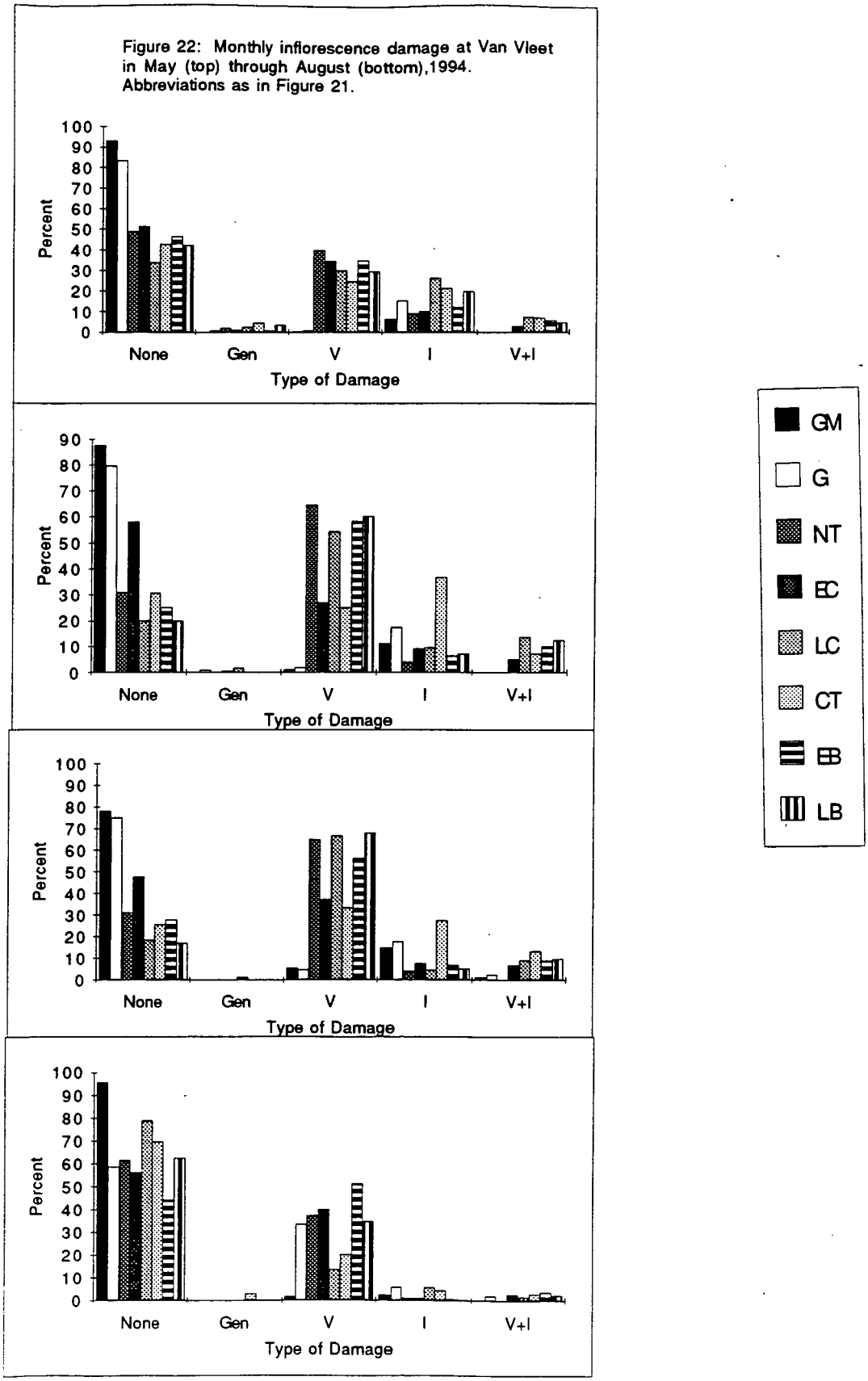


Figure 23: A site comparison of inflorescence damage in 1993 for Clear Creek, Deer Creek, Prospect Park, and Van Vleet. Abbreviations as in Figures 6 and 7.

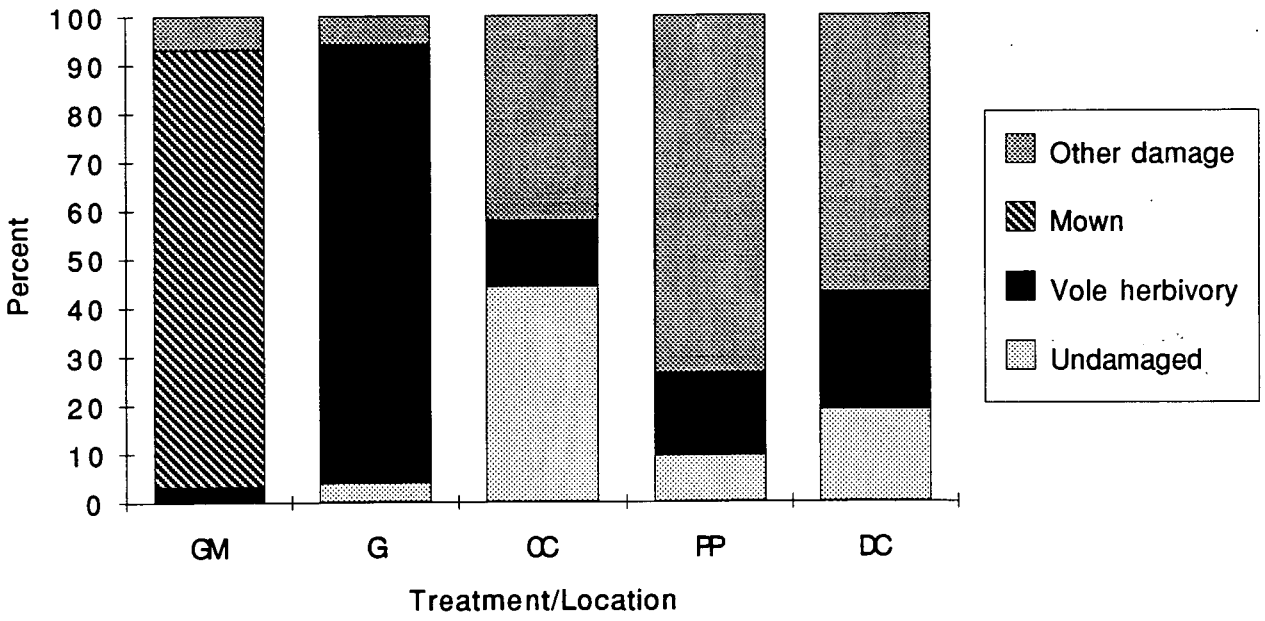


Figure 24: A site comparison of inflorescence damage in 1994 for Clear Creek, Deer Creek, Prospect Park, and Van Vleet. Abbreviations as in Figures 6 and 7.

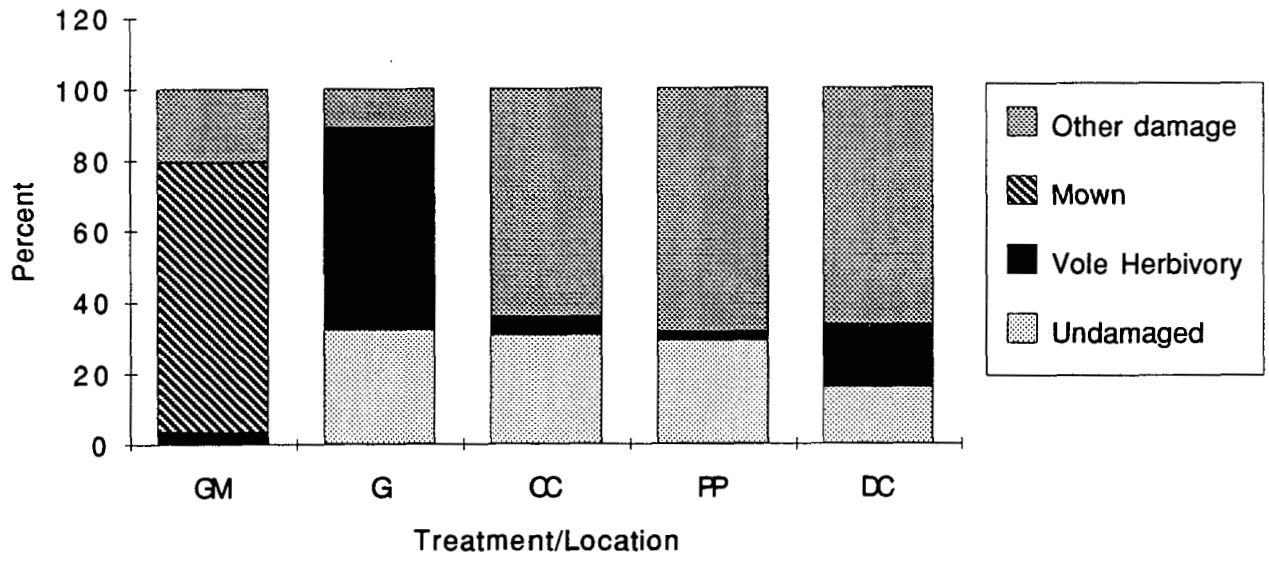


Figure 25: Average leaf area (top) and number of basal leaves (bottom) for 1992 at Van Vleet. Treatment abbreviations as in Figure 6.

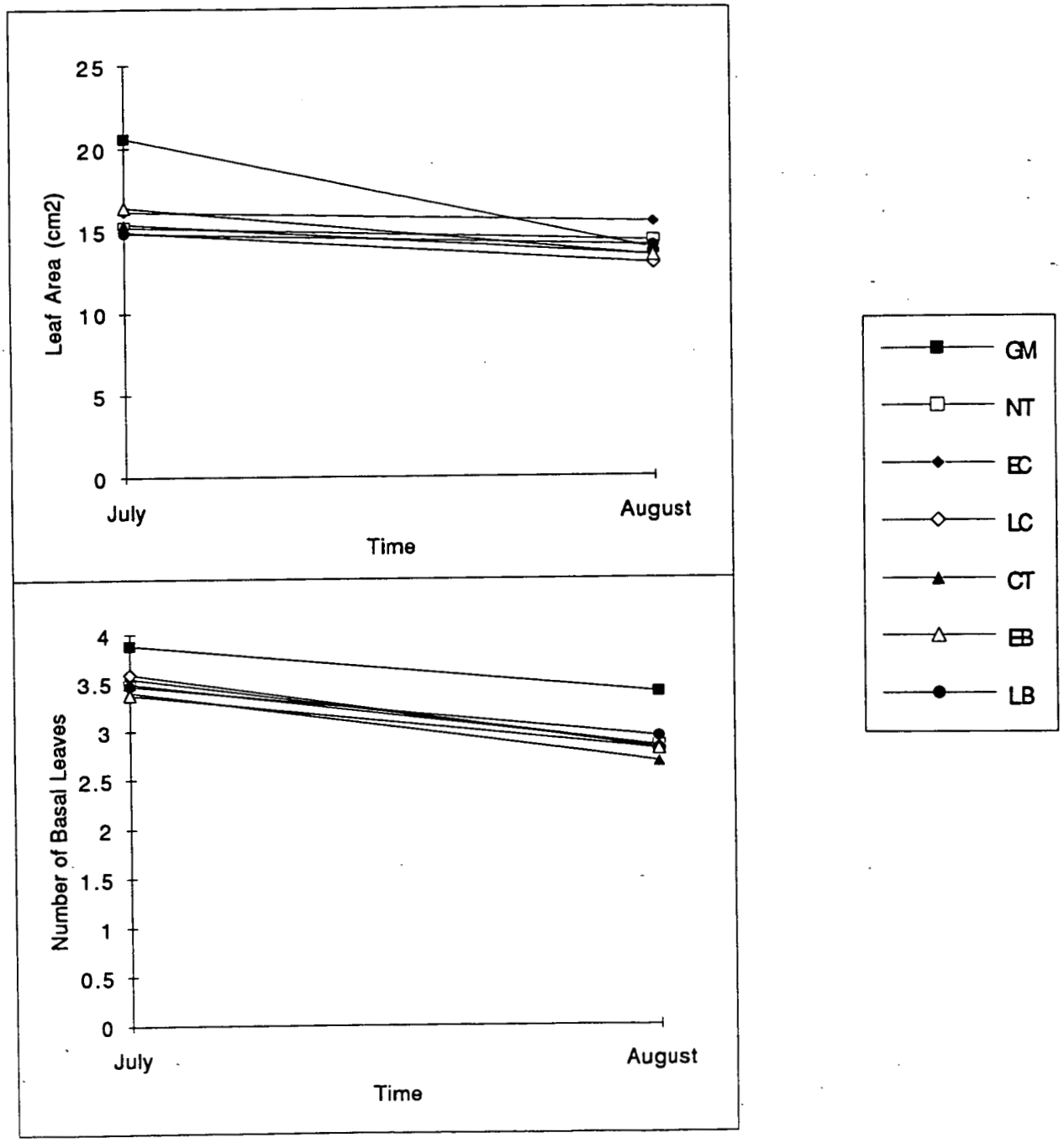


Figure 26: Average leaf area (top) and number of basal leaves (bottom) for 1993 at Van Vleet. Treatment abbreviations as in Figure 6.

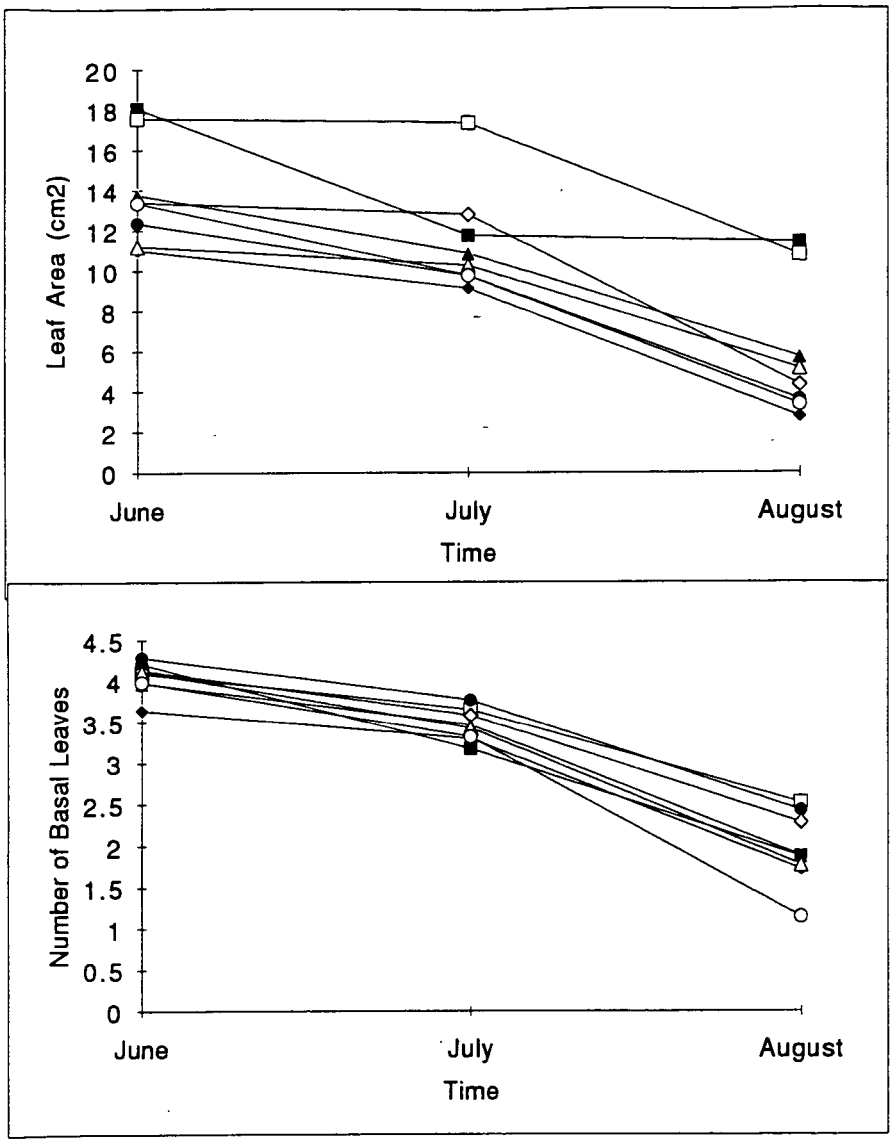


Figure 27: Average leaf area (top) and number of basal leaves (bottom) for 1994 at Van Vleet. Treatment abbreviations as in Figure 6.

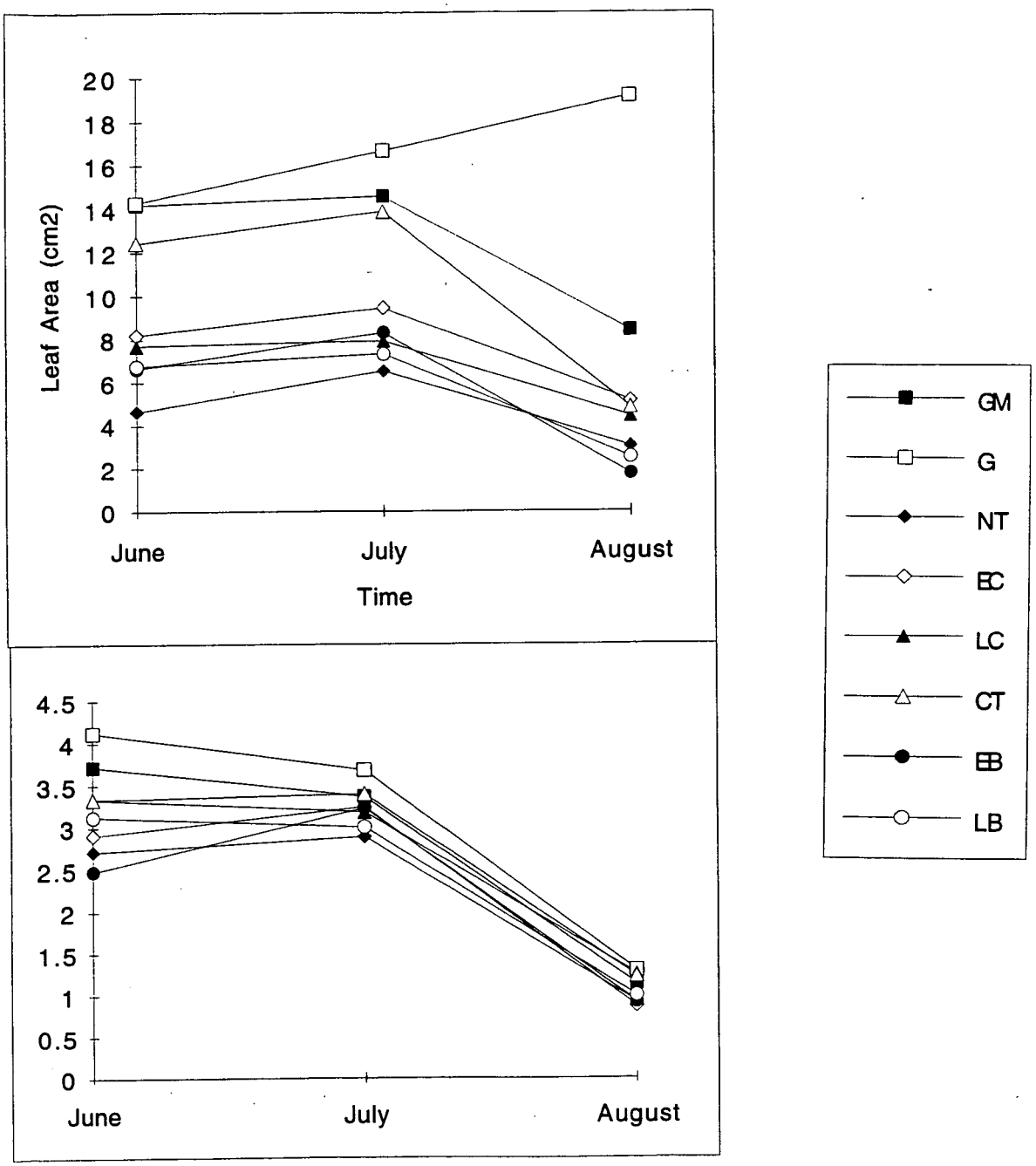


Figure 28: Ground water well monitoring for 1993 at Van Vleet Ranch. Wells two and six (highlighted) are near orchid patches.

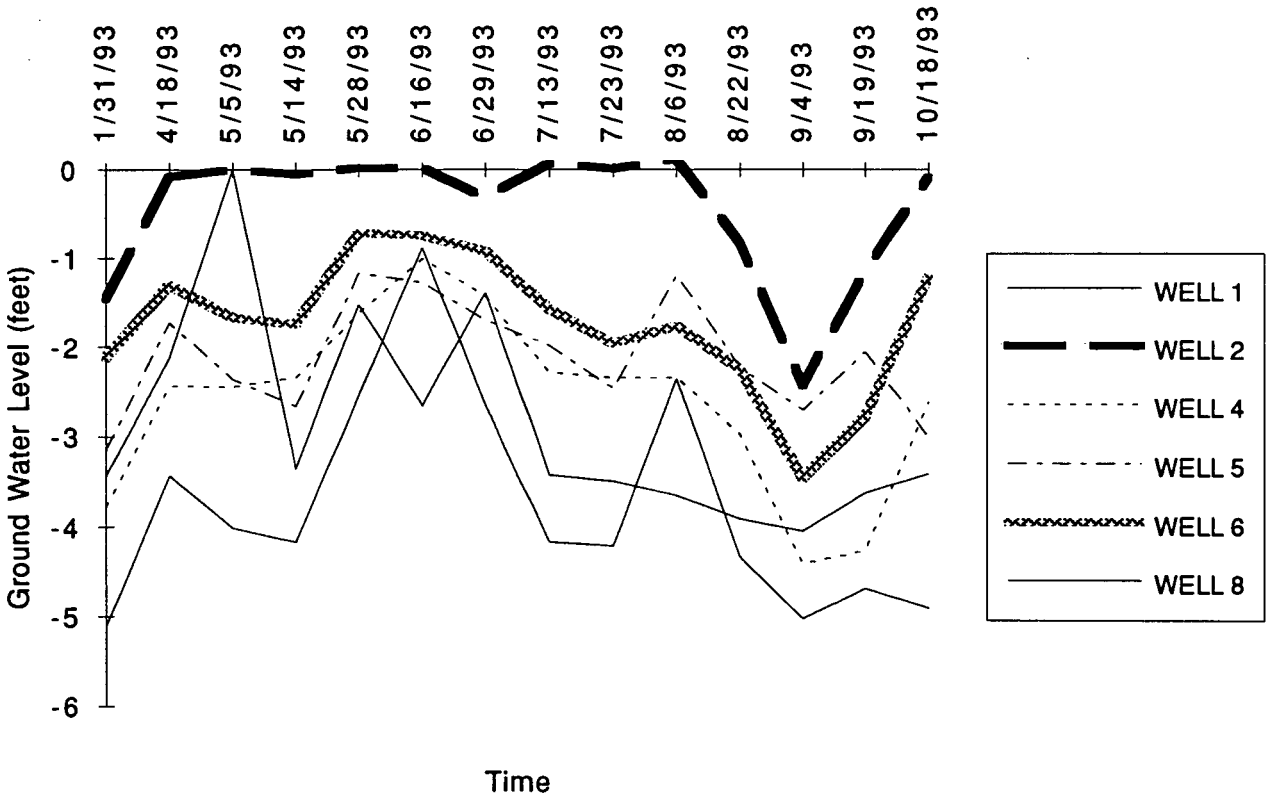


Figure 29: Soil moisture as measured by gravimetric analysis at the Van Vleet Ranch during the 1993 field season. Abbreviations as in Figure 6.

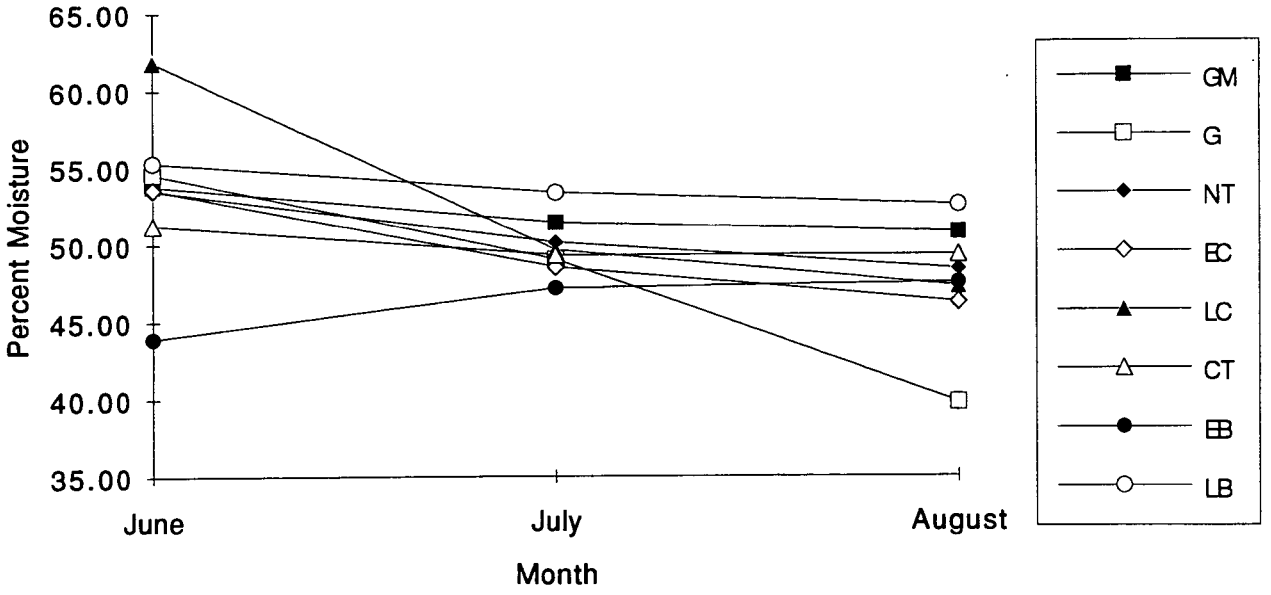


Figure 30: Soil moisture as measured by gravimetric analysis at the Van Vleet Ranch during the 1994 field season. Abbreviations as in Figure 6.

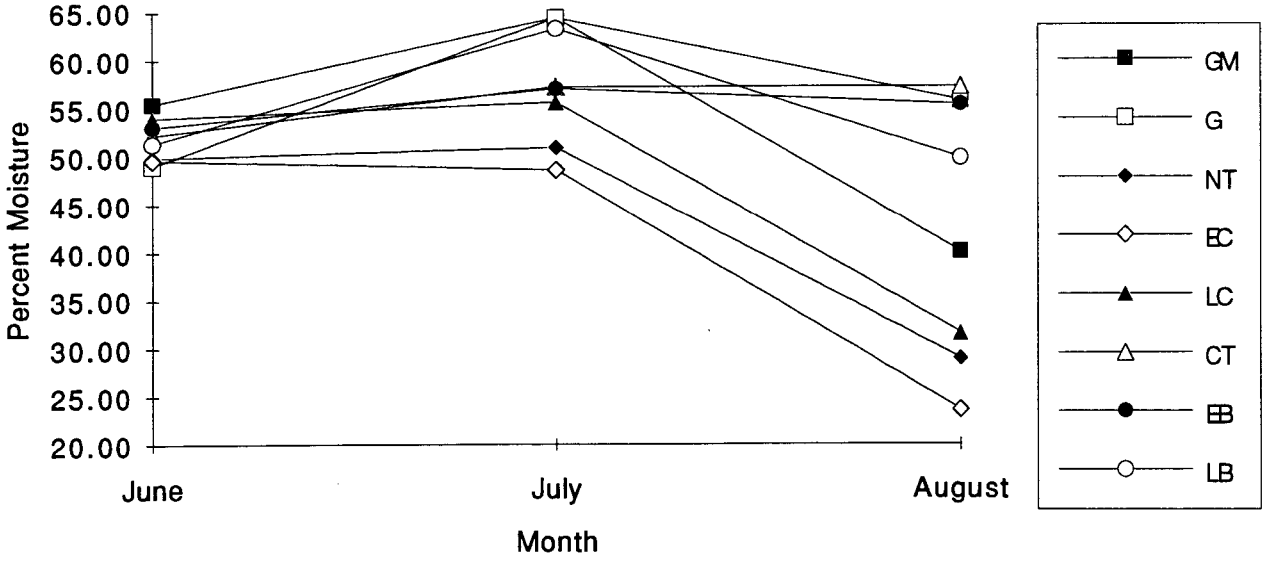


Figure 31: Volumetric soil moisture as measured using Time Domain Reflectometry (TDR) at Van Vleet during 1993. Abbreviations as in Figure 6.

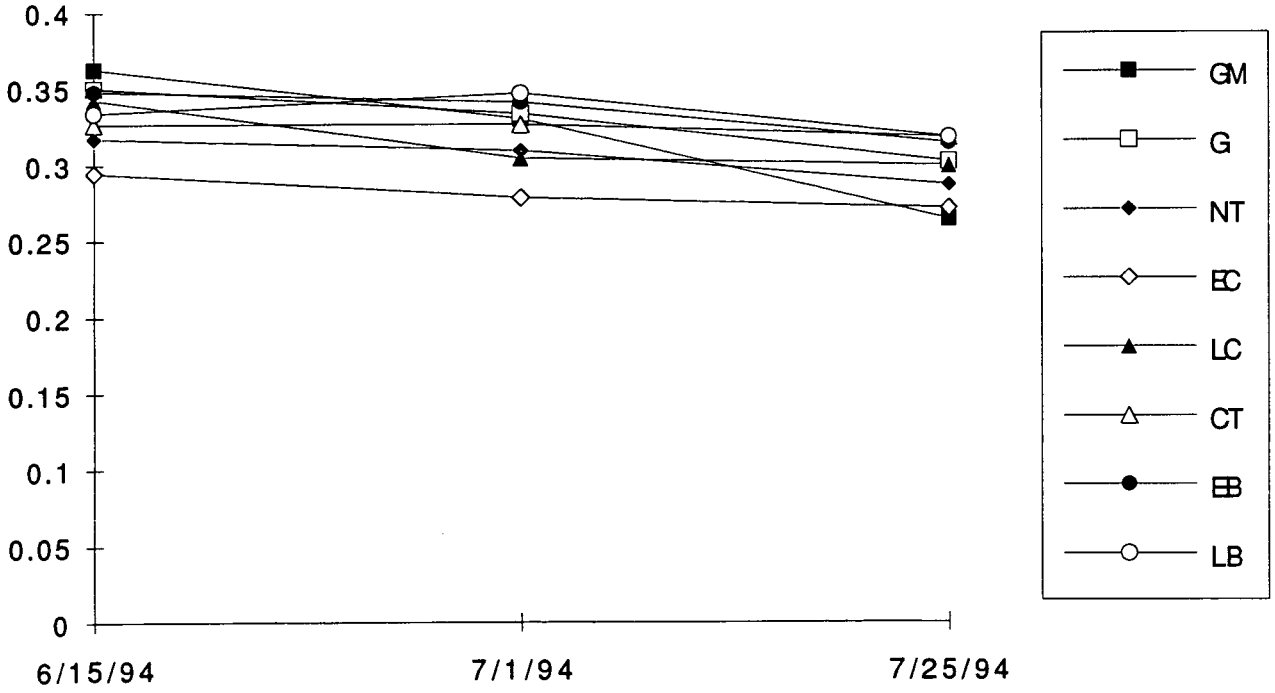


Figure 32: Volumetric soil moisture as measured using Time Domain Reflectometry at Van Vleet during 1994. Abbreviations as in Figure 6.

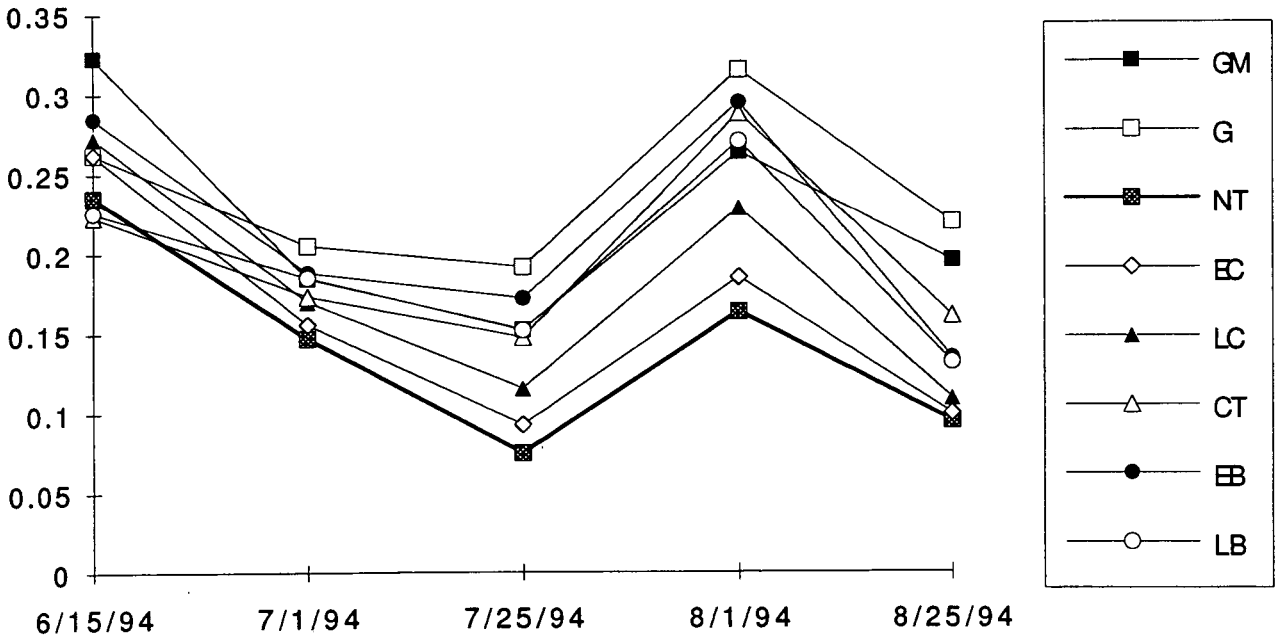


Figure 33: Percent bare cover measured at Van Vleet during June, 1993. Abbreviations as in Figure 6.

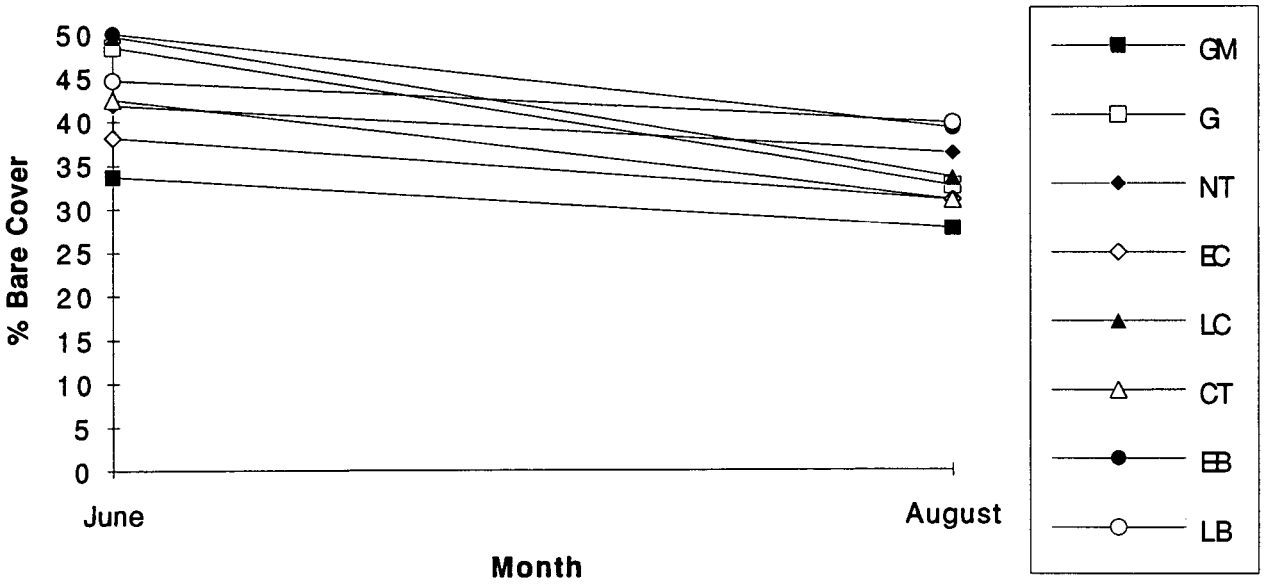


Figure 34: Percent bare cover measured monthly at Van Vleet from June through August, 1994. Abbreviations as in Figure 6.

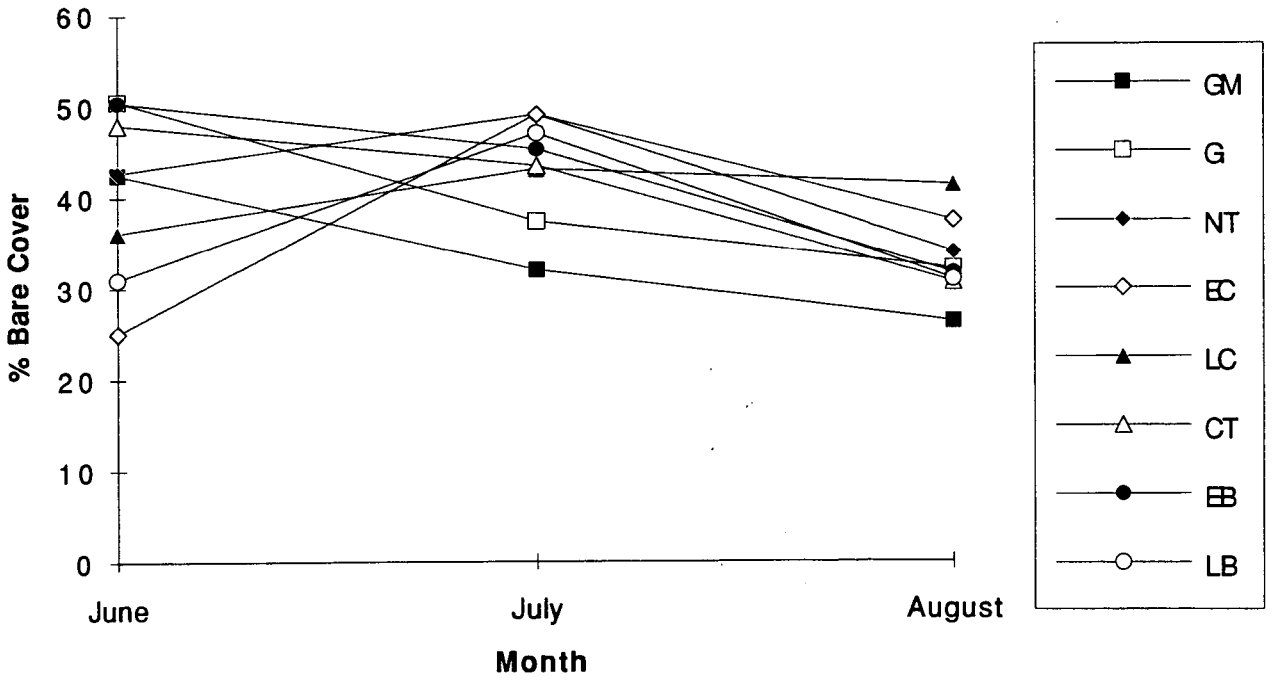


Figure 35: Average percentage of Canada Thistle in quadrats for treatment plots at Van Vleet in June, 1994. Abbreviations as in Figure 6.

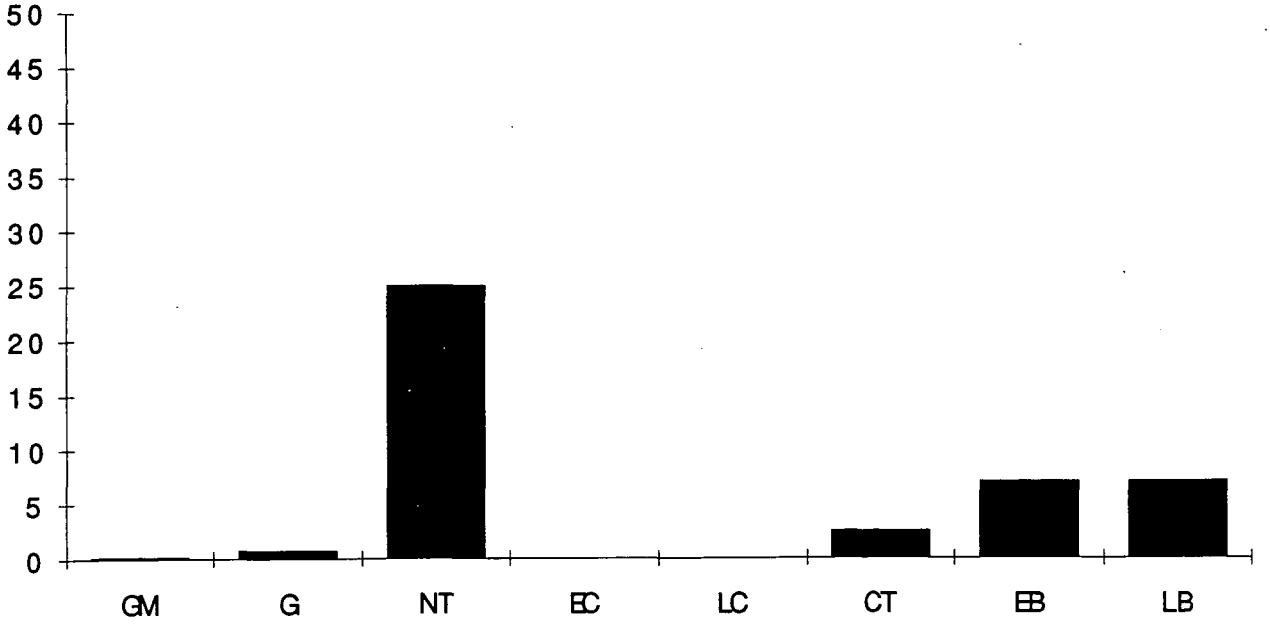


Figure 36: Photosynthetically active radiation measured in August, 1993 at the Van Vleet Ranch. Treatments are abbreviated as in Figure 6.

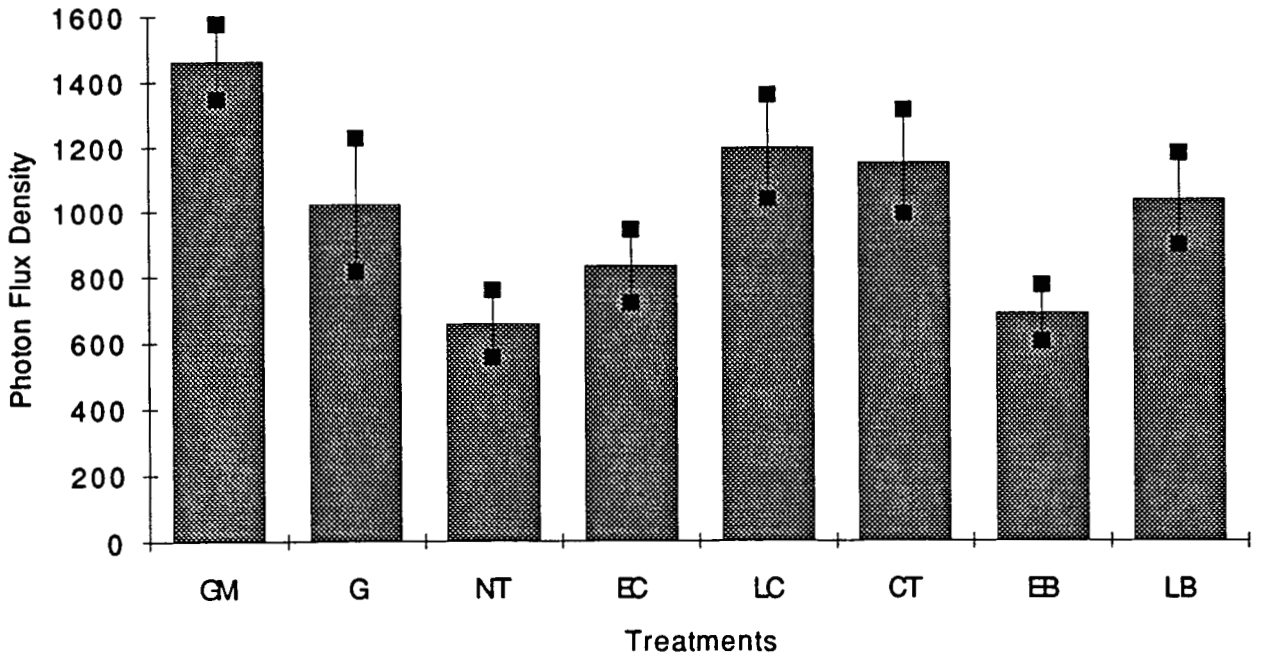
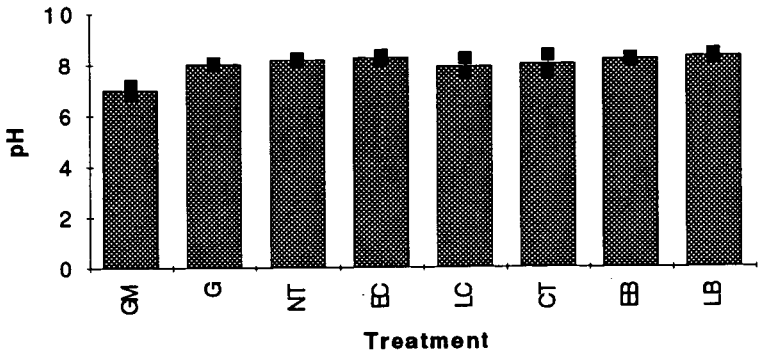
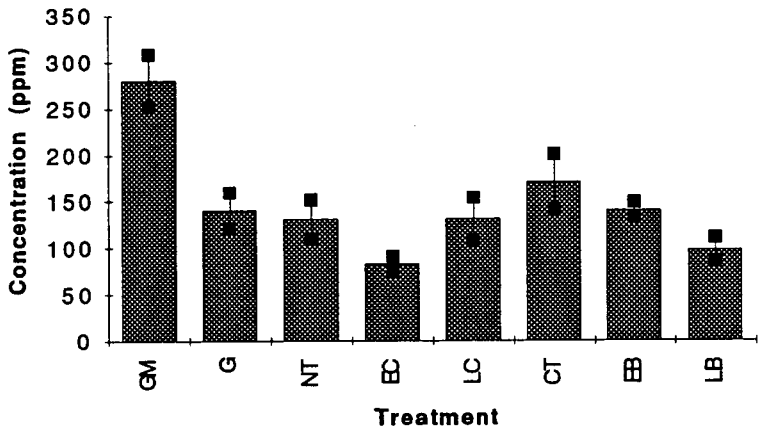


Figure 37: Average soil pH (top), iron concentration (middle), and manganese concentration (bottom) at Van Vleet site for all treatment groups, June, 1994. Abbreviations as in Figure 6.





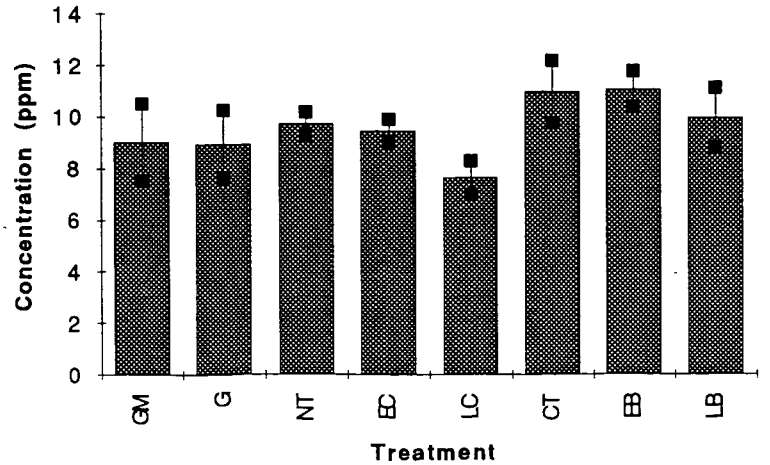
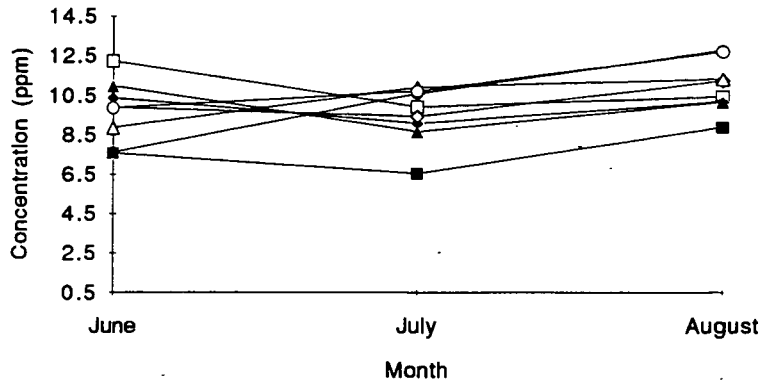
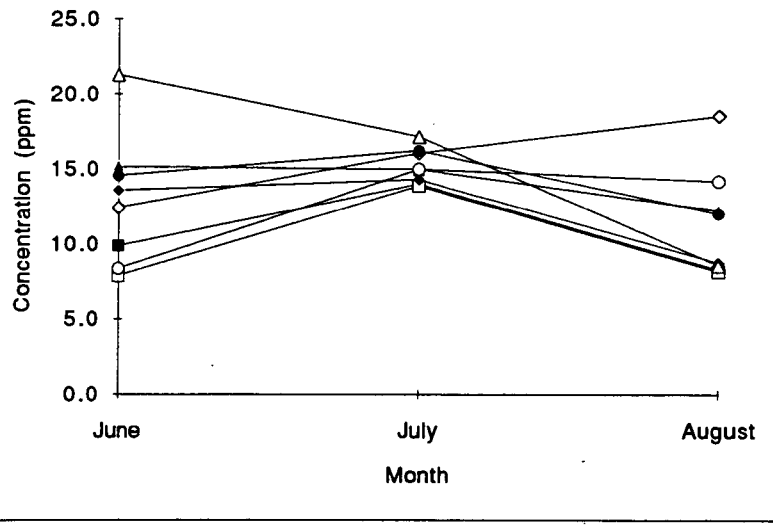


Figure 38: Average ammonium concentration at Van Vleet in 1994 for all treatment groups. Abbreviations as in Figure 6.





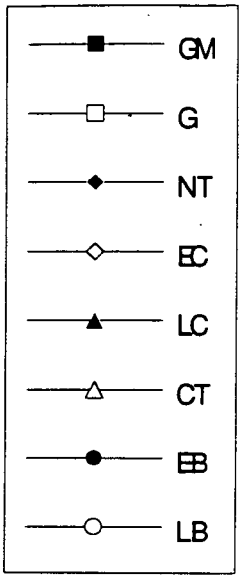


Figure 39: A comparison of soil characteristics for Clear Creek, Deer Creek, Prospect Park, d Van Vleet in June 1994. Abbreviations as in Figures 6 and 7.

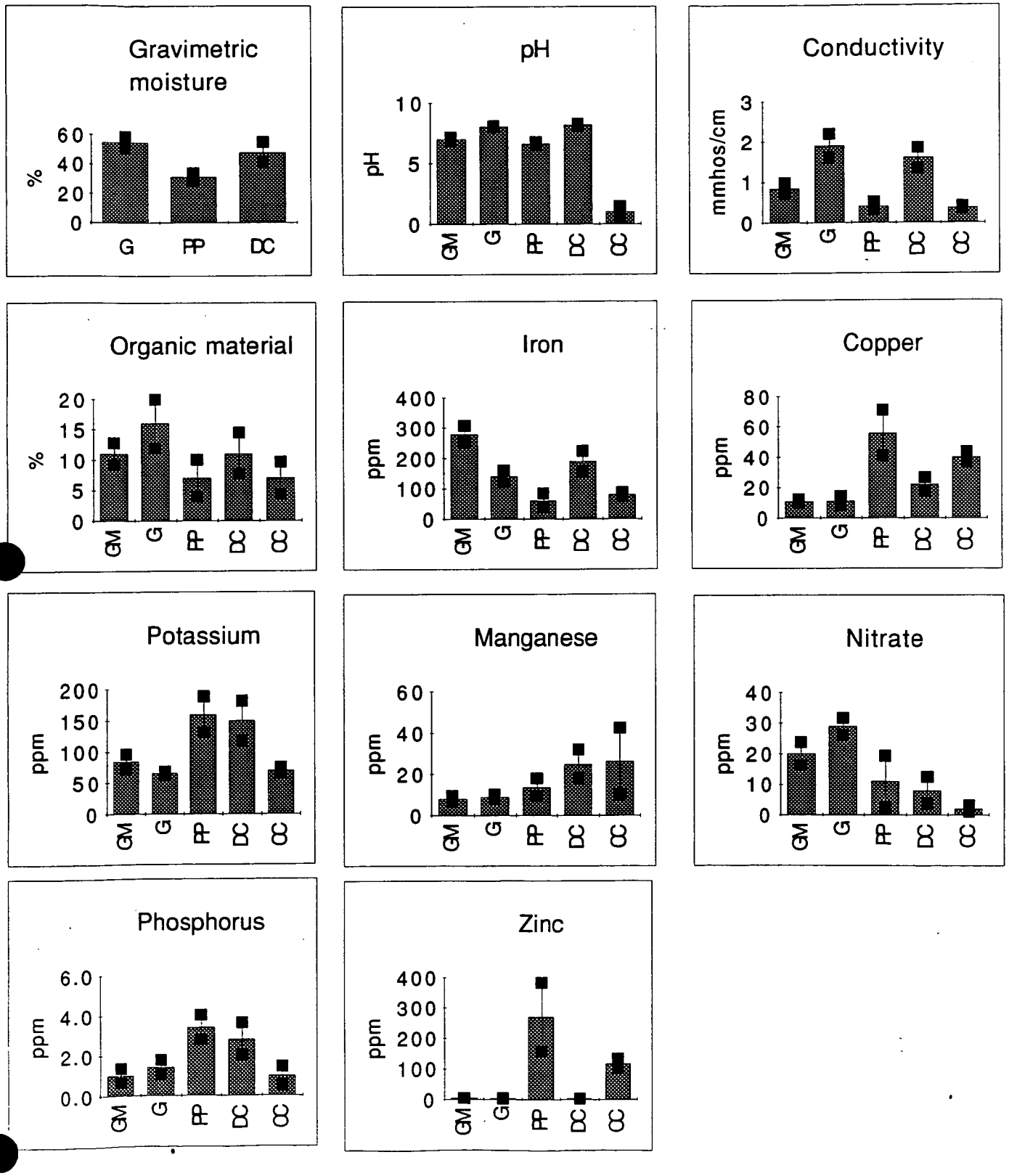


Figure 40: Chart of the first two canonical axes using phenological states as predictors for treatment at Van Vleet, 1994.

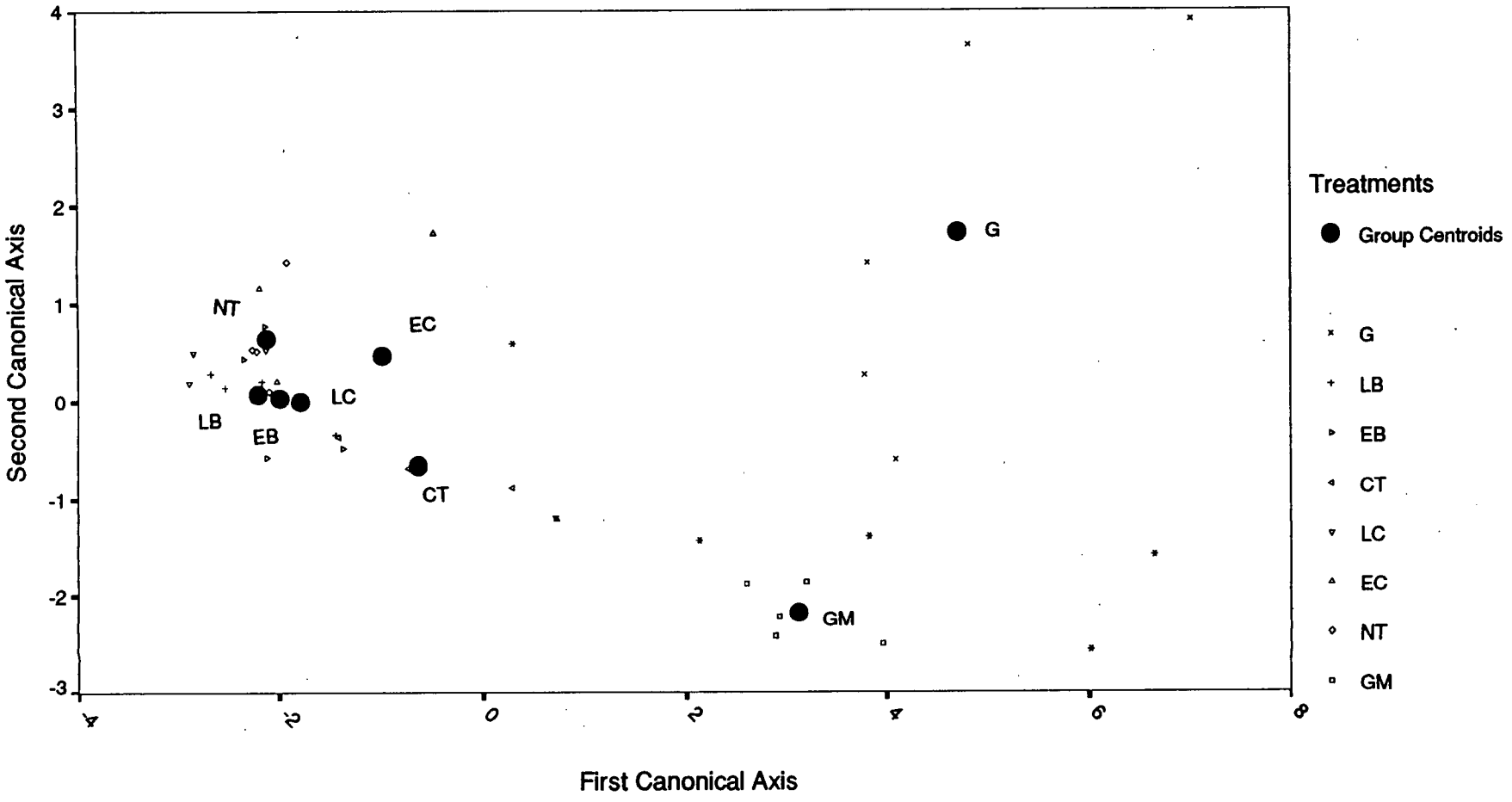


Figure 41: Plot of the first two canonical axes using environmental variables as predictors for treatment at Van Vleet, 1994

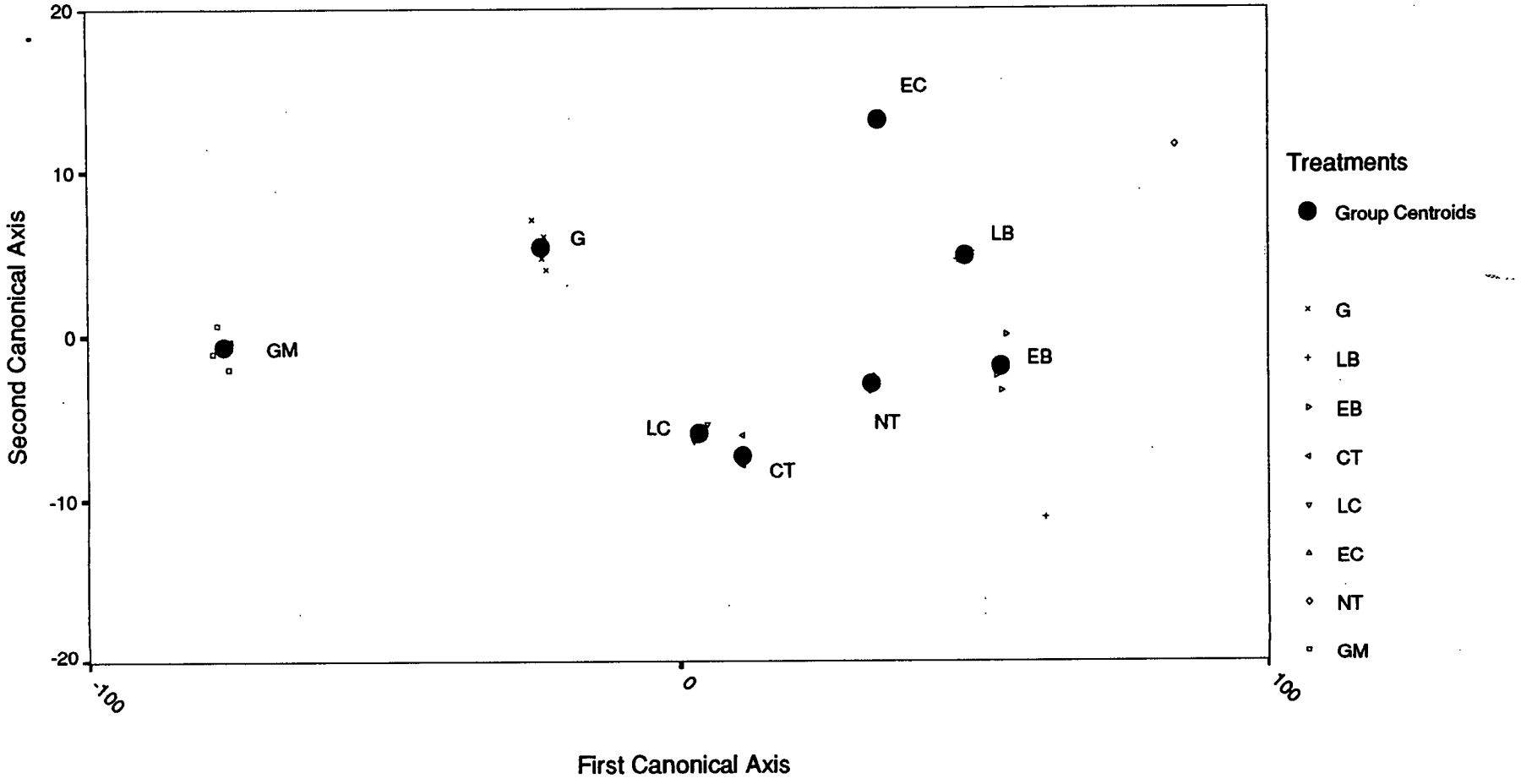


Figure 42: Plot of the first two canonical axes using phenological states as predictors for sites, 1994.

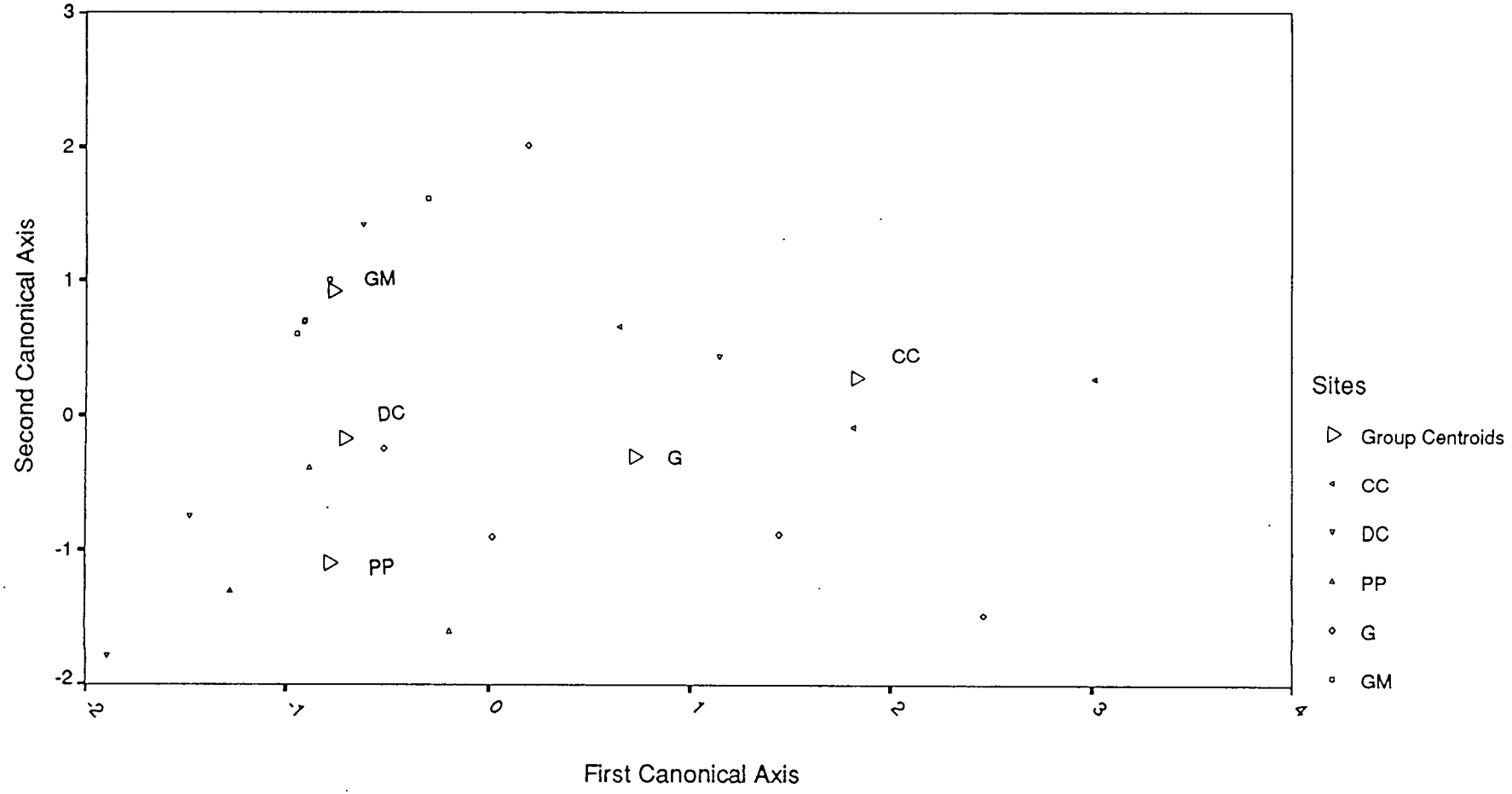


Figure 43: Plot of the first two canonical axes using environmental variables as predictors for site, 1994.

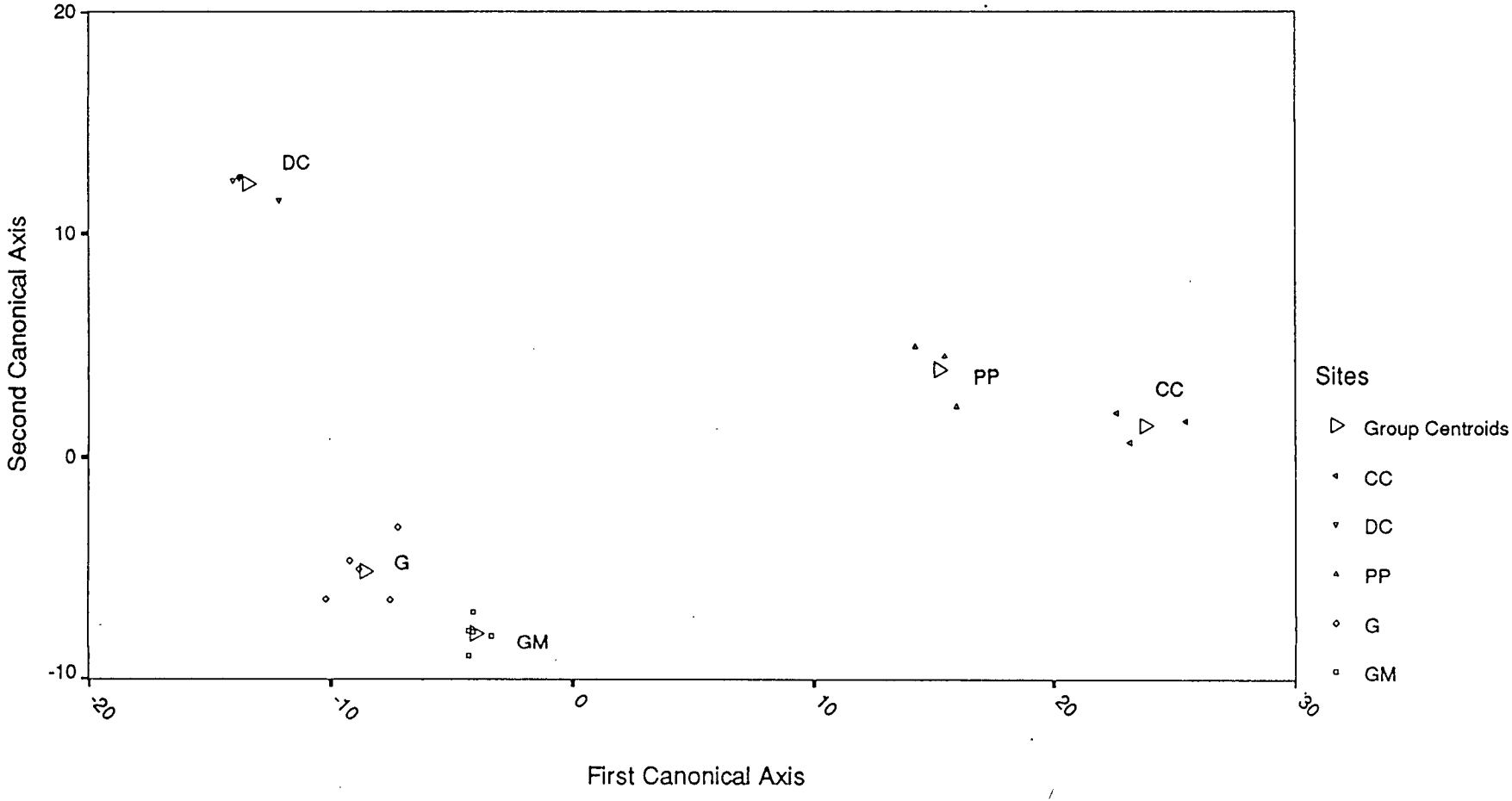


Figure 44: Location of study sites in Colorado and Utah. Abbreviations as in Figure 7 with the addition of Van Vleet (VV).

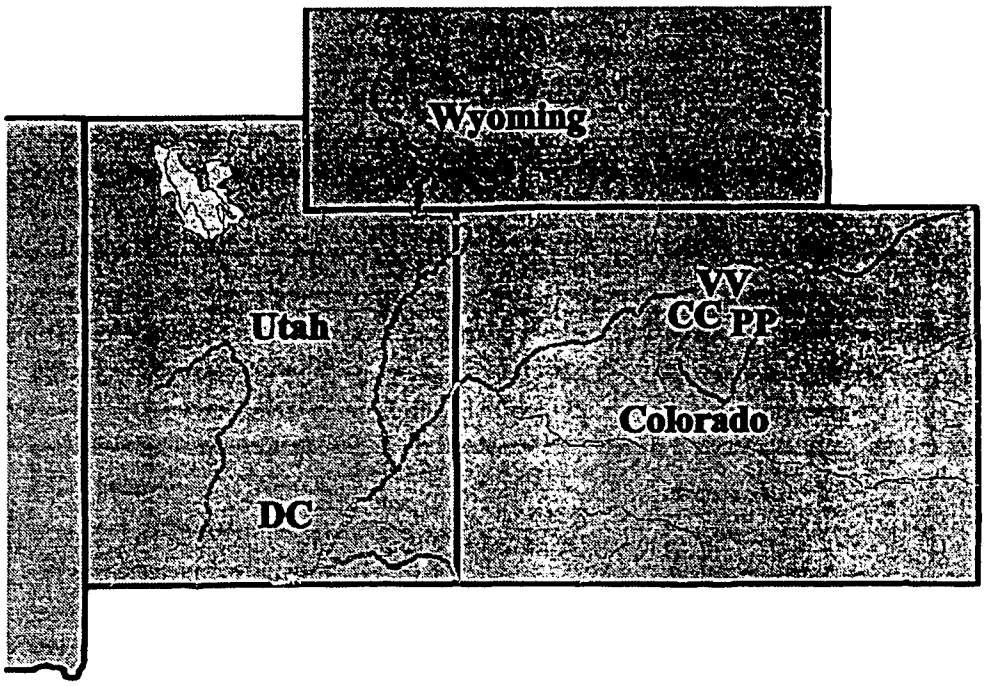
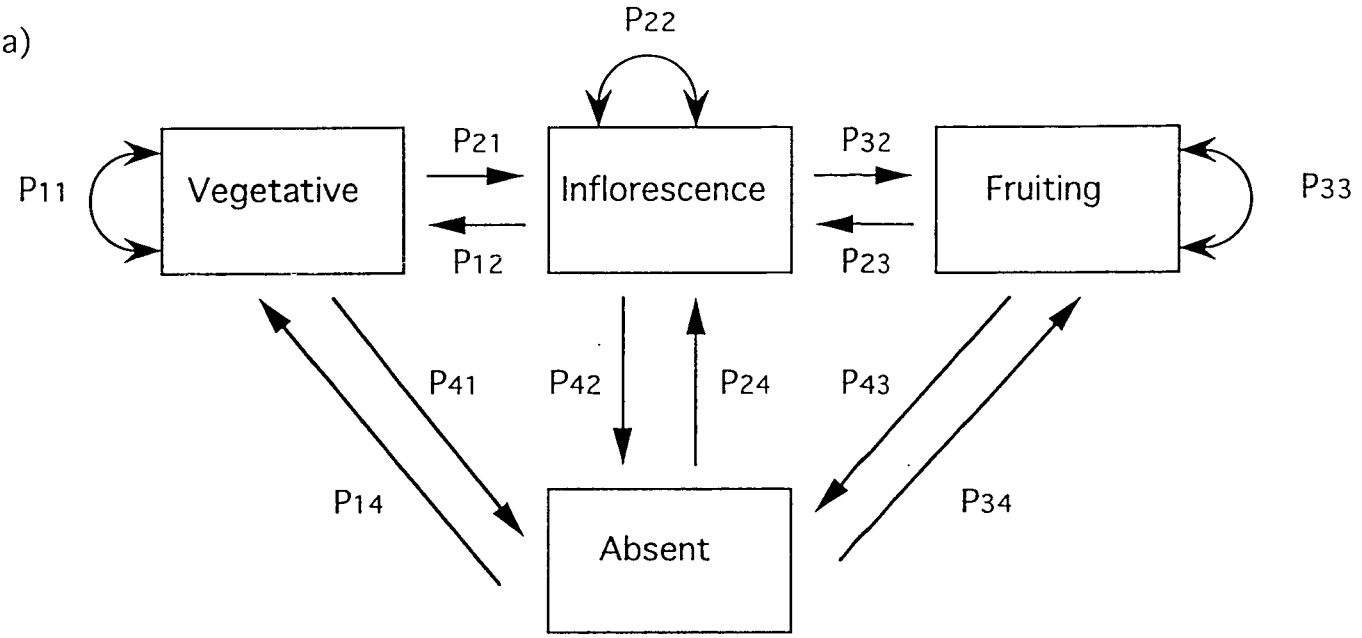


Figure 45: (a) Transition model for populations of Spiranthes diluvialis. (b) Corresponding projection matrix for the life cycle shown in (a). Abbreviation are: reproductive value (R); proportion of individuals vegetative (X1), producing an inflorrescence and not setting fruit (X2), producing an inflorescence and setting fruit (X3), and absent or with no above-ground foliage (X4).

(a)



(b)

$$\begin{bmatrix} P11 & P12 & P13+vR & P14 \\ P21 & P22 & P23+iR & P24 \\ P31 & P32 & P33+fR & P34 \\ P41 & P42 & P43+aR & 0 \end{bmatrix} \qquad \begin{bmatrix} X1 \\ X2 \\ X3 \\ X4 \end{bmatrix}$$

Table 1: Summary of the species of Spiranthes sampled, their location, and the number of individuals collected from each population. Species abbreviations are: SC, cernua; SDe, delitescens; SD, diluvialis; SL, lucida; SM, magnicamporum; SOc, ochroleuca; SOd, odorata; SP, porrifolia SR, romanzoffiana; and SV, vernalis.

Spiranthes species	Location (County & State)	Sample size
SC	Galveston, Texas	35
	Greene,New York	33
	Greene, New York	36
	Greene, New York	36
SDe	Santa Cruz, Arizona	24
	Santa Cruz, Arizona	32
	Santa Cruz, Arizona	46
SD	Utah, Utah	21
	Uintah, Utah	45
	Uintah, Utah	19
	Daggett, Utah	49
	Jefferson, Colorado	70
	Boulder, Colorado	53
	Garfield, Utah	74
	Utah, Utah	23
	Duchesne, Utah	73
	Utah, Utah	33
	Uintah, Utah	56
	Boulder, Colorado	135
SL	Chenango, New York	21
SM	Sante Fe, New Mexico	35
	Howell, Missouri	27
SOc	Alleghany, Pennsylvania	36
	Hamilton, New York	27
	Hamilton, New York	43
SOd	Dade, Florida	33
SP	Humboldt, California	23
	Humboldt, California	28
SR	Boulder, Colorado	38
	Boulder, Colorado	26
	Clear Creek, Colorado	26
	Park, Colorado	49
	Duchesne, Utah	32
SV	Galveston, Texas	38
	Madison, Missouri	25

Table 2: Allele frequencies for nine species of Spiranthes. Species abbreviations as in Table 1.

	SL	SP	SR	SD	SDe	SM	SC	SOc	SOd	SV
Adh										
1	-	0.506	0.586	0.386	0.505	0.100	0.063	-	-	-
2	-	0.152	0.042	0.548	0.333	0.950	0.141	-	-	0.909
3	1.000	0.014	-	0.330	0.159	-	0.359	0.705	1.000	0.091
4	-	0.328	0.302	-	-	-	-	-	-	-
5	-	0.007	0.012	0.062	-	-	0.391	0.295	-	-
6	-	0.014	0.072	0.139	0.004	-	-	-	-	-
7	-	-	0.006	-	-	-	-	-	-	-
9	-	-	-	-	-	-	0.055	-	-	-
Dia										
1	*	-	-	0.124	-	0.078	0.189	0.537	-	-
2	*	0.724	0.942	0.875	0.722	0.860	0.617	0.463	1.000	0.605
3	*	0.171	0.009	0.182	-	0.063	-	-	-	0.395
4	*	0.106	0.034	-	0.278	-	0.068	-	-	-
5	*	-	0.010	0.017	-	-	-	-	-	-
6	*	-	0.004	0.057	-	-	0.102	-	-	-
7	*	-	-	-	-	-	0.023	-	-	-
Est-1										
1	1.000	0.420	0.445	0.492	-	-	-	0.447	0.500	-
2	-	0.420	0.445	0.492	-	-	-	0.447	0.500	-
3	-	0.040	0.110	0.047	1.000	-	1.000	-	-	1.000
4	-	0.020	-	-	-	-	-	0.079	-	-
5	-	0.109	-	0.053	-	-	-	-	-	-
6	-	-	-	-	-	1.000	-	-	-	-
Est-2										
1	-	0.200	0.152	0.615	0.045	0.512	0.562	-	-	-
2	-	0.200	0.579	0.432	0.487	0.250	0.195	-	-	-
3	-	0.367	0.237	0.040	0.464	-	-	-	-	-
4	-	-	0.005	-	-	-	-	-	-	-
5	1.000	-	0.027	-	0.004	0.239	0.181	0.449	-	-
6	-	0.234	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	0.062	0.058	-	-
8	-	-	-	-	-	-	-	0.493	-	-
9	-	-	-	-	-	-	-	-	1.000	1
Fe-1										
1	0.860	0.046	0.130	0.409	0.025	0.401	0.408	0.265	1.000	0.5
2	-	0.454	0.601	0.617	0.606	0.340	0.156	0.010	-	-
3	-	0.454	0.112	0.039	0.365	-	-	-	-	-
5	-	0.004	0.002	0.007	-	-	0.144	-	-	-
6	-	0.004	0.149	0.007	-	-	0.120	-	-	-
7	0.140	-	-	-	-	-	0.168	0.607	-	0.5
8	-	0.046	-	-	0.004	-	-	-	-	-
9	-	-	-	-	-	0.260	-	0.112	-	-
Lap-1										
1	*	-	0.030	0.502	0.318	0.914	0.138	-	0.500	0.654
2	*	1.000	0.927	0.473	-	0.077	-	-	0.500	-
3	*	-	-	0.086	0.205	0.010	0.681	0.133	-	0.295
4	*	-	0.028	0.073	0.394	-	-	-	-	-
5	*	-	0.015	-	-	-	-	-	-	0.051
6	*	-	-	-	-	-	0.181	0.867	-	-
7	*	-	0.027	-	-	-	-	-	-	-

Table 2: continued from previous page.

	SL	SP	SR	SD	SDe	SM	SC	SOc	SOd	SV
Mdh-1										-
0	-	-	1.000	0.019	-	-	-	0.141	-	-
1	1.000	-	-	0.996	-	1.000	0.760	0.008	-	-
2	-	-	-	0.008	0.667	-	-	0.508	0.500	0.824
3	-	0.046	-	-	0.275	-	0.240	0.109	0.500	-
4	-	0.091	-	-	0.059	-	-	0.234	-	-
6	-	0.864	-	-	-	-	-	-	-	0.176
Mdh-2										
1	1.000	0.577	0.998	1.000	-	-	-	-	-	-
2	-	0.409	0.002	-	1.000	-	-	-	-	-
3	-	0.014	-	-	-	-	-	-	-	-
6	-	-	-	-	-	1.000	1.000	1.000	1.000	1
Mdh-3										
1	1.000	0.322	0.998	1.000	-	-	-	-	-	-
2	-	0.679	-	-	1.000	-	-	-	-	0.179
3	-	-	0.002	-	-	-	-	-	-	-
6	-	-	-	-	-	1.000	1.000	1.000	1.000	0.821
Mdh-4										
1	1.000	0.305	0.713	1.000	0.291	1.000	1.000	1.000	-	0.192
2	-	0.294	0.105	-	0.709	-	-	-	-	0.708
3	-	0.329	0.095	-	-	-	-	-	-	0.051
4	-	0.074	0.035	-	-	-	-	-	-	0.051
5	-	-	0.073	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	1.000	-
Mnr										
1	1.000	1.000	1.000	0.501	0.500	0.500	-	-	-	-
2	-	-	-	0.493	0.500	0.500	0.788	0.806	1.000	1
4	-	-	-	0.077	-	-	-	0.194	-	-
5	-	-	-	-	-	-	0.212	-	-	-
Pgi										
1	-	-	0.153	0.626	0.500	0.750	0.101	0.944	-	1
2	-	0.500	0.348	0.374	0.333	0.250	-	-	-	-
3	0.950	-	-	-	-	-	-	-	1.000	-
4	-	-	0.287	0.009	0.167	-	0.848	-	-	-
5	0.050	0.500	0.217	-	-	-	-	-	-	-
6	-	-	0.007	-	-	-	-	-	-	-
7	-	-	-	-	-	-	0.051	0.056	-	-
Tpi-1										
1	-	1.000	0.752	0.997	1.000	0.965	-	0.974	1.000	-
2	-	-	0.191	0.017	-	0.036	-	-	-	-
3	-	-	0.048	-	-	-	-	-	-	-
4	-	-	0.009	-	-	-	-	-	-	-
5	-	-	-	0.014	-	-	-	0.026	-	-
7	1.000	-	-	-	-	-	-	-	-	-
Tpi-2										
1	-	-	0.063	0.514	-	0.320	0.826	0.806	0.500	0.05
2	-	1.000	0.871	0.486	0.951	0.070	-	0.194	0.500	0.95
3	-	-	0.003	-	0.049	-	0.070	-	-	-
4	-	-	0.058	-	-	-	-	-	-	-
5	-	-	0.005	-	-	-	-	-	-	-
6	-	-	-	-	-	0.500	0.105	-	-	-
7	1.000	-	-	-	-	-	-	-	-	-

Table 3: Summary of genetic variability in ten species of Spiranthes: total number of alleles per taxon (At), mean number of alleles per locus (A), mean number of alleles per polymorphic locus (Ap), percentage of loci polymorphic using the 99% criterion (P), expected heterozygosity (He), observed heterozygosity (Ho), and the mean sample size per locus per population (N). Species abbreviations as in Table 1.

	At	Ap	P	He	Ho	N
SC	36.0	2.7	71.4	0.350	0.286	109.7
SDe	27.7	2.0	61.9	0.302	0.398	23.9
SD	25.0	1.8	63.1	0.282	0.460	54.0
SL	14.0	1.1	16.7	0.030	0.007	11.0
SM	19.5	1.4	39.3	0.149	0.179	20.7
SOc	32.0	2.3	78.6	0.307	0.231	71.4
SOd	18.0	1.3	28.6	0.146	0.250	23.2
SP	32.5	2.3	60.7	0.307	0.347	15.5
SR	33.0	2.4	62.9	0.273	0.237	23.6
SV	24.0	1.8	57.1	0.199	0.260	28.4

Table 4: Alleles present at 14 locl in nine species of Spiranthes. Abbreviations as in Table 1.

Taxa	SL	SP	SR	SD	SDe	SM	SC	SOc	SOd	SV
Chromosome#	44	44	44	74	74	30	30/60	30	30	30
Adh										
1		X	X	X	X	X	X			
2		X	X	X	X	X	X			X
3	X	X		X	X		X	X	X	X
4		X	X							
5		X	X	X			X	X		
6		X	X	X	X					
7			X							
9							X			
Dia										
1				X		X	X	X		
2		X	X	X	X	X	X	X	X	X
3		X	X	X		X				X
4		X	X		X		X			
5			X	X						
6			X	X			X			
7							X			
Est-1										
1	X	X	X	X				X	X	
2		X	X	X				X	X	
3		X	X	X	X		X			X
4		X						X		
5		X		X						
6						X				
Est-2										
1		X	X	X	X	X	X			
2		X	X	X	X	X	X			
3		X	X	X	X					
4			X							
5	X		X		X	X	X	X		
6		X								
7							X	X		
8								X		
9									X	X
Fe-1										
1	X	X	X	X	X	X	X	X	X	X
2		X	X	X	X	X	X	X		
3		X	X	X	X					
5		X	X	X			X			
6		X	X	X			X			
7	X						X	X		X
8		X			X					
9						X		X		
Lap-1										
1			X	X	X	X	X		X	X
2		X	X	X		X			X	
3				X	X	X	X	X		X
4			X	X	X					
5			X							X
6							X	X		
7			X							

Table 4: continued from previous page.

Taxa	SL	SP	SR	SD	SDe	SM	SC	SOc	SOd	SV
Chromosome#	44	44	44	74	74	30	30/60	30	30	30
Mdh-1										
Missing			X	X				X		
1	X			X		X	X	X		
2				X	X			X	X	X
3		X			X		X	X	X	
4		X			X			X		
6		X								X
Mdh-2										
Missing						X	X	X	X	X
1	X	X	X	X						
2		X	X		X					
3		X								
Mdh-3										
Missing						X	X	X	X	X
1	X	X	X	X						
2		X			X					X
3			X							
Mdh-4										
1	X	X	X	X	X	X	X	X		X
2		X	X		X					X
3		X	X							X
4		X	X							X
5			X							
6									X	
Mnr										
1	X	X	X	X	X	X				
2				X	X	X	X	X	X	X
4				X				X		
5							X			
Pgi										
1			X	X	X	X	X	X		X
2		X	X	X	X	X				
3	X								X	
4			X	X	X		X			
5	X	X	X							
6			X							
7							X	X		
Tpi-1										
Missing							X	X		X
1		X	X	X	X	X			X	
2			X	X		X				
3			X							
4			X							
5				X				X		
7	X									
Tpi-2										
1			X	X		X	X	X	X	X
2		X	X	X	X	X		X	X	X
3			X		X		X			
4			X							
5			X							
6						X	X			
7	X									

Table 5: Mean genetic identities (Nei, 1978) between pairs of populations within species (diagonal) and between pairs of species within Spiranthes (off-diagonal; top panel). A summary of the average genetic identities (I) within and among the two chromosomal series (bottom panel). Abbreviations as in Table 1.

Population	SD	SDe	SL	SP	SR	SC	SM	SOc	SOd	SV
SD	0.962									
SDe	0.455	0.900								
SL	0.530	0.097	***							
SP	0.576	0.580	0.301	0.756						
SR	0.727	0.453	0.434	0.795	0.928					
SC	0.577	0.314	0.305	0.265	0.335	0.901				
SM	0.619	0.362	0.261	0.300	0.334	0.664	0.781			
SOc	0.467	0.305	0.255	0.231	0.273	0.782	0.571	0.935		
SOd	0.387	0.325	0.301	0.300	0.290	0.601	0.453	0.596	***	
SV	0.413	0.595	0.066	0.308	0.271	0.500	0.563	0.556	0.627	0.920

*** only one population sampled

Series	I	Range
30-30	0.591	0.453,0.782
30-44	0.273	0.066,0.335
44-44	0.510	0.301,0.795

Table 6: Populations of Spiranthes diluvialis sampled for isozyme analysis including the location, basin, watershed, population size, and the number of individuals sampled from each population.

Population	Location (County & State)	Basin	Watershed	Sample size
American Fork	Utah, Utah	Great Basin	Utah Lake	21
Ashley Creek	Uintah, Utah	Uintah	Uintah	45
Big Brush Creek	Uintah, Utah	Uintah	Uintah	19
Brown's Park	Daggett, Utah	Uintah	Mainstem Green River	49
Clear Creek	Jefferson, Colorado	Platte	Clear Creek	70
Cherryvale	Boulder, Colorado	Platte	Boulder Crk/St. Vrain	53
Deer Creek	Garfield, Utah	Colorado R.	Escalante	74
Diamond Fork	Utah, Utah	Great Basin	Utah Lake	23
Duchesne River	Duchesne, Utah	Uintah	Uintah	73
Powell Slough	Utah, Utah	Great Basin	Utah Lake	33
Uintah River	Uintah, Utah	Uintah	Uintah	56
Van Vleet Ranch	Boulder, Colorado	Platte	Boulder Crk/St. Vrain	135

Table 7: Allele frequencies for twelve populations of Spiranthes diluvialis. Population abbreviations: VV = Van Vleet, AF = American Fork, ASH = Ashley , BBC = Big Brush Creek, BP = Brown's Park, CC = Clear Creek, CV = Cherryvale, DC = Deer Creek, DF = Diamond Fork, DR = Duchesne River, PS = Powell Slough, and UR = Uintah River. An asterisk (*) indicates a population unique allele.

Locus/ Allele	Allele frequency											
Adh	VV	AF	ASH	BBC	BP	CC	CV	DC	DF	DR	PS	UR
1	0.478	0.337	0.480	0.500	0.170	0.511	0.459	0.085	0.321	0.460	0.270	0.558
2	0.522	0.663	0.520	0.500	0.680	0.489	0.541	0.320	0.679	0.519	0.716	0.429
3	-	-	-	-	-	-	-	0.330	-	-	-	-
5	-	-	-	-	0.150	-	-	-	-	0.022	0.014	-
6	-	-	-	-	-	-	-	0.265	-	-	-	0.014
Dia												
1	0.034	0.368	-	-	-	0.012	-	-	-	-	0.189	0.017
2	0.929	0.526	0.984	1.000	1.000	0.959	1.000	0.828	0.500	1.000	0.811	0.966
3	0.037	-	-	-	-	0.029	-	0.163	0.500	-	-	-
5	-	-	0.016	-	-	-	-	-	-	-	-	0.017
6	-	0.105	-	-	-	-	-	0.010	-	-	-	-
Est-1												
1	0.469	0.500	0.500	0.500	0.500	0.500	0.500	0.475	0.500	0.474	0.485	0.500
2	0.469	0.500	0.500	0.500	0.500	0.500	0.500	0.475	0.500	0.474	0.485	0.500
3	0.063	-	-	-	-	-	-	0.050	-	-	0.030	-
5	-	-	-	-	-	-	-	-	-	0.053	-	-
Est-2												
1	0.858	0.048	0.598	0.614	0.795	-	0.458	0.988	0.643	0.659	0.328	0.781
2	0.142	0.952	0.402	0.386	0.205	1.000	0.542	0.012	0.357	0.301	0.672	0.219
3	-	-	-	-	-	-	-	-	-	0.040	-	-
Fe-1												
1	0.543	0.109	0.505	0.576	0.448	0.444	0.173	0.373	0.477	0.430	-	0.426
2	0.457	0.891	0.495	0.424	0.552	0.556	0.760	0.627	0.523	0.560	1.000	0.560
3	-	-	-	-	-	-	0.067	-	-	0.010	-	-
5	-	-	-	-	-	-	-	-	-	-	-	0.007
6	-	-	-	-	-	-	-	-	-	-	-	0.007
Lap-1												
1	0.504	0.475	0.488	0.500	0.583	0.500	0.529	0.500	0.500	0.500	0.441	0.500
2	0.493	0.525	0.512	0.500	0.417	0.500	0.286	0.500	0.500	0.385	0.559	0.500
3	-	-	-	-	-	-	-	-	-	0.086	-	-
4	0.004	-	-	-	-	-	0.186	-	-	0.029	-	-
Mdh-1												
1	0.992	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.989	1.000	0.973
3	0.008	-	-	-	-	-	-	-	-	-	-	-
6	0.008	-	-	-	-	-	-	-	-	0.011	-	0.027
Mdh-2												
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-3												
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-4												
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mnr												
1	0.508	0.500	0.500	0.462	0.500	0.500	0.500	0.500	-	0.500	0.500	0.538
2	0.492	0.500	0.500	0.462	0.500	0.500	0.500	0.500	-	0.500	0.500	0.463
4	-	-	-	0.077	-	-	-	-	-	-	-	-
Pgi												
1	0.791	0.641	0.537	0.533	0.511	0.500	0.500	0.849	0.632	0.661	0.826	0.527
2	0.209	0.359	0.463	0.467	0.489	0.500	0.500	0.142	0.368	0.339	0.174	0.473
4	-	-	-	-	-	-	-	0.009	-	-	-	-
Tpi-1												
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.969	-	1.000	1.000	1.000
2	-	-	-	-	-	-	-	0.017	-	-	-	-
5	-	-	-	-	-	-	-	0.014	-	-	-	-
Tpi-2												
1	0.500	0.500	0.500	0.500	0.549	0.500	0.492	0.631	-	0.500	0.500	0.500
2	0.500	0.500	0.500	0.500	0.451	0.500	0.508	0.369	-	0.500	0.500	0.500

Table 8: Summary of genetic variability in twelve populations of Spiranthes diluvialis: total number of alleles per population (At), mean number of alleles per locus (A), mean number of alleles per polymorphic locus (Ap), percentage of loci polymorphic using the 99% criterion (P), and the mean sample size per locus (N).

	At	A	Ap	P	N
American Fork	23	1.7	2.6	64.3	20.6
Ashley Creek	23	1.6	2.6	64.3	45.3
Big Brush Creek	23	1.6	2.8	57.1	20.0
Brown's Park	23	1.6	2.8	57.1	48.9
Clear Creek	23	1.6	2.8	57.1	69.9
Cherryvale	24	1.7	3.0	57.1	52.6
Deer Creek	30	2.1	3.3	71.4	73.9
Diamond Fork	23	1.6	2.6	64.3	21.1
Duchesne	29	2.1	3.0	64.3	72.7
Powell Slough	24	1.7	3.0	57.1	33.0
Uintah River	28	2.0	3.0	71.4	55.6
Van Vleet	27	1.9	2.9	71.4	134.9
AVERAGE	25	1.8	2.9	63.1	54.0

Table 9: Summary of alleles present at polymorphic loci in twelve populations of Spiranthes diluvialis. Each number (1-6) represents a different allele.

	Adh	Dia	Est-1	Est-2	Fe-1	Lap-1	Mdh-1	Pgi	Tpi-1
American Fork	1,2	1,2,6	1,2	1,2	1,2	1,2	1	1,2	1
Ashley Creek	1,2	2,5	1,2	1,2	1,2	1,2	1	1,2	1
Big Brush Creek	1,2	2	1,2	1,2	1,2	1,2	1	1,2	1
Brown's Park	1,2,5	2	1,2	1,2	1,2	1,2	1	1,2	1
Clear Creek	1,2	1,2,3	1,2	2	1,2	1,2	1	1,2	1
Cherryvale	1,2	2	1,2	1,2	1,2,3	1,2,4	1	1,2	1
Deer Creek	1,2,3,5	2,3,6	1,2,3	1,2	1,2	1,2	1	1,2,4	1,2,5
Diamond Fork	1,2	2,3	1,2	1,2	1,2	1,2	1	1,2	1
Duchesne	1,2,5	2	1,2,5	1,2,3	1,2,3	1,2,3,4	1,6	1,2	1
Powell Slough	1,2,5	1,2	1,2,3	1,2	2	1,2	1	1,2	1
Uintah River	1,2,6	1,2,5	1,2	1,2	1,2,5,6	1,2	1,6	1,2	1
Van Vleet	1,2	1,2,3	1,2,3	1,2	1,2	1,2,4	1,3	1,2	1

Table 10: Observed heterozygosity across populations and loci. Abrreviations as in Table 7 and as follows: A, average observed heterozygosity across all loci; AP, average observed heterozygosity across polymorphic loci; He, average expected heterozygosity for under Hardy-Weinberg equilibrium for the observed allele frequencies.

	VV	AF	ASH	BBC	BP	CC	CV	DC	DF	DR	PS	UR
Adh	0.838	0.826	1.000	1.000	0.560	0.947	0.892	0.460	0.714	0.975	0.730	0.892
Dia	0.031	0.105	0.032	-	-	0.058	-	0.388	1.000	-	-	-
Est-1	0.672	0.722	1.000	0.077	1.000	1.000	0.870	0.542	0.857	0.931	0.903	0.868
Est-2	0.210	0.095	0.630	0.182	0.227	-	0.667	0.023	0.762	0.596	0.655	0.313
Fe-1	0.358	0.217	0.830	0.783	0.758	0.804	0.519	0.479	0.864	0.773	-	0.662
Lap	0.986	1.000	1.000	1.000	0.813	1.000	0.614	0.950	1.000	1.000	1.000	1.000
Mdh-1	0.016	-	-	-	-	-	-	-	-	0.011	-	0.027
Mdh-2	-	-	-	-	-	-	-	-	-	-	-	-
Mdh-3	-	-	-	-	-	-	-	-	-	-	-	-
Mdh-4	-	-	-	-	-	-	-	-	-	-	-	-
Mnr	0.984	1.000	1.000	0.923	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.975
Pgi	0.528	0.813	1.000	0.933	1.000	1.000	1.000	0.362	0.947	0.797	0.424	0.946
Tpi-1	-	-	-	-	-	-	-	0.091	-	-	-	-
Tpi-2	1.000	1.000	1.000	1.000	1.000	1.000	0.983	0.811	1.000	1.000	1.000	1.000
Average	0.402	0.413	0.535	0.421	0.454	0.486	0.467	0.365	0.582	0.506	0.408	0.477
Expected H	0.271	0.276	0.290	0.297	0.274	0.258	0.288	0.275	0.318	0.295	0.252	0.286

Table 11: Mean genetic identities (Nei, 1978) between pairs of twelve populations of Spiranthes diluvialis. Population averages are calculated from all the pairwise comparisons for a particular population. Abbreviations as in Table 7.

Population	AF	A	BBC	BP	CC	CV	DC	DF	DR	PS	LR	VV
American Fork	***											
Ashley	0.941	***										
Brush Creek	0.936	1.000	***									
Brown's Park	0.916	0.993	0.995	***								
Clear Creek	0.974	0.968	0.969	0.933	***							
Cherryvale	0.963	0.989	0.933	0.978	0.973	***						
Deer Creek	0.876	0.954	0.956	0.967	0.873	0.934	***					
Diamond Fork	0.941	0.979	0.98	0.976	0.939	0.963	0.958	***				
Duchesne	0.934	1.000	1.000	0.993	0.954	0.988	0.966	0.977	***			
Powell Slough	0.99	0.958	0.952	0.948	0.956	0.976	0.926	0.953	0.962	***		
Uintah River	0.921	1.000	1.000	0.956	0.943	0.984	0.964	0.974	0.998	0.947	***	
Van Vleet	0.905	0.989	0.994	0.986	0.922	0.963	0.976	0.976	0.995	0.942	0.993	***
Average	0.936	0.979	0.974	0.967	0.946	0.968	0.941	0.965	0.979	0.955	0.971	0.967

Table 12: Pairwise comparisons of populations of Spiranthes diluvialis for Fst and Nm. Population abbreviations as in Table 7.

Colorado-Colorado		
Populations	Fst	Nm
VV-CC	0.100	1.13
VV-CV	0.046	2.59
CC-CV	0.037	3.25
Average	0.061	2.32

Utah-Utah		
populations	Fst	Nm
AF-AS	0.072	1.61
AF-BC	0.000	Infinity
AF-BP	0.100	1.13
AF-DC	0.140	0.77
AF-DF	0.073	1.59
AF-DR	0.079	1.46
AF-PS	0.018	6.82
AF-UR	0.093	1.22
AS-BC	0.000	infinity
AS-BP	0.010	12.38
AS-DC	0.055	2.15
AS-DF	0.028	4.34
AS-DR	0.002	62.38
AS-PS	0.056	2.11
AS-UR	0.003	41.54
BC-BP	0.008	15.50
BC-DC	0.051	2.33
BC-DF	0.028	4.34
BC-DR	0.001	124.88
BC-PS	0.062	1.89
BC-UR	0.002	62.38
BP-DC	0.040	3.00
BP-DF	0.030	4.04
BP-DR	0.009	13.76
BP-PS	0.068	1.71
BP-UR	0.012	10.29
DC-DF	0.047	2.53
DC-DR	0.041	2.92
DC-PS	0.095	1.19
DC-UR	0.043	2.78
DF-DR	0.029	4.19
DF-PS	0.059	1.99
DF-UR	0.031	3.91
DR-PS	0.049	2.43
DR-UR	0.002	62.38
PS-UR	0.069	1.69
Average	0.042	13.81

Colorado-Utah		
Populations	Fst	Nm
VV-AF	0.113	0.98
VV-AS	0.016	7.69
VV-BC	0.013	9.49
VV-BP	0.017	7.23
VV-DC	0.030	4.04
VV-DF	0.030	4.04
VV-DR	0.007	17.73
VV-PS	0.078	1.48
VV-UR	0.009	13.76
AF-CC	0.036	3.35
AF-CV	0.046	2.59
AS-CC	0.044	2.72
AS-CV	0.015	8.21
BC-CC	0.046	2.59
BC-CV	0.019	6.45
BP-CC	0.086	1.33
BP-CV	0.027	4.50
CC-DC	0.149	0.71
CC-DF	0.074	1.56
CC-DR	0.06	1.96
CC-PS	0.062	1.89
CC-UR	0.074	1.56
CV-DC	0.077	1.50
CV-DF	0.040	3.00
CV-DR	0.015	8.21
CV-PS	0.032	3.78
CV-UR	0.021	5.83
Average	0.046	4.75

Table 13: List of species found within demographic plots of S. diluvialis at Van Vleet. Native species are indicated by an asterisk (*). Voucher specimens have been placed in the University of Colorado Herbarium.

Species	Common name
Fern Allies	
Equisetum arvense*	field horsetail
Hippochaete laevigata*	scouring rush
Forbs	
Aster hesperius*	aster
Cirsium arvense	Canada thistle
Dianthus armeria	pink
Limnorchis hyperborea*	northern bog-orchid
Lobelia siphilitica*	great lobelia
Lotus tenuis	birdfoot trefoil
Lycopus americanus*	horehound
Plantago eriopoda*	redwool plantain
Plantago lanceolata*	english plantain
Prunella vulgaris *	heal-all
Ranunculus sp.	buttercup
Sisyrinchium montanum*	blue-eyed-grass
Trifolium pratense	clover
Trifolium repens	clover
Verbena hastata*	blue vervain
Graminoids	
Agropyron smithii*	western wheat grass
Agrostis gigantea	red-top
Andropogon gerardii*	big bluestem
Carex nebraskensis*	sedge
Carex aurea*	sedge
Carex douglasii*	sedge
Carex emoryi*	sedge
Carex lanuginosa*	sedge
Carex scoparia*	sedge
Carex vulpinoidea*	sedge
Eloeocharis acicularis*	spike rush
Eleocharis elliptica*	spike rush
Eleocharis palustris	spike rush
Festuca arundinacea	fescue
Festuca pratensis*	meadow fescue
Juncus articus*	rush
Juncus confusus*	Colorado rush
Juncus dudleyi*	rush
Juncus longistylis*	rush
Juncus torreyi*	rush
Panicum virgatum*	switchgrass
Poa pratensis	Kentucky blue grass
Triglochin maritimum*	arrow-grass

Table 14: Correlations between plant variables and environmental variables. Direction of correlation, if significant, is indicated as positive (P) or negative (N). Plant variables include growth, phenology (phen), absence (abx), vegetative (vegx), produced an inflorescence (inx), set fruit (frx), plant damage due to vole herbivory (vole) or mowing (mow), and rosette formation (rosette). Environmental variables include gravimetric soilmoisture (G. moisture), microsite soil moisture (TDR moisture), percent bare cover, light, soil conductivity, soil pH, lime content, and soil concentrations of phosphorus, iron, ammonium, and nitrate. See text for explanation.

	growth	phen	abx	vegx	inx	frx	vole	mow	rosette
G. moisture	N								
TDR moisture	P					P			
% bare cover	N	N		P	N		P		
light	N							P	
soil conductivity						P			
soil pH	N	N		P	N		P		N
lime content	N	N	P	P	N				N
Phosphorus	P								P
Iron	P	P		N	P		N		P
Ammonium		N		P	N				
Nitrate	P								

Table 15: Summary of the canonical variables with the first two canonical axes and percentage of correct a posteriori classification (%) for the treatments at Van Vleet and the three riparian sites. Abbreviations as in Table 14 with the addition of percent bare cover (bcov), soil pH (pH), soil conductivity (conduc), soil iron concentration (fe),ammonium (a), percent organic material (om), copper (cu), nitrate (nn), potassium (k), phosphorous (p), and zinc (zn). Numbers after the predictors indicate the month and/or year the variable was measured.

Van Vleet Ranch							
Phenology				Environmental			
Predictor	Canonical Variables 1	2	%	Predictor	Canonical Variables 1	2	%
phen	5.0181	1.7823	62	bcov794	29.6594	0.4175	93
abx	2.9879	1.2366		pH694	12.2972	1.6721	
vegx	2.4389	1.6756		conduc694	10.4757	11.1455	
frx	-1.2535	0.3125		bcov894	-6.5158	-3.5069	
				bcov693	0.0711	-6.6587	
All Sites							
Phenology				Environmental			
Predictor	Canonical Variables 1	2	%	Predictor	Canonical Variables 1	2	%
frx94	0.8408	-0.5922	55	pH694	-5.1977	0.2577	100
inx94	0.3797	0.9557		fe694	-4.1968	-0.1210	
				a694	3.9487	0.7782	
				om694	3.5468	0.0351	
				cu694	2.3953	2.3953	
				nn694	2.2346	2.7964	
				k694	-1.8329	-0.4546	
				cond694	1.3074	2.4021	
				p694	-0.8079	0.7758	
				zn694	0.4999	0.7259	

Table 16: Transition probabilities at Van Vleet for eight different management treatments for 1992-1993 and 1993-1994 and at the three riparian sites for 1993-1994. Abbreviations for Van Vleet are GM, grazed and mown; G, grazed only; N, control with no external treatment; EC, early clip; LC, late clip; CT, clip twice; EB,early burn; and LB, late burn. Riparian areas are DC, Deer Creek; CC, Clear Creek; and PP, Prospect Park.Stages are vegetative, V; produced an inflorescence but did not set fruit, I; produced an inflorescence and set fruit, F; and absent or did not produce above-ground foliage, A.

1992-1993

		from GM			
		V	I	F	A
to	V	0.169	0.127	0.143	0.097
	I	0.761	0.873	0.857	0.161
	F	0.000	0.000	0.000	0.000
	A	0.070	0.000	0.000	0.742

		from NT			
		V	I	F	A
to	V	0.644	0.568	0.438	0.390
	I	0.327	0.405	0.531	0.000
	F	0.000	0.000	0.000	0.000
	A	0.029	0.027	0.031	0.610

		from EC			
		V	I	F	A
to	V	0.709	0.367	0.545	0.736
	I	0.266	0.617	0.455	0.208
	F	0.000	0.000	0.000	0.000
	A	0.025	0.017	0.000	0.057

		from LC			
		V	I	F	A
to	V	0.571	0.219	0.000	0.531
	I	0.418	0.781	1.000	0.204
	F	0.000	0.000	0.000	0.000
	A	0.010	0.000	0.000	0.265

		from CT			
		V	I	F	A
to	V	0.610	0.271	0.000	0.556
	I	0.390	0.729	1.000	0.139
	F	0.000	0.000	0.000	0.000
	A	0.000	0.000	0.000	0.306

		from EB			
		V	I	F	A
to	V	0.595	0.340	0.430	0.425
	I	0.311	0.660	0.571	0.250
	F	0.000	0.000	0.000	0.000
	A	0.095	0.000	0.000	0.325

		from LB			
		V	I	F	A
to	V	0.377	0.250	0.267	0.540
	I	0.594	0.708	0.733	0.400
	F	0.000	0.000	0.000	0.020
	A	0.028	0.042	0.000	0.040

1993-1994

		from GM			
		V	I	F	A
to	V	0.578	0.290	0.000	0.545
	I	0.311	0.683	0.000	0.318
	F	0.000	0.005	0.000	0.023
	A	0.111	0.022	0.000	0.114

		from G			
		V	I	F	A
to	V	0.583	0.299	0.333	0.878
	I	0.200	0.350	0.500	0.082
	F	0.083	0.254	0.167	0.041
	A	0.133	0.096	0.000	0.000

		from NT			
		V	I	F	A
to	V	0.769	0.848	0.000	0.500
	I	0.037	0.076	0.000	0.000
	F	0.000	0.000	0.000	0.000
	A	0.194	0.076	0.000	0.500

		from EC			
		V	I	F	A
to	V	0.698	0.623	0.000	0.355
	I	0.054	0.247	0.000	0.125
	F	0.000	0.000	0.000	0.063
	A	0.248	0.130	0.000	0.125

		from LC			
		V	I	F	A
to	V	0.865	0.740	0.000	0.750
	I	0.034	0.182	0.000	0.167
	F	0.000	0.000	0.000	0.000
	A	0.101	0.078	0.000	0.083

		from CT			
		V	I	F	A
to	V	0.863	0.549	0.000	1.000
	I	0.125	0.394	0.000	0.000
	F	0.000	0.000	0.000	0.000
	A	0.013	0.056	0.000	0.000

		from EB			
		V	I	F	A
to	V	0.726	0.662	0.000	0.222
	I	0.060	0.127	0.000	0.000
	F	0.000	0.000	0.000	0.000
	A	0.214	0.211	0.000	0.778

		from LB			
		V	I	F	A
to	V	0.827	0.811	1.000	0.455
	I	0.012	0.115	0.000	0.182
	F	0.000	0.000	0.000	0.000
	A	0.160	0.074	0.000	0.364

1993-1994

		from DC			
		V	I	F	A
to	V	0.467	0.269	0.333	0.833
	I	0.400	0.462	0.667	0.167
	F	0.033	0.077	0.000	0.000
	A	0.100	0.192	0.000	0.000

		from CC			
		V	I	F	A
to	V	0.120	0.043	0.192	0.414
	I	0.520	0.553	0.346	0.483
	F	0.320	0.362	0.308	0.103
	A	0.040	0.043	0.154	0.000

		from PP			
		V	I	F	A
to	V	0.535	0.216	0.250	0.839
	I	0.256	0.486	0.250	0.129
	F	0.070	0.189	0.250	0.000
	A	0.140	0.108	0.250	0.032

Table 17: Mortality rates under the eight different management regimes at Van Vleet and the three riparian sites. Abbreviations as in Table 16.

Site/Treatment	Vegetative	Inflorescence	Fruiting	Mean/Treatment
GM	8.4	1.0	0.0	3.1
G	7.4	5.4	0.0	4.3
N	10.3	4.8	1.5	5.5
EC	12.6	6.7	0.0	6.4
LC	5.1	3.6	0.0	2.9
CT	0.6	2.6	0.0	1.1
EB	14.2	9.7	0.0	8.0
LB	8.7	5.3	0.0	4.7
DC	9.2	17.7	0.0	9.0
CC	3.7	3.9	14.2	7.3
PP	12.8	9.9	0.2	7.6

Table 18: Reproductive values for each treatment at Van Vleet and the three riparian areas. Abbreviations as in Table 16.

92-93	GM	G	NT	EC	LC	CT	EB	LB
V	0.169	0.231	0.165	0.234	0.156	0.139	0.084	0.142
I	0.281	0.084	0.000	0.064	0.060	0.035	0.049	0.105
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005
A								
93-94	GM	G	NT	EC	LC	CT	EB	LB
V	0.288	0.394	0.165	0.234	0.176	0.174	0.133	0.180
I	0.153	0.046	0.000	0.043	0.039	0.000	0.000	0.072
F	0.009	0.018	0.000	0.021	0.000	0.000	0.000	0.000
A								
93-94	DC	CC	PP					
V	2.194	0.170	0.926					
I	4.387	0.306	0.926					
F	0.000	0.272	0.926					
A	0.000	0.136	0.926					

Table 19: Projection matrices at Van Vleet for eight different management treatments for 1992-1993 and 1993-1994 and at the three riparian sites. Abbreviations as in Table 16.

1992-1993

		from GM			
		V	I	F	A
to	V	0.165	0.124	0.308	0.095
	I	0.742	0.851	1.117	0.157
	F	0	0	0	0
	A	0.068	0	0	0.723

		NT			
		V	I	F	A
to	V	0.59	0.52	0.566	0.357
	I	0.3	0.371	0.486	0
	F	0	0	0	0
	A	0.027	0.025	0.028	0.559

		EC			
		V	I	F	A
to	V	0.638	0.33	0.725	0.662
	I	0.239	0.555	0.452	0.187
	F	0	0	0.021	0
	A	0.023	0.015	0	0.051

		LC			
		V	I	F	A
to	V	0.545	0.209	0.156	0.507
	I	0.399	0.746	1.015	0.195
	F	0	0	0	0
	A	0.01	0	0	0.253

		CT			
		V	I	F	A
to	V	0.519	0.23	0.139	0.473
	I	0.332	0.62	0.885	0.118
	F	0	0	0	0
	A	0	0	0	0.26

		EB			
		V	I	F	A
to	V	0.522	0.298	0.461	0.373
	I	0.273	0.579	0.55	0.219
	F	0	0	0	0
	A	0.083	0	0	0.285

		LB			
		V	I	F	A
to	V	0.354	0.235	0.393	0.508
	I	0.558	0.666	0.794	0.376
	F	0	0	0.005	0.019
	A	0.026	0.039	0	0.038

1993-1994

		from GM			
		V	I	F	A
to	V	0.564	0.283	0.169	0.531
	I	0.303	0.666	0.153	0.31
	F	0	0.005	0.009	0.022
	A	0.108	0.021	0	0.111

		G			
		V	I	F	A
to	V	0.558	0.286	0.713	0.84
	I	0.191	0.335	0.524	0.078
	F	0.079	0.243	0.178	0.039
	A	0.127	0.092	0	0

		NT			
		V	I	F	A
to	V	0.704	0.777	0.165	0.458
	I	0.034	0.07	0	0
	F	0	0	0	0
	A	0.178	0.07	0	0.458

		EC			
		V	I	F	A
to	V	0.628	0.561	0.234	0.32
	I	0.049	0.222	0.043	0.113
	F	0	0	0.021	0.057
	A	0.223	0.117	0	0.113

		LC			
		V	I	F	A
to	V	0.826	0.707	0.176	0.716
	I	0.032	0.174	0.039	0.159
	F	0	0	0	0
	A	0.096	0.074	0	0.079

		CT			
		V	I	F	A
to	V	0.85	0.541	0.174	0.985
	I	0.123	0.388	0	0
	F	0	0	0	0
	A	0.013	0.055	0	0

		EB			
		V	I	F	A
to	V	0.637	0.581	0.133	0.195
	I	0.053	0.111	0	0
	F	0	0	0	0
	A	0.188	0.185	0	0.682

		LB			
		V	I	F	A
to	V	0.777	0.762	1.12	0.428
	I	0.011	0.108	0.072	0.171
	F	0	0	0	0
	A	0.15	0.07	0	0.342

1993-1994

		from DC			DC
		V	I	F	A
to	V	0.427	0.246	2.498	0.762
	I	0.366	0.422	4.997	0.152
	F	0.03	0.07	0	0
	A	0.091	0.176	0	0

		CC			CC
		V	I	F	A
to	V	0.113	0.04	0.352	0.391
	I	0.491	0.523	0.633	0.456
	F	0.302	0.342	0.563	0.098
	A	0.038	0.04	0.281	0

		PP			PP
		V	I	F	A
to	V	0.479	0.194	1.15	0.751
	I	0.229	0.436	1.15	0.116
	F	0.063	0.17	1.15	0
	A	0.125	0.097	1.15	0.029

Table 20: Summary of eigenvalues (lambda) and their bootstrapped 95% confidence intervals for eight treatments at the Van Vleet site and three riparian habitats (top) and the relationship of eigenvalues among treatments and sites (bottom). Abbreviations as in Table 16.

	1992 - 1993		1993 - 1994	
	eigenvalue	confidence interval	eigenvalue	confidence interval
GM	0.980	0.979-0.990	0.957	0.931-0.969
G	*	*	1.006	0.975-1.099
NT	0.919	0.912-0.927	0.915	0.914-0.916
EC	0.902	0.892-0.912	0.894	0.854-0.913
LC	0.959	0.956-0.964	0.954	0.951-0.964
CT	0.982	0.978-0.987	0.992	0.988-0.993
EB	0.887	0.884-0.914	0.885	0.879-0.897
LB	0.939	0.932-0.952	0.926	0.923-0.933
DC	*	*	1.142	0.891-1.503
CC	*	*	1.180	1.647-2.115
PP	*	*	1.657	1.284-2.009
GM = CT > LC > LB > NT = EC = EB			G = CT > GM = LC = LB > NT > EC = EB CC = PP CC > GM, G, NT, EC, LC, CT, EB, LB, DC PP = CC, DC PP > GM, G, NT, EC, LC, CT, EB, LB DC = GM, G, NT, EC, LC, CT, EB, LB	

Table 21: The stage distribution of the population at equilibrium (right eigenvector) and the contribution of each stage to population growth (left eigenvector) for eight treatments at Van Vleet and three riparian areas, with lambda=1.00. The values shown are the proportion of the individuals in each stage class. Abbreviations as in Figure 16.

		1992-1993			1993-1994			1993-1994	
		RIGHT	LEFT		RIGHT	LEFT		RIGHT	LEFT
GM	V	0.135	0.212	V	0.428	0.272	V	0.635	0.126
	I	0.856	0.230	I	0.510	0.303	I	0.755	0.141
	F	0.000	0.334	F	0.005	0.131	F	0.058	0.976
	A	0.009	0.225	A	0.057	0.294	A	0.155	0.113
G	V	*	*	V	0.525	0.207	V	0.250	0.422
	I	*	*	I	0.259	0.237	I	0.785	0.431
	F	*	*	F	0.129	0.342	F	0.546	0.710
	A	*	*	A	0.088	0.214	A	0.153	0.365
NT	V	0.586	0.225	V	0.811	0.301	V	0.691	0.085
	I	0.322	0.240	I	0.031	0.316	I	0.527	0.155
	F	0.000	0.291	F	0.032	0.054	F	0.379	0.983
	A	0.093	0.245	A	0.157	0.329	A	0.351	0.051
EC	V	0.563	0.211	V	0.710	0.288			
	I	0.416	0.233	I	0.090	0.309			
	F	0.000	0.317	F	0.015	0.093			
	A	0.021	0.239	A	0.185	0.310			
LC	V	0.339	0.229	V	0.854	0.304			
	I	0.658	0.235	I	0.056	0.309			
	F	0.000	0.297	F	0.000	0.069			
	A	0.003	0.240	A	0.090	0.319			
CT	V	0.412	0.241	V	0.814	0.317			
	I	0.588	0.234	I	0.166	0.308			
	F	0.000	0.281	F	0.000	0.056			
	A	0.000	0.244	A	0.020	0.320			
EB	V	0.470	0.221	V	0.776	0.300			
	I	0.487	0.233	I	0.051	0.317			
	F	0.000	0.291	F	0.000	0.045			
	A	0.044	0.254	A	0.073	0.339			
LB	V	0.299	0.222	V	0.810	0.223			
	I	0.663	0.232	I	0.058	0.231			
	F	0.001	0.305	F	0.000	0.303			
	A	0.037	0.242	A	0.132	0.244			

Table 23: Elasticities for eight management regimes at Van Vleet and three riparian habitats. Abbreviations as in Table 16.

1992-1993						1993-1994					1993-1994				
		from					from					from			
	GM					GM					DC				
		V	I	F	A		V	I	F	A		V	I	F	A
	V	0.02	0.102	0	3E-04	V	0.223	0.144	0.001	0.035	V	0.114	0.071	0.068	0.055
to	I	0.097	0.763	0	0.006	I	0.134	0.379	8E-04	0.023	I	0.109	0.136	0.151	0.012
	F	0	0	0	0	F	0	0.001	2E-05	8E-04	F	0.063	0.156	0	0
	A	0.009	0	0	0	A	0.046	0.012	0	0	A	0.022	0.045	0	0
						G					OC				
							V	I	F	A		V	I	F	A
						V	0.275	0.071	0.091	0.075	V	0.012	0.013	0.07	0.024
to						I	0.108	0.095	0.079	0.008	I	0.052	0.172	0.129	0.029
						F	0.065	0.1	0.038	0.006	F	0.052	0.185	0.189	0.01
						A	0.065	0.024	0	0	A	0.003	0.011	0.049	0
	NT					NT					PP				
		V	I	F	A		V	I	F	A		V	I	F	A
	V	0.387	0.199	0	0.018	V	0.602	0.028	0	0.169	V	0.033	0.011	0.046	0.028
to	I	0.21	0.152	0	0.006	I	0.03	0.003	0	0	I	0.029	0.043	0.083	0.008
	F	0	0	0	0	F	0	0	0	0	F	0.05	0.106	0.529	0
	A	0.019	0.01	0	0	A	0.166	0.003	0	0	A	0.005	0.003	0.027	0
	BC					BC									
		V	I	F	A		V	I	F	A					
	V	0.37	0.151	0	0.018	V	0.48	0.006	0.004	0.162					
to	I	0.153	0.28	0	0.006	I	0.04	0.025	7E-04	0.031					
	F	0	0	0	0	F	0	0	1E-04	0.005					
	A	0.015	0.008	0	0	A	0.184	0.013	0	0					
	LC					LC									
		V	I	F	A		V	I	F	A					
	V	0.188	0.143	0	0.002	V	0.73	0.042	0	0.077					
to	I	0.141	0.521	0	9E-04	I	0.029	0.01	0	0.017					
	F	0	0	0	0	F	0	0	0	0					
	A	0.004	0	0	0	A	0.09	0.005	0	0					
	CT					CT									
		V	I	F	A		V	I	F	A					
	V	0.259	0.161	0	0	V	0.707	0.09	0	0.02					
to	I	0.161	0.419	0	0	I	0.1	0.063	0	0					
	F	0	0	0	0	F	0	0	0	0					
	A	0	0	0	0	A	0.011	0.009	0	0					
	BB					BB									
		V	I	F	A		V	I	F	A					
	V	0.262	0.163	0	0.03	V	0.533	0.034	0	0.19					
to	I	0.144	0.334	0	0.019	I	0.047	0.007	0	0					
	F	0	0	0	0	F	0	0	0	0					
	A	0.048	0	0	0	A	0.178	0.012	0	0					
	LB					LB									
		V	I	F	A		V	I	F	A					
	V	0.106	0.161	3E-04	0.021	V	0.652	0.047	0	0.1					
to	I	0.174	0.476	7E-04	0.016	I	0.01	0.007	0	0.042					
	F	0	0	6E-06	0.001	F	0	0	0	0					
	A	0.009	0.03	0	0	A	0.138	0.005	0	0					

Table 22: Observed population structure at Van Vleet for eight treatment regimes from 1992-1994. Abbreviations as in Table 16.

Treatment	Stage	1992	1993	1994
GM	V	0.378	0.191	0.382
	I	0.585	0.788	0.570
	F	0.037	0.000	0.000
	A	0.000	0.021	0.048
G	V	*	0.238	0.556
	I	*	0.736	0.339
	F	*	0.027	0.000
	A	*	0.000	0.105
NT	V	0.601	0.654	0.784
	I	0.214	0.322	0.048
	F	0.185	0.000	0.000
	A	0.000	0.024	0.168
EC	V	0.491	0.613	0.671
	I	0.373	0.373	0.126
	F	0.137	0.000	0.000
	A	0.000	0.014	0.203
LC	V	0.748	0.533	0.803
	I	0.244	0.461	0.107
	F	0.008	0.000	0.000
	A	0.000	0.006	0.090
CT	V	0.611	0.530	0.738
	I	0.381	0.470	0.232
	F	0.008	0.000	0.000
	A	0.000	0.000	0.030
EB	V	0.548	0.515	0.671
	I	0.348	0.442	0.085
	F	0.104	0.000	0.000
	A	0.000	0.043	0.244
LB	V	0.658	0.388	0.796
	I	0.155	0.584	0.079
	F	0.186	0.005	0.000
	A	0.000	0.024	0.125

Table 24: Deterministic and stochastic modeling of Van Vleet treatment regimes and riparian sites. Level of environmental stochasticity (ES) is the maximum level resulting in an exinction probability of less than 5%. Abbreviations as in Table 16 and an asterisk (*) indicates a model could not be projected since only one transition year was recorded.

Treatment	Deterministic		Stochastic	Level of ES
	1992-1993	1993-1994	1992-1993	1993-1994
GM	Extinct	Extinct	Extinct	0
G	*	Not Extinct	*	0.0024
NT	Extinct	Extinct	Extinct	0
EC	Extinct	Extinct	Extinct	0
LC	Extinct	Extinct	Extinct	0
CT	Extinct	Not Extinct	Extinct	0.0005
EB	Extinct	Extinct	Extinct	0
LB	Extinct	Extinct	Extinct	0
DC	*	Not Extinct	*	0.1997
CC	*	Not Extinct	*	0.0799
PP	*	Not Extinct	*	0.4999