# Inventory and Status Report of American Ground Nut (Apios americana Medicus) in Colorado



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## **Abstract**

The American ground nut, *Apios americana*, is exceedingly rare in the state of Colorado (Colorado Natural Heritage Program 1999, Weber and Wittman 1996). Using the Natural Heritage ranking system, it has been assigned global and state rarity ranks of G5S1, indicating that this species is globally secure but critically imperiled in the state of Colorado. It is the only representative of the genus *Apios* in Colorado. Five occurrences of *Apios americana* are currently documented in the state, all within Boulder County in areas managed by the City of Boulder (CNHP 2000). Two other historical occurrences have been previously documented, in Denver and Yuma counties. Research by CNHP botanists and volunteers in the summer of 2000 was intended to 1) assess the status of the known occurrences, 2) assess the overall status of the species in Colorado, 3) search for previously unknown occurrences, 4) conduct preliminary research on the reproductive biology and pollination ecology, and 5) generate management suggestions based on the available data.

The known occurrences do not appear to have declined in recent years, and all supported robust, healthy plants in the summer of 2000. Flowering occurred at all of the occurrences in 2000 despite drought conditions, and three of the five known occurrences flowered prolifically. No new occurrences were identified in this study. The historic occurrence from Yuma County was not relocated in one day of searching, and may well be extirpated, as is almost certainly the case with the Denver County occurrence.

Many populations of *Apios americana* farther east are incapable of sexual reproduction because they are triploid, containing an extra copy of chromosomes in their genome (Seabrook and Dionne 1976, Bruneau 1986, Bruneau and Anderson 1988). Bruneau (1986) developed morphometric methods of inferring the ploidy of *Apios americana* using pollen stainability, guard cell size, and flower color. In the present study, Bruneau's methodology was used to assess the ploidy of the Colorado occurrences. The pollen, guard cell, and flower color data for the Colorado occurrences strongly indicate triploidy for all of the Colorado occurrences. A lack of any observations or collections of fruit in Colorado also suggests triploidy. As such, the species in Colorado is not only extremely rare but is probably also incapable of sexual reproduction and dispersal by seed.

The only observed insect visitor to the flowers of *Apios americana* that appeared to be pollinating the flowers was *Apis mellifera*, the honey bee. These insects were observed on two occasions entering the flowers for periods exceeding 1.5 minutes. After the visits, flowers entered by bees were tripped but it is not known if this was the result of the bee's entry. Previous studies claim that megachilid (leaf cutter) bees are the only legitimate pollinators of *Apios americana* (Bruneau and Anderson 1986, Bruneau and Anderson 1994). No leaf cutter bees were observed in the present study, but they have been observed in the study area (A. Armstrong, pers. com. 2001) and evidence of Megachilid activity is present on a 1948 specimen from White Rocks at the CU Herbarium.

Given the current rarity and reproductive limitations of *Apios americana* in Colorado, the probability of extirpation due to human impacts, floods, competitive exclusion by exotic species, management changes, and stochastic processes is extremely high. Because the long term viability of this species is not secure in Colorado, special attention must be paid to careful management, which to this date has been excellent in the City of Boulder Open Space. Recommendations for a conservation action plan include: continued management for *Apios americana*, limiting human impacts in and around the occurrences, preventing grazing in the occurrences, modifying the haying regime at the Cherryvale occurrence, maintaining the appropriate groundwater regime at White Rocks, managing weed infestations closely, and conducting further research on the species in Colorado.

# **Objectives**

The overall objective of this study was to identify all extant populations of *Apios americana* in Colorado and assess their status. This included revisiting known populations in the City of Boulder Open Space, searching for new occurrences, and investigating historical populations known for Denver and Yuma counties. The City of Boulder Open Space and the private lands in the vicinity of Boulder contain extensive potential habitat for *Apios americana*. Because *Apios americana* can be very difficult to see, and because there is a great deal of seemingly suitable habitat for it, further intensive searching was warranted in the City of Boulder Open Space. The proximity of occurrences in Kansas and Nebraska and the presence of extensive potential habitat in eastern Colorado also suggest a need for further inventory work in this little studied area.

In order to assist land managers and land use planners in protecting *Apios americana*, Potential Conservation Areas (PCAs) were delineated and mapped. These areas represent our best estimate of the area required to support the long term viability of *Apios americana*. These areas confer no legal protection to the occurrences. The PCA documentation includes an evaluation of threats or potential threats and management recommendations are offered to address these.

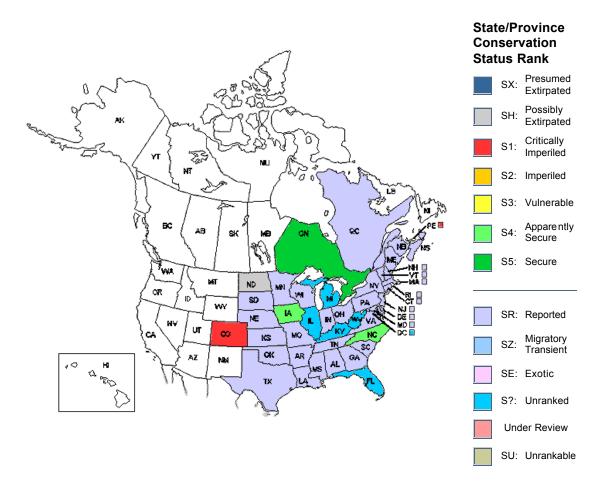
As a secondary objective, preliminary research was done on the reproductive biology of *Apios americana*, with an emphasis on information that would assist in the development of a conservation action plan for this species. The primary mode of reproduction was investigated to help refine conservation action plans for this species. Since the mode of reproduction is contingent largely on whether the species is diploid or triploid, the ploidy of the Colorado plants was investigated in this study.

The pollination ecology of *Apios americana* has been a matter of some disagreement among ecologists (Westerkamp 1997, Bruneau and Anderson 1994, Westerkamp and Paul 1993, Bruneau and Anderson 1986) and it remains uncertain whether the species' legitimate pollinators are flies or megachilid bees. Thus, a preliminary study of the insect visitors of *Apios americana* in Colorado was warranted.

# **Species information**

#### **Present Formal Status**

Apios americana is common throughout much of the eastern U.S. and Canada, and is thus considered "globally secure" (G5) by the Association for Biodiversity Information. Map 1 (following page) shows the distribution and Heritage Ranks for *Apios americana* throughout North America. In Colorado it is exceedingly rare and is ranked "critically imperiled" (S1) by the Colorado Natural Heritage Program. It is not classified as a sensitive species by any federal agencies. The Kansas Natural Heritage Inventory, Nebraska Natural Heritage Program, and the South Dakota Natural Heritage Data Base all consider this species sufficiently common in the eastern portion of their jurisdictions that it is not tracked.



Map 1. Distribution of *Apios americana* by state/province throughout North America (from the NatureServe website- www.natureserve.org).

# **Description**

The ground nut, *Apios americana* Medicus (Fabaceae) is a perennial twining or trailing herbaceous vine, 1 to 5 meters long, with tuberous roots. Its alternate leaves have small stipules and 5 to 7 leaflets. The leaflets are ovate, acute, and 2 to 7 cm long. Plants can be glabrous to pubescent. The flowers are pedicellate and born in axillary racemes. The calyx is green or redtinted. Flowers are 8 to 10 millimeters long with a papillionaceous corolla. The standard is reflexed, whitish-pink dorsally and reddish-maroon ventrally with brown and green markings in the throat. The wings are pinkish-red and white around the distal edge. See Great Plains Flora Association (1986) and Harrington (1954) for more complete descriptions.

# **Geographical Distribution**

*Apios americana* ranges widely from Nova Scotia south to Florida, and East to Minnesota, Texas, and Colorado (Seabrook and Dionne 1976; USDA NRCS 1999). It is one of two representatives of the genus *Apios* in the New World, along with *A. priceana*, another tuberous perennial vine (Bruneau 1986, USDA NRCS 1999).

The only extant populations currently known in Colorado are in Boulder County within the City of Boulder Open Space. A specimen was collected by Alice Eastwood in 1887 in Denver County, but it has almost certainly been extirpated from this locality. Dr. William Weber (pers. com. 2000) reports another historic account of this species from the bluffs south of the town of Wray in Yuma County on the north fork of the Republican River.

The Colorado populations are disjunct, with more than 200 miles separating them from another occurrence. The nearest known occurrence to those in Colorado is in Kansas near the Colorado border, in Stanton County (McGregor and Barkley 1977, *USDA NRCS* 1999). Other proximal occurrences are found in western Nebraska on the Niobrara River (G. Steinauer, pers. com. 2000), and in the Black Hills of South Dakota (D. Ode, pers. com. 2000).

#### **Environment and Habitat**

Apios americana is a plant of mesic woodlands, riparian areas, sloughs, and banks of streams and ponds (Bruneau 1986, *USDA NRCS* 1999, pers. com. C. Freeman 2000). In Colorado, one of the known occurrences is located on a river bank, three are adjacent to irrigation ditches, and one is on a shaded, south-facing sandstone cliff on a seep (City of Boulder Open Space 2000, CNHP 2000). It is ranked as a facultative wetland species (FACW) in Colorado and throughout most of its range by the U.S. Fish and Wildlife Service (USFWS 1988).

# **Historic and Contemporary Values**

*Apios americana* has been an extremely valuable plant to humans in North America for thousands of years. The seeds and tuberous roots have been important food sources among many Native American tribes (*USDA NRCS* 1999). It is widely credited as having saved the pilgrims at Plymouth Rock from starvation in the winter of 1620 (Gibbons 1962, Blackmon and Reynolds 1986, Ode 1990).

Apios americana has been studied extensively in other parts of its range due to its potential as a food crop (Blackmon and Reynolds 1986, Reynolds et al. 1988, Holcomb 1990, Valverde et al. 1990). It is one of the few species known that can fix atmospheric nitrogen in root nodules, and produces an edible tuber. The tubers are a good source of carbohydrates and contain between 13 and 17 percent protein, about three times more than potatoes (*USDA NRCS* 1999). Thus, it has enormous potential as a food crop in the developing world. The tubers have recently been found to contain an anti-carcinogenic compound and may harbor other biologically active substances (Krishnan 1998).

At its current rarity, the probability of local extinction of *Apios americana* in the near future is very high. Disjunct populations often harbor genotypes that are not found in the larger population. With the development of modern gene sequencing technology it has become evident that the loss of such populations, though not as tragic as the loss of an entire species, still represents a significant loss of biodiversity. As a crop plant, the natural variation contained within the genomes of the Colorado populations of *Apios americana* could serve to produce more vigorous hybids when it is cultivated. Thus, by helping to insure the long-term viability of the Colorado occurrences, we incur significant benefits to public health, biodiversity, Colorado's natural heritage, and the economy.

# **Reproductive Biology**

With regard to the Colorado occurrences, the reproductive biology of *Apios americana* is particularly interesting. In other parts of its range, Seabrook and Dionne (1976), and Bruneau and Anderson (1988) found that individuals can be either diploid (two copies of each chromosome per cell) or triploid (three copies of each chromosome per cell). Triploid plants are morphologically similar to diploid plants but can only reproduce asexually with tubers. Flowers are produced but they never bear seeds. Diploid plants are capable of both asexual reproduction with tubers, and sexual reproduction with seeds. Apparently, the ploidy of the Colorado populations and all other western populations had not been investigated prior to this study. Because triploids have such a limited reproductive potential relative to diploids, knowledge of the ploidy of the Colorado populations is crucial when assessing the level of imperilment for these occurrences. An understanding of the reproductive biology of *Apios americana* in Colorado will help in designing and implementing effective conservation action plans.

The identity of the legitimate pollinator for *Apios americana* has been a topic of considerable disagreement among scientists, with strong arguments having been made naming either flies (Westerkamp and Paul 1993, Westerkamp 1997) or megachilid bees as the legitimate pollinators (Bruneau and Anderson 1986, Bruneau and Anderson 1994).

#### Methods

#### **Inventory Methods**

Botanists from the Colorado Natural Heritage Program conducted field surveys for *Apios americana* in Boulder and Yuma Counties for 15 days in August and September 2000. The following sections provide details regarding the methodology used during the inventory phase of this project.

# **Collect Information**

Prior to field surveys, CNHP databases were updated with information regarding the known locations *Apios americana*. A variety of information sources were searched for this information. Local herbaria were searched (UCM, CS, City of Boulder Open Space) for information on locations, habitat, and associated species. Local experts on *Apios americana* (Dr. W.A. Weber, Lynn Riedel, Nancy Neupert, Bill Jennings) were contacted for advice and recommendations regarding this project. All available literature regarding this species was obtained and incorporated into CNHP's databases.

# Identify Targeted Inventory Areas

Survey areas were chosen based on their likelihood of harboring unknown populations of *Apios americana*. Areas that appeared to contain the ecological and geological features found in known occurrences of *Apios americana* were selected using GIS layers and drawn on topographic maps. The areas thus delineated were referred to as Targeted Inventory Areas (TIAs), and visiting these areas served as a work plan for the field crew when searching for *Apios americana*. Targeted Inventory Area selection was not influenced by land ownership patterns.

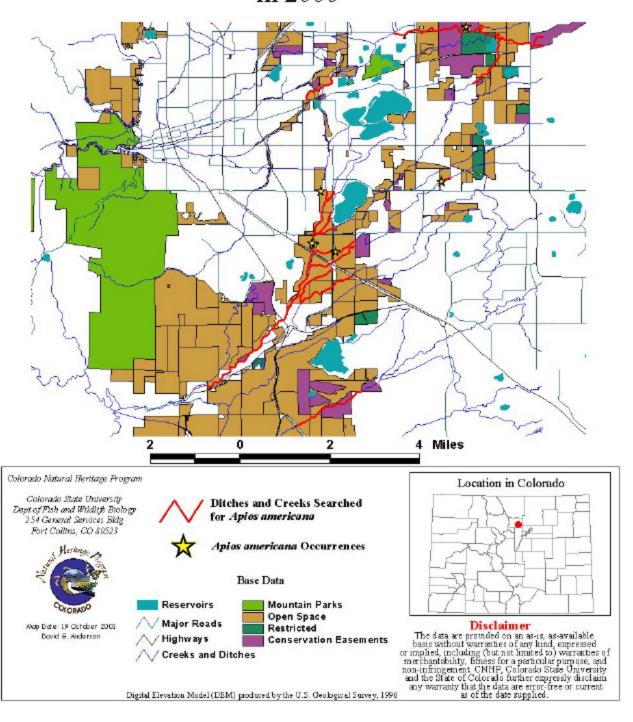
# **Conduct Field Surveys**

All the known occurrences of *Apios americana* in Colorado were visited during this survey. Voucher specimens were collected from two of the occurrences and will be deposited at the University of Colorado Museum Herbarium and at the Colorado State University Herbarium. The UTM coordinates of each occurrence was determined using a Garmin GPS 12CX unit. Other data recorded at each occurrence included numbers observed, associated taxa, exotic species, habitat description, disturbance features, observable threats, and potential protection and management needs. The overall significance of each occurrence relative to others was estimated by rating the quality (size, vigor, etc.) of the population, the condition or naturalness of the habitat, the estimated long-term viability of the population, and the landscape context of the occurrence. These factors are combined into an element occurrence rank, useful in refining conservation priorities. See the section on Natural Heritage Methodology for additional information about element occurrence ranking (Appendix 3).

Targeted Inventory Areas (TIAs) were visited throughout the City of Boulder Open Space and City of Wray during the survey phase of this project. Known occurrences were visited first so the field botanists could develop skills in recognizing the species. Initially, TIAs were surveyed broadly to assess the suitability of habitat for the species. This involved driving through as much of a given TIA as possible. Areas within a given TIA that appeared to contain suitable habitat for the species were surveyed intensively on foot. The measure of habitat suitability was defined by the ecological conditions present within the known occurrences of *Apios americana*. Priority search areas were (in order of priority) South Boulder Creek, cliffs in the White Rocks Natural Area, Coal Creek, irrigation ditches, and lake margins (See Map 2 for areas searched).

Map 2

# Areas Searched for Apios americana in the City of Boulder Open Space in 2000



#### Delineate Potential Conservation Areas

In order to prioritize conservation efforts, a Potential Conservation Area (PCA) was delineated. A PCA is an estimation of the minimum area needed to ensure persistence of the element occurrence. In order to ensure the preservation of *Apios americana*, the ecological processes that support the species must be preserved. Preliminary conservation planning boundaries are meant to include features on the surrounding landscape that provide these functions. Data collected in the field are essential to delineating such a boundary, but other sources of information such as geologic and topographic maps are also used refine the boundaries. In developing PCA boundaries, a number of factors were considered, including specific locations of *Apios americana* and surrounding habitat needed to protect significant ecological processes at each PCA. The delineation of conservation planning boundaries in this report does not confer any regulatory protection. The boundaries are solely intended to facilitate land use planning and decision-making for the conservation of *Apios americana*.

# **Ploidy Analysis**

Because no single morphological character can be used to consistently differentiate a diploid plant from a triploid plant, several morphological characters were observed to determine ploidy. Following the methodology of Bruneau (1986), pollen stainability, guard cell size, and floral morphology were analyzed to determine ploidy indirectly. Plant material for the analysis was collected from one or two individuals in each subpopulation. Due to the rarity of this species, a minimal amount of plant material was removed to reduce potential negative impacts. Using data for percent of pollen grains unstained and guard cell length from Bruneau (1986) and from the present study, a stepwise multiple linear regression model was fitted to predict the ploidy of the Colorado occurrences. The SAS statistical software package (version 8.1)was used for this analysis.

#### Pollen

Bruneau (1986) showed that pollen size and stainability are good indicators of ploidy in *Apios americana*. In diploids, pollen is uniform and a vast majority of the grains are stained by tetrazolium, whereas triploids have great variability in pollen size with many poorly stained, undersized grains which result from meiotic malfunction. At each population, a flower was removed from one or more plants and stored at approximately 5 degrees C until analysis (within two days). Two anthers were removed from each flower and macerated in a cavity slide in a 0.1% solution of tetrazolium. The pollen was incubated in the stain for approximately one hour prior to counting. All grains were counted as either stained or unstained at 400X. Duplicate slides were also made using MTT stain to see if stainability of pollen grains was consistent between the two stains. Two anthers from the same flowers used in the tetrazolium analysis were macerated in cavity slides in MTT stain (0.1% MTT, 30% sucrose) and incubated for 24 hours at 5 degrees C. Ratios of stained versus unstained pollen were similar between the two stains, and in some cases the MTT stain provided better results. However, the tetrazolium stained material was used in the final analysis because it allows the results to be compared to those of Bruneau (1986), who also used tetrazolium to stain the pollen of *Apios americana*. The

sample taken on August 8 at the Cherryvale occurrence dried up before counting, and another sample was not obtained before flowering was complete at this occurrence.

#### Guard Cell Size

Bruneau (1986) showed that large guard cells showed some correlation with triploidy, although this was not statistically significant. Guard cells were measured from one or two plants from each known occurrence to compare measurements with those of Bruneau. One terminal leaflet was removed per occurrence and stored in a sealed container at approximately 5 degrees C until analysis (within two days). Clear nail polish was applied to the abaxial leaf surface and allowed to dry, then peeled off and permanently mounted on a microscope slide. 20 guard cells per leaf were measured in two dimensions (X and Y) (Figure 1) on a video monitor connected to an objective microscope. These measurements were converted to micrometers using a stage micrometer.

#### Flower Color

Cytotypes have been distinguished from each other somewhat consistently by floral characteristics in other parts of the range of *Apios americana*, although this alone was not a reliable indicator of ploidy (Bruneau 1986). A "dark" and "light" color form were recognized by Bruneau (1986) among plants observed in New England, Ontario, Ohio, and Tennessee. The light form was most often seen in triploid plants, while the dark form was more characteristic of diploid plants. The two forms differ primarily in the coloration of the wings, standard, and calyx. Flowers from each population and subpopulation in the City of Boulder Open Space were collected and photographed, and diagnostic characters were noted.

#### Flower visitation by potential pollinators

Groups of flowers were observed for half hour periods in which all insect visits were documented by species. An insect visit was defined as any time an insect touched a flower, either by walking on it or by flying to it. If a flower was visited twice by the same insect, this was still counted as two visits. Insects that had visited the flowers during the timed observations were caught following the observation period and mounted following the methodology of Cranshaw and Kondratieff (1995). Insect specimens were identified by Dr. Boris Kondratieff, Entomology Department, Colorado State University.

Insect visitation rate (IVR) was calculated using the following equation:

$$IVR = (10)OV/F$$

where OV is the number of visits observed during the observation period, and F is the number of flowers watched by the observer during the observation period. The numerator is multiplied by 10 to adjust the visitation rate to represent visits per 10 flowers per half hour.

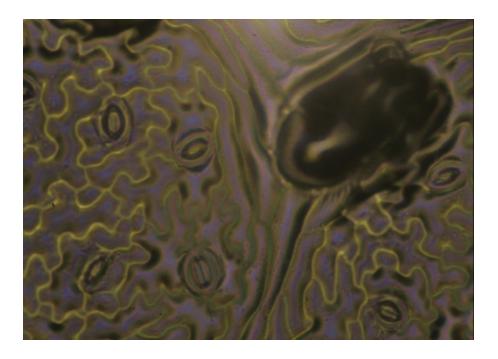


Figure 1. Photograph of the epidermis of *Apios americana* at 400X. The doughnut-shaped objects are the leaf stomates. The large dark structure is a trichome. These features are within a matrix of leaf epidermal cells (small cells with reticulate margins). One guard cell from each of 20 stomates was measured in the long dimension (X) and short dimension (Y).

#### Results

#### **Distribution in Colorado**

Precise locational data was obtained for each known occurrence (Table 1) to facilitate relocation for future monitoring or research projects. Despite extensive searching, no new occurrences of *Apios americana* were found in this study.

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Table I	Locational	intormation	on the known	occurrences of $A$	lpios americana.
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Code	Reference in this report	UTM coordinates		
APAM01	Van Vleet population A	13S 481154E 4425728N		
APAM1B	Van Vleet population B	13S 481208E 4425695N		
APAM1C	Van Vleet population C	13S 481227E 4425673N		
APAM1D	Van Vleet population D	13S 481154E 4425728N		
APAM02	Baseline Road	13T 481470E 4427572N		
APAM03	Spicer	13T 485758E 4427923N		
APAM04	Cherryvale Road	13S 481997E 4425422N		
APAM05	White Rocks	13T 486631E 4433413N		

Due to habitat alteration and downcutting, much appropriate habitat has evidently been lost in the Boulder area. Many of the promising areas along South Boulder Creek turned out to be unsuitable. In many places the creek banks rise almost straight up from the water with a 1 to 3 meter edge that makes it difficult to walk out of the creek for hundreds of meters. Thus, the vegetation adjacent to the creek is in many places more characteristic of upland vegetation, interspersed with remnant old cottonwoods.

In some respects the potential habitat along Coal Creek in Boulder County seemed promising for *Apios americana*. Many associated species were found (*Salix exigua, Toxicodendron rydbergii, Populus deltoides*) and the creek was not excessively downcut in many places. However, the creek was completely dry when visited on August 17, 2000 and the constant moisture required by *Apios americana* is probably not available in late summer in many years in this drainage. The upper soil horizons in the riparian area were quite dry in most places.

Many areas along irrigation ditches in Boulder County appeared suitable for *Apios americana*. Given the range of habitats occupied by the known occurrences along irrigation ditches (Spicer, Cherryvale, and Baseline Road), the lack of more occurrences on irrigation ditches is probably only a function of its inability to disperse to these locations (see Conclusions-Implications of Triploidy).

Two potential habitat areas were identified in the vicinity of Wray in Yuma County. One was the bluffs south of Wray, on city property. Historically, the bluffs were used as a garbage dump, and much of the colluvial area at the base of the bluffs was covered by the remains of old cars, bottles, and other trash. The resulting degradation of the area, alterations in groundwater levels that dried up seeps from the cliffs, or resedential development on the south edge of Wray may

have extirpated the occurrence. Several moist seepy areas with *Celtis reticulata* were found during the search that were reminiscent of the occurrence at White Rocks, but no *Apios americana* plants were found (Figure 2). However, the complex, dendritic pattern of the bluffs (Figure 3) creates a large surface area, and all the potential habitat was not searched.

The other potential habitat in the vicinity of Wray is the riparian area of the Republican River. Although the historic occurrence is reported from the bluffs south of the town, the river appeared more promising. Many of the associated plant species in the Boulder County occurrences were represented here as well. These include *Solidago canadensis*, *Spartina pectinata*, *Androsaemum cannabinum*, *Salix exigua*, *Glycyrrhiza lepidiota*, and *Populus deltoides*. The area near the softball fields in town appears quite promising. This area was searched for one hour but no *Apios americana* were located.

#### **Status of the Known Occurrences**

Overall the population size, condition, and landscape context has not changed significantly in any of the known occurrences relative to information available from previous years. All occurrences appeared to support robust, healthy plants. Qualitatively the occurrences do not seem to be in decline. Natural Heritage Element Occurrence Ranks were assigned based on comparison between the five Colorado occurrences, not in comparison with occurrences rangewide. Typically, ranks are not assigned this way, but no occurrences have been ranked by other heritage programs in other parts of the range of *Apios americana* and element occurrence rank specifications have not been developed. Please see Appendix 3 for interpretation of the Element Occurrence Ranks.

Due to the growth form of *Apios americana*, an accurate census of any of the occurrences in Colorado would require either digging up the plants or a genetic analysis. A single plant may consist of an extensive network of 120 tubers and rhizomes weighing a total of 3.7 kg (Blackmon and Reynolds 1986). Numerous stems emanate from this network. Following them all to the surface would result in a heavy impact on the plant due to trampling and inadvertent breaking of stems, and would still not provide an accurate count. In the heavy overgrowth typical in all the occurrences, it is virtually impossible to find the beginning and end of a stem, so even this cannot be used to infer a count. This makes monitoring and status assessment difficult and renders population estimates purely subjective. Prior estimates of population size do not specify if the observer counted stems, patches, or if a "ballpark figure" was used. It is likely that most occurrences are made up of one or a few individuals that have proliferated vegetatively via tubers and rhizomes.

Although all occurrences appear to be doing well overall, their long-term viability is dubious due to small population size. With the possible exception of the White Rocks occurrence, all are located in highly facile, fragile, and largely human-controlled environments. Using the 50/500 rule for minimum population viability as a guideline, none of the known occurrences in Colorado approach the minimum criteria for long-term viability, or even for short-term maintenance of genetic variability. The level of imperilment is greatly elevated when the limitations imposed by triploidy are taken into account (see Conclusions-Implications of Triploidy).

Previously documented threats to *Apios americana* in Colorado include competitive exclusion by weeds, disturbance (erosion of ditch banks, ditch cleaning), and grazing (CNHP 1999). As a part of this inventory, other threats or potential threats were documented. Management suggestions are offered to address these threats in Conclusions- Assessment and Recommendations. Below are comments specific to each occurrence, including notes on signs of disease, herbivory, and threats. For the complete element occurrence information see Appendix 5- Element Occurrence Records for *Apios americana*.



Figure 2. A moist, seepy alcove in the bluffs south of Wray, Colorado with Hackberry (*Celtis reticulata*). This site was reminiscent of the occurrence of *Apios americana* at White Rocks. Cars that have been pushed off the cliff into this alcove long ago are visible.



Figure 3. The bluffs south of Wray, Colorado.

#### Van Vleet

# Natural Heritage Element Occurrence Rank: B

**Location:** T1S R70W S10NW. Van Vleet Property, City of Boulder Open Space. North Bank of South Boulder Creek, approximately 400 meters south of S. Boulder Road, behind the "L" barn and continuing upstream approximately 30 meters in four sub-occurrences.

**Status:** Plants in the main population at this occurrence were among the largest and healthiest in Colorado. This is also one of only two natural occurrences in Colorado. This occurrence consists of four suboccurrences (A through D), two of which were first identified during this survey by Nancy Neupert, Lynn Riedel, and the primary author. The northern-most suboccurrence (A) is by far the largest of the four. Plants at this suboccurrence flowered prodigiously and produced an extensive net of vines over an area of approximately 7 by 3 meters during the summer of 2000, consisting of perhaps 5 plants (Figure 4). The second suboccurrence (B) consisted of perhaps 4 to 7 plants in a patch 2 meters in diameter. The third (C) consists of 4 to 8 robust plants, which also flowered prodigiously. Flowering occurred on all but one of the suboccurrences (D), which consisted of a single small patch (probably one plant) approximately 1 meter across. Previous population estimates cite three individuals for the entire occurrence, but this estimate does not appear to include all of the suboccurrences.

**Threats:** No threats, predation, or injury have been previously documented to plants at this occurrence. The leaves of many plants showed signs of insect herbivory at suboccurrence A (Figure 5), probably by grasshoppers or beetles (pers. com. B. Kondratieff 2000). Grasshoppers were abundant at this occurrence.

On many flowers in suboccurrence A, the keel, stamens, and gynoecium had been removed, evidently selectively eaten by an insect. The perpetrator of this herbivory was never identified. This probably has a minimal effect on the health and viability of the occurrence if the plants are triploid.

Population B, C, and D are very close to the creek, and a major flood event could conceivably wash these occurrences away. *Apios americana* is able to reestablish itself vegetatively in such events, but in such a small population with little appropriate habitat downstream, the probability of successful reestablishment is very low (see Conclusions- Implications of Triploidy).

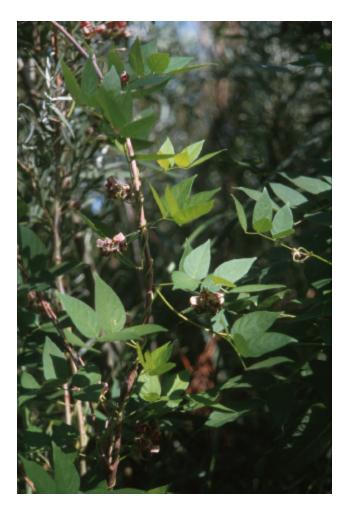


Figure 4. Apios americana climbing on Salix exigua at the Van Vleet occurrence.



Figure 5. Insect damage to the leaves of *Apios americana* at the Van Vleet occurrence.

#### Baseline Road

# Natural Heritage Element Occurrence Rank: C

Location: T1S R70W S3. Burke 1 Property, City of Boulder Open Space. Between an irrigation ditch and the shoulder on the south side of Baseline Road, where the ditch goes under the road, approximately 100 meters west of South Boulder Creek and the Bobolink Trailhead.

**Status:** The Baseline Road occurrence consists of two distinct patches, probably representing two or three individuals. Prolific flowering was observed on August 8, 2000 and continued through at least August 21, 2000. No evidence of decline was observed at this site from previous years. Two individuals were estimated to occur here in 1996; presumably these are the same plants. The ecological conditions that support this occurrence are anthropogenically created and maintained.

**Threats:** Due to its small population size and proximity to Baseline Road, this occurrence is the least secure of all the known populations. Past management along the transportation corridor has been very good and great attention has been paid to having minimal impacts on this occurrence. It is potentially threatened by alterations to the road, ditch management, mowing, and roadside weed spraying if current management practices change. Trampling, toxic runoff from the road are other potential threats that could be more difficult to manage for. Numerous exotic species were noted that threaten the occurrence: Chinese elm (*Ulmus pumila*), Russian olive (*Eleagnus angustifolia*), prickly lettuce (*Lactuca serriola*), chickory (*Chicorum intybus*), smooth brome (*Bromus inermis*), ragweed (*Ambrosia psylostachya*), yellow sweetclover (*Melilotus album*), and teasel (*Dipsacus fullonum*). Particularly menacing to this occurrence are *Dipsacus fullonum* and *Breea arvensis* (*Breea* was not seen here but is abundant along Baseline Road). The threats cited here are meant to take into account long-term probabilities rather than current management.

This occurrence was depredated in a similar fashion to the Van Vleet and Cherryvale occurrences, with the keel, stamens and gynoecium removed on several flowers. the banner was also partially removed on one flower. No insect damage to the leaves was noted.

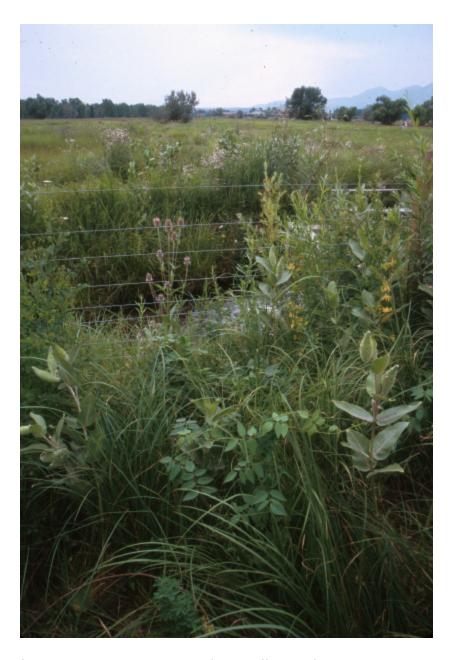


Figure 6. *Apios americana* at the Baseline road occurrence. Note weedy species (*Dipsacus fullonum, Breea arvensis*) in the pasture behind the occurrence.

# Spicer

# Natural Heritage Element Occurrence Rank: C

**Location:** T1N R69W S31W2SW4. Spicer Property, City of Boulder Open Space. Along north bank of the South Boulder Canyon Ditch, 100 meters north of the property line.

**Status:** Approximately seventeen individuals were seen in 1994, although the census method was not recorded (CNHP 2000). On August 3, 2000 plants were found in two large, sprawling clumps, consisting of perhaps 3 to 12 individuals. The western clump was approximately 2 meters in diameter, and the eastern clump was approximately 4 meters in diameter. Plants were observed flowering prolifically on August 8 (23 racemes total). From the available data a population trend cannot be deduced at this occurrence. The ecological conditions that support this occurrence are anthropogenically created and maintained.

**Threats:** Erosion of the ditch bank, ditch cleaning, weed invasion, and changes in management are all potential threats to this occurrence.



Figure 7. *Apios americana* on the Spicer property.

### Cherryvale Road

# Natural Heritage Element Occurrence Rank: C

**Location:** T1S R70W S10 NW4NW4SE4. Van Vleet Property, City of Boulder Open Space. On the south bank of the South Boulder Canyon Ditch approximately 100 meters (300 feet) east of Cherryvale Road, northeast of the barnyard complex.

**Status:** This occurrence may be expanding vegetatively. Between 40 and 70 shoots were counted in August, 2000 at this site, 33 of which were in the haymeadow (Figure 9). It appears that the network of tubers has grown out into the haymeadow, but the above ground shoots are mowed off before they can obtain significant above-ground biomass. All shoots were between 5 and 20 cm in length in this part of the occurrence. If this small portion of the haymeadow was allowed to grow until mid-September before being cut, the quality of this occurrence would probably improve.

Poor flowering was observed overall at this occurrence in 2000. Only one shoot was observed with flowers on August 7. During a revisit on August 9, the occurrence had been disturbed, evidently browsed, and the flowering vine was gone. By August 16 some vines were beginning to senesce, possibly due to drought conditions. No flowering was observed at this occurrence after August 9.

The ecological conditions that support this occurrence are anthropogenically created and maintained

**Threats:** Browsing by herbivores (perhaps by livestock, although the perpetrator was not observed; raccoons could conceivably break vines and cause the damage seen also) in the portion of the occurrence near the fence may be impacting the occurrence. Haying in the part of the meadow adjacent to the fence is probably having the biggest impact on the occurrence. Weed invasion and potential alterations to the ditch are also conceivable threats. Road widening may affect the occurrence if it results in alteration of the flow of water in the South Boulder Canyon Ditch

On August 7, flowers were observed with the keel, stamens, and gynoecium removed as seen at the Baseline Road and Van Vleet occurrences (Figure 10).



Figure 8. *Apios americana* at the Cherryvale Road occurrence.



Figure 9. Small shoots of *Apios americana* in the haymeadow approximately 3 meters south of the fence at the Cherryvale Road occurrence.



Figure 10. A flower of *Apios americana* with the keel, stamens, and gynoecium removed, presumeably by an insect.

#### White Rocks

# Natural Heritage Element Occurrence Rank: A

**Location:** T1N R69W S18. White Rocks Natural Area, Ertl 1 Property (Conservation easement held by City of Boulder Open Space). Above the north bank of South Boulder Creek, on Laramie sandstone, in a cove with a perennial seep.

**Status:** This is the largest and highest quality occurrence of *Apios americana* in Colorado. It consists of many individuals (50 have been previously estimated), the bulk of all the plants in Colorado. Plants at this occurrence flowered prodigiously at this occurrence, and on August 17, 2000 all plants appeared green and healthy. By August 28 some plants on the eastern edge of the occurrence had begun to senesce, and only six racemes were found in the entire occurrence. The senescence may be the result of the atypical drought conditions.

**Threats:** This is the most secure population in Colorado, due to its large population size, natural and legal protection, and low human visitation. However, it is vulnerable to alterations in groundwater levels that might affect the flow of water in the seep that sustains it. As residential development progresses on the bluff above the occurrence to the north and east, this is becoming a very real threat. If visitation rates are allowed to increase, human visitation is another potential impact. Current management practices for the area are helping to ensure the security of this occurrence.

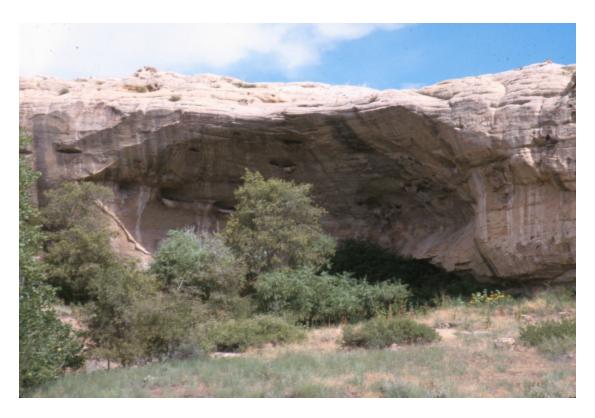


Figure 11. Habitat of the *Apios americana* occurrence at White Rocks Natural Area.



Figure 12. Close-up of the occurrence at White Rocks.

### **Ploidy Analysis**

The weight of evidence from the analysis of ploidy suggests that Colorado plants are triploids. A stepwise linear regression model using percent of unstained pollen and guard cell length as independent variables was highly significant ( $r^2 = .91$ , F = 122.33, P < .0001). At all occurrences (except Cherryvale where no pollen data were obtained), the model predicted triploidy (Table 2).

Table 2. Ploidy predictions for 4 occurrences based on a stepwise linear regression model.

Occurrence	Ploidy predicted by the model		
Van Vleet pop. A	3.4		
Van Vleet pop. B	3.2		
Van Vleet pop. C	3.3		
Baseline Road	3.6		
Spicer	2.8		
White Rocks	3.2		

# Failure to fruit

No fruits were observed at any of the occurrences in 2000, nor have any been previously observed (pers. com. L. Riedel 2000) or collected. The flowers, after blooming, wither and drop off with the pedicel (Figure 13). The flowers drop following the formation of an abscission layer at the base of the pedicel, leaving behind only the rachis of each inflorescence (Figure 14).

#### Pollen

The pollen from all specimens was highly heterogeneous, with numerous small, underdeveloped grains (Figure 15). The percent of unstained or poorly stained grains was uncharacteristically high for typical viable pollen (Table 3). Pollen stainability documented in this study correlates well with that observed by Bruneau (1986) in triploid plants, and is perhaps the strongest evidence thus far that the Colorado plants are triploid. A simple linear regression using only pollen data from Bruneau (1986) and Colorado predicted triploidy for all occurrences ( $r^2 = .81$ , F = 209.21, P < .0001).

Table 3. Pollen stainability data of the Colorado occurrences.

Site	Collection Date	% unstained	number of grains observed	Predicted ploidy
Van Vleet pop. A	8-Aug	67.5	203	3.2
Van Vleet pop. B	17-Aug	76.5	68	3.4
Van Vleet pop. C	8-Aug	63.8	309	3.2
Baseline Rd.	8-Aug	60.4	280	3.1
Spicer	8-Aug	58.3	216	3.0
Cherryvale Rd.	8-Aug	-	-	-
White Rocks	17-Aug	63.7	193	3.2
White Rocks	17-Aug	63.0	595	3.2



Figure 13. Flowers have withered and fallen off of this plant from the occurrence at the Van Vleet occurrence.



Figure 14. A fully senesced rachis of an inflorescence with no remains of flowers, fruits, or pedicels. Photo taken at the Van Vleet occurrence.

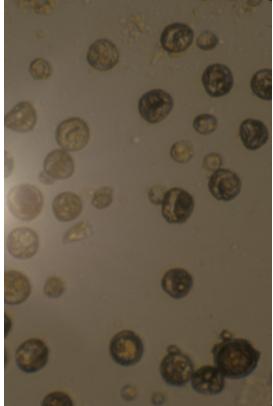


Figure 15. Tetrazolium stained pollen of *Apios americana* from the White Rocks occurrence viewed at 400X. Note the heterogeneity in grain size and stainability.

# Guard Cell Size

Average guard cell size is large in the Colorado plants (Table 4). Guard cell length correlates well with that of triploids in Bruneau (1986), although width does not. Width was probably measured differently in the two studies, with the cutin layer on the inside of the stomatal opening included by Bruneau (1986).

Table 4. Guard cell size data (average of 20 cells each) of the Colorado occurrences.

		x mm	y mm	x(y)
Van Vleet pop. A	AVE:	26.48	6.32	166.06
	SD:	2.80	1.22	30.52
Van Vleet pop. B	AVE:	23.84	4.87	116.44
	SD:	1.70	0.69	21.37
Van Vleet pop. C	AVE:	26.29	6.59	174.77
	SD:	4.95	1.32	52.15
<b>Baseline Road</b>	AVE:	29.84	6.75	194.76
	SD:	5.73	1.55	29.29
Spicer plant 1	AVE:	22.07	6.05	133.69
	SD:	2.57	1.17	30.36
Spicer plant 2	AVE:	24.52	5.67	138.99
	SD:	2.08	0.56	17.50
Cherryvale Road	AVE:	26.99	5.00	134.12
	SD:	2.13	0.74	16.21
White Rocks	AVE:	25.59	5.35	137.05
	SD:	2.44	0.88	27.01

### Flower Color

Overall, flower color is mostly but not entirely suggestive of triploidy. Flowers at all occurrences were relatively light in color as are most eastern triploids, with a light pink banner externally (Figure 16). The interior banner surface also shows triploid coloration, with a green-streaked throat and dark reddish-brown coloration (Figure 17). The wings are dark red with a narrow white periphery (Figure 18). However, Bruneau (1986) states that triploids she examined had a "completely green" calyx. Flowers at all but one occurrence had a reddish-pink tint to the calyx, which is typical of diploid plants. This minor variation in triploid flower color has not previously been documented for *Apios americana*, and may be indicative of some degree of genetic drift away from a ancestral eastern populations. At Van Vleet population B, flowers were found that were entirely green on the calyx.



Figure 16. Close-up of flower of *Apios americana* showing light, triploid color morph (Spicer). This flower has been tripped by an insect- note the curled stamens and pistil below the keel.



Figure 17. Interior banner surface of *Apios americana* showing green streaks in the throat and the dark reddish-brown coloration (Cherryvale).



Figure 18. Flower close-up showing the narrow white band on the distal portion of the wings. This flower is untripped.

# Flower visitation by potential pollinators

A total of eight half hour observation periods were conducted in August 2000. The observations were conducted at the Van Vleet (populations A and C) and Cherryvale occurrences. Insect visitors were recorded during only four of the observation periods (Table 5). Insect visitors included two species of ants (*Formica spp.*), beetles (*Boisea trivittatus* Say and *Bothrotes plumbeus* LeConte), and honey bees (*Apis melifera* L.). Ants were observed climbing around on the racemes, evidently uninterested in entering the flowers. Beetles were also observed either sitting on flowers or walking among them, but not entering the corollas or showing any particular interest in the flowers themselves. No beetles were observed during the timed observations, so no visitation rates were calculated for them.

Honey bee visits were observed on three occasions at Van Vleet to flowers in populations A and C. A typical honey bee visit lasted sometimes 1.5 minutes or longer. The bees climbed into the flowers, forcing themselves between the banner and keel. On August 15, 2000 at population A, two flowers that had been visited by a bee were inspected after the half hour observation period and both were tripped. It is not known if the flowers were tripped previously, and thus the bee visit may or may not have caused the flowers to trip. Numerous tripped flowers seen at all populations suggests that an insect visitor, perhaps honey bees, is able to trip the flowers, since the flowers cannot trip themselves (Bruneau 1986). At the Van Vleet occurrence, 43% of all flowers observed were tripped. Many insects including honey bees were observed at White Rocks, but none were observed to enter the flowers. Many flowers had been tripped at this location on August 17 and 8/28.

Table 5. Insect Visitation Rates (Visits per half hour per 10 flowers) for the Van Vleet occurrence. \* anecdotal observation.

Location	Date	Time	Insect Visitation Rate	
			Ants	Bees
Population A	8/7/2000	17:00	2.26	-
Population A*	8/9/2000	7:00		2 visits observed
Population A	8/15/2000	9:15	2.24	1.37
Population A	8/16/2000	9:10	0.78	-
Population C	8/16/2000	10:00	-	1.00

# **Conclusions**

#### **Distribution in Colorado**

The failure in this inventory to find new populations during the intensive search of the City of Boulder Open Space, and to relocate the historic population in the area south of Wray confirms that this species is indeed extremely rare in the state of Colorado and has probably declined as a result of human impacts. However, extensive habitat for *Apios americana* is present in eastern Colorado, and this area of the state has not been extensively investigated by botanists or thoroughly searched. Areas on which to focus future searches for this species statewide in order of priority are the Republican River (north fork and mainstem), South Platte River, Arkansas River, and the Smoky Hill River. Downstream in Kansas and Nebraska, all of these watersheds support populations of *Apios americana*.

It is rather unlikely that other populations of *Apios americana* are yet to be discovered within the City of Boulder Open Space. Areas that should be searched further include the unsearched sections of South Boulder Creek to the east and unsearched irrigation ditches in the City of Boulder Open Space and on private land. Private lands in the vicinity with apparently suitable habitat offer strong hope that there are other undiscovered populations residing in the Boulder area.

If the Colorado plants are triploid, the distribution of *Apios americana* in the City of Boulder Open Space is difficult to explain, since three of the populations (Baseline Road, Cherryvale Road, and Spicer) have clearly been established recently. Before human alteration of the landscape that created the irrigation ditches, these areas were upland prairie and did not contain appropriate habitat for *Apios americana*. In the absence of dispersal by seed, it is difficult to imagine how the plants got there and a parsimonious explanation is wanting. The following hypothesis is offered as a possible explanation.

Historically there may have been several or many large populations of *Apios americana* along South Boulder Creek. When South Boulder Creek was channelized by steam shovels in the 1940's, all but one of these occurrences were extirpated. The steep river banks created by channelization and intense disturbance of the riparian area destroyed most of the plants and made the riparian area largely unsuitable for *Apios americana* (Figure 19). Under this hypothesis, the population surviving at Van Vleet is all that remains of what was once a more extensive population. Although this hypothesis provides a parsimonious explanation for the current rarity of *Apios americana* in the Boulder vicinity, there is no historic data to suggest that this species was once more common in the area. The earliest known collection in the area was made by Dr. Joseph Ewan in 1940 at White Rocks, and prior to 1994 no collections had been made anywhere but there. With a long history of so many excellent botanists residing and collecting in the area, it seems unlikely that this species could have gone unnoticed for so long.

The three populations in irrigation ditches may have become established by either of two mechanisms. Water dispersal from South Boulder Creek could have brought the tubers to their locations in the ditches. Or, a more plausible explanation may be that tubers were transported in soil stuck to the treads of a steam shovel that had been used in South Boulder Creek before being used in excavation or cleaning of the ditch in which the tubers were deposited. This explanation

best explains the occurrence at Spicer, where the plants are growing high above the water in the ditch. However, a combination of factors might also explain this, if the tuber or tubers were washed to this location first, then deposited on the berm next to the ditch when the ditch was cleaned.

The White Rocks population may have been established long ago when the river bank was higher, or may have been planted by Native Americans, who may have brought the species to Colorado in the first place. This begs the larger question of the origin of *Apios americana* in Colorado, which also generates numerous plausible and interesting hypotheses. Comparative genetic analyses of *Apios americana* here and in other parts of its range could shed light on these questions.



Figure 19. South Boulder Creek just south of the Highway 36 bridge. Note the raised bank in the foreground resulting from channelization of the creek. This area may once have supported *Apios americana* but is now unsuitable.

# **Implications of Triploidy**

There are several implications for the conservation of *Apios americana* in Colorado if our populations are indeed triploid. Overall, the probability of long-term survival is lower if the Colorado populations are triploid. Triploid plants have relatively low rates of genetic change and adaptation within populations, no potential for outcrossing, limited dispersal ability, and low reproductive potential. Since populations are also extremely small at present, it is believed by the authors that the Colorado populations are in great danger of extirpation. Human impacts not withstanding, natural stochastic processes alone could eradicate this species in Colorado at its current degree of rarity.

Since triploids are sterile and have no means of sexual reproduction in this species, point mutation is the only means of evolution in response to environmental change. Populations of asexually reproducing individuals evolve extremely slowly relative to sexually reproducing populations of organisms.

The potential for the establishment of new populations in Colorado is contingent on the mobility of propagules. The present study suggests that the establishment of new populations is extremely unlikely on a human timescale, since all populations presently known in the state appear to be capable only of vegetative reproduction through proliferation of tubers. The only conceivable means of dispersal for the Colorado plants is the movement of these tubers by flooding or human activity. This is a rather effective means of dispersal if there are a large number of tubers to be dispersed as in the larger, more secure populations in the east. However, due to its rarity in Colorado, potential dispersal events are more likely to extirpate occurrences of Apios americana than to disperse them. The probability that a given tuber washed away in a flood will successfully reestablish itself is undoubtedly extremely low, even under ideal conditions. There are long odds that the tuber in question will actually be deposited in appropriate habitat downstream, and then successfully avoid predation to resprout. Where populations are large and tubers are abundant, an asymptotic survivorship curve can still result in successful dispersal and reproduction by random deposition of tubers. But in very small populations, stochastic processes such as this bring the statistical probability of successful dispersal to almost zero, and of extinction very near to P=1. In Colorado, the only tubers that could potentially be dispersed in this way are from plants on the bank of South Boulder Creek on the Van Vleet property. If there are 100 tubers present that make up the entire population at this site, and if 50 are washed away in a flood, the probability that any one of these tubers will survive and resprout is so low that this would probably result only in a loss of half of the population at the Van Vleet site and no successful dispersal.

It is possible that there is very little genetic variation between individual plants in Colorado. Since there is no potential for outcrossing or sexual reproduction, it is possible that all of the plants in Colorado arose from one or a few common ancestors. A genetic analysis of each of the populations would probably go a long way towards explaining the origin and history of *Apios americana* in Colorado.

#### **Assessment and Recommendations**

Given the current rarity and reproductive limitations to *Apios americana* in Colorado, the probability of extirpation due to human impacts, floods, competitive exclusion by exotic species, management changes, and stochastic processes is extremely high. Because the long term viability of this species is not secure in Colorado, special attention must be paid to careful management. Excellent management by the City of Boulder Open Space is probably the only reason the species still exists in Colorado at all. The following suggestions are offered based on observations made in this study. In many cases they probably require no changes be made in current management practices.

- 1. Continue to manage for the conservation of *Apios americana*. Current routing of trails and management practices by City of Boulder Open Space favor the persistence of this species.
- 2. Keep human visitation to an absolute minimum in areas with *Apios americana*. Continued attention to limiting human impacts in the vicinity of Van Vleet and White Rocks Natural Area will help increase the probability of long term viability at these occurrences.
- 3. Prevent livestock from entering occurrences of *Apios americana*. This species is highly palatable to horses and cattle and does not respond well to grazing (USDA NRCS 1999).
- 4. At the Cherryvale occurrence, *Apios americana* shoots were found in the haymeadow as far as three meters south from the fence. This occurrence would probably benefit from allowing the grass in that small part of the meadow to grow all summer without haying. This would allow the tubers in this part of the occurrence to produce long shoots in the summer and generate more photosynthate for further tuber development. Currently, tubers under the haymeadow can only produce very short shoots that probably contribute very little towards gross biomass accumulation. After senescence of the above-ground portions in the fall, the grass in the occurrence could also be hayed without affecting the plants.
- 5. Conduct additional research on *Apios americana* in Colorado. Inventory for new occurrences and updates of known occurrences should be conducted. Suitable habitat on private land should also be inventoried where possible. A low-impact monitoring program should be implemented that can assess long-term trends. Chromosomal counts should be conducted for all the Colorado populations. Electrophoretic data on the variability between genomes in Colorado and comparisons with other populations in other parts of the range of *Apios americana* could shed light on the origins our populations.
- 6. Manage weed infestations in the vicinity of *Apios americana* very closely. Two aggressive weed species, Canada thistle (*Breea arvensis*) and Teasel (*Dipsacus fullonum*) have a high affinity for *Apios americana* habitat and could competitively exclude it if left unchecked.

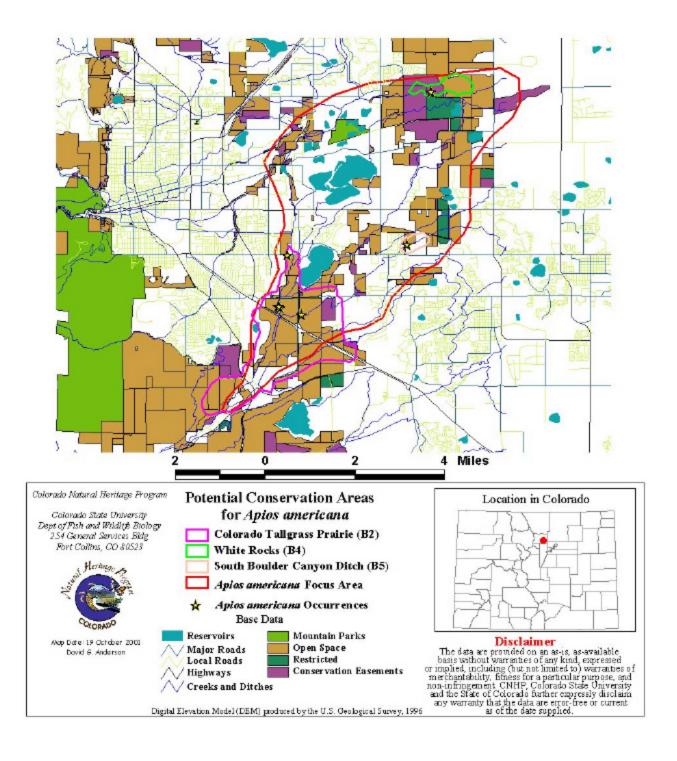
7. Efforts should be made to prevent groundwater alterations that would affect the flow of water in the seep that supports the White Rocks occurrence. If the flow of water from this seep stops, this occurrence will probably desiccate due to its southern exposure. Seeping water from this cliff also supports an occurrence of *Asplenium andrewsii*, a widely disjunct fern that is rare in Colorado. Many other interesting plants and plant associations are found here that would also be affected by groundwater alterations.

# Potential Conservation Areas for Apios americana

Three Potential Conservation Areas (PCAs) are currently drawn to include all the known occurrences of *Apios americana* and other natural heritage elements in the vicinity (Map 3- see following page). These areas represent our best estimate of the areas where conservation effort is needed to help sustain the known occurrences in the Boulder vicinity.

Also included on the map is a larger polygon that incorporates all the known occurrences and all the identified potential habitat for *Apios americana* in the Boulder vicinity. This "focus area" represents the area in which to base future search efforts and management for the species. Many areas, particularly those on private land, remain to be searched within this area.

Potential Conservation Areas for *Apios americana* in the City of Boulder Open Space



# **Specimens**

## City of Boulder Open Space Herbarium:

Boulder Co. T1S R70W S3 NW4. On Burke 1 property. By fence line on south side of Baseline Road west of South Boulder Creek by ditch. Elev. ca 5280ft/1609m. Wet meadow, at edge of road. With *Thalictrum dasycarpum, Spartina pectinata*, and grasses. Height 1.5m, flrs brownpurple. 17 Aug 1999. Nancy Neupert 5

Boulder Co. T1S R70W S3 NW4. On Burke I property. By fence line on south side of Basline Road, west of South Boulder Creek by ditch. Elev. ca. 5280ft/1609m. Wet meadow, at edge of road. With *Thalictrum dasycarpum, Spartina pectinata*, and grasses. Height 1.5 m, flowers brown-purple. 17 Aug 1999. Nancy Neupert 6

Boulder Co. T1N R69W S31 W2SW4. Spicer Property. On north bank of South Boulder Canyon ditch approximately 400ft east of driveway to 7763 Baseline Rd. Disturbed riparian ditch-bank with *Bromus inermis, Spartina pectinata, Apocynum androsaemifolium, Thalictrum, Salix fragilis*. Partial shade to open. Approximately 17 plants, only 1 in flower. Elev. ca. 5350ft. Native 24 Aug 1994 T. Nauman and N. Williams 413

Boulder Co. T1S R70W S10 NW4SE4. Van Vleet property. Along fence south of South Boulder Canyon ditch behind barnyard complex on east side of Cherryvale Road, approximately 1800ft. south of South Boulder Road, 300ft. east of Cherryvale Road, Irrigated wet meadow. Elev. ca. 5400ft. 11 June 1997 Nancy Neupert 2

#### University of Colorado Museum Herbarium:

Denver Co. Denver near Valverde Bridge. Aug 1887 A. Eastwood Accession # 23993

Boulder Co. In shady thicket, seepage area at base of cliffs, White Rocks, 8 mi. NE of Boulder. July 1, 1948 W.A. Weber 4211

Boulder Co. Spicer property, City of Boulder Open Space, west bank of irrigation ditch. Elevation 5350 ft. Niwot quad. Disturbed soil of an irrigation ditch bank. Associated species: *Apocynum cannabinum, Panicum virgatum, Elymus* sp., *Salix exigua, Asclepias speciosa, Aster eriocephala, Helianthus nuttallii, Spartina pectinata, Dactylis glomerata*. Magenta wings and standard, 3 to 12 individuals. 3 Aug 2000 D. Anderson, L. Riedel, N. Neupert. DA0049 (to be deposited)

#### Colorado State University Herbarium:

Boulder Co. In shady thicket, seepage area at base of cliffs, White Rocks, 8 mi. n.e. of Boulder. July 1, 1948 W.A. Weber 4211

Boulder Co. Ertl Property, City of Boulder Open Space, White Rocks Natural Area. Elevation: 5180 ft. Niwot quad. Seepy, south facing sandstone cliff. In partial shade and moist to saturated soil. Associated species: *Eupatorium maculosa, Asclepias speciosa, Solidago canadensis*,

*Thalictrum dasycarpum, Toxicodendron rydbergii.* 18 Aug 2000 D. Anderson, L. Riedel, B. Jennings. DA0051 (to be deposited)

Insect specimens will be deposited at the CSU Entomology Department.

# Tulane University Herbarium:

Boulder Co. Scarce, climbing over brush and weeds about seep. White Rocks, near Valmont. 10 August 1940. Ewan 12256.

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# **Appendix 1- The Natural Heritage Network and Methodology**

Colorado is well known for its rich diversity of geography, wildlife, plants, and plant communities. However, like many other states, it is experiencing a loss of much of its flora and fauna. This decline in biodiversity is a global trend resulting from human population growth, land development, and subsequent habitat loss. Globally, the loss in species diversity has become so rapid and severe that Wilson (1988) has compared the phenomenon to the great natural catastrophes at the end of the Paleozoic and Mesozoic eras.

The need to address this loss in biodiversity has been recognized for decades in the scientific community. However, many conservation efforts made in this country were not based upon preserving biodiversity; instead, they primarily focused on preserving game animals, striking scenery, and locally favorite open spaces. To address the absence of a methodical, scientifically-based approach to preserving biodiversity, Dr. Robert Jenkins, in association with The Nature Conservancy, developed the Natural Heritage Methodology in 1978.

Recognizing that rare and imperiled species are more likely to become extinct than common ones, the Natural Heritage Methodology ranks species according to their rarity or degree of imperilment. The ranking system is scientifically based upon the number of known locations of the species as well as its biology and known threats. By ranking the relative rareness or imperilment of a species, the quality of its populations, and the importance of associated Potential Conservation Areas, the methodology can facilitate the prioritization of conservation efforts so the most rare and imperiled species may be preserved first. As the scientific community began to realize that plant communities are equally important as individual species, this methodology has also been applied to ranking and preserving rare plant communities, as well as the best examples of common communities.

The Natural Heritage Methodology is used by Natural Heritage Programs throughout North, Central, and South America, forming an international database network. Natural Heritage Network data centers are located in each of the 50 U.S. states, five provinces of Canada, and 13 countries in South and Central America and the Caribbean. This network enables scientists to monitor the status of species from a state, national, and global perspective. It also enables conservationists and natural resource managers to make informed, objective decisions in prioritizing and focusing conservation efforts.

#### What is Biological Diversity?

Protecting biological diversity has become an important management issue for many natural resource professionals. Biological diversity at its most basic level includes the full range of species on Earth, from species such as bacteria, and protists, through multicellular kingdoms of plants, animals, and fungi. At finer levels of organization, biological diversity includes the genetic variation within species, both among geographically separated populations and among individuals within a single population. On a wider scale, diversity includes variations in the biological communities in which species live, the ecosystems in which communities exist, and the interactions between these levels. All levels are necessary for the continued survival of species and plant communities, and all are important for the well-being of humans. It stands to reason that biological diversity should be of concern to all people.

The biological diversity of an area can be described at four levels:

- 1. **Genetic Diversity** -- the genetic variation within a population and among populations of a plant or animal species. The genetic makeup of a species is variable between populations within its geographic range. Loss of a population results in a loss of genetic diversity for that species and a reduction of total biological diversity for the region. This unique genetic information cannot be reclaimed.
- 2. **Species Diversity** -- the total number and abundance of plant and animal species and subspecies in an area.

- 3. **Community Diversity** -- the variety of plant communities within an area that represent the range of species relationships and inter-dependence. These communities may be diagnostic or even restricted to an area. It is within communities that all life dwells.
- 4. **Landscape Diversity** -- the type, condition, pattern, and connectedness of natural communities. A landscape consisting of a mosaic of natural communities may contain one multifaceted ecosystem, such as a wetland ecosystem. A landscape also may contain several distinct ecosystems, such as a riparian corridor meandering through shortgrass prairie. Fragmentation of landscapes, loss of connections and migratory corridors, and loss of natural communities all result in a loss of biological diversity for a region. Humans and the results of their activities are integral parts of most landscapes.

The conservation of biological diversity must include all levels of diversity: genetic, species, community, and landscape. Each level is dependent on the other levels and inextricably linked. In addition, and all too often omitted, humans are also linked to all levels of this hierarchy. We at the Colorado Natural Heritage Program believe that a healthy natural environment and human environment go hand in hand, and that recognition of the most imperiled elements is an important step in comprehensive conservation planning.

# Appendix 2- Colorado's Natural Heritage Program

To place this document in context, it is useful to understand the history and functions of the Colorado Natural Heritage Program (CNHP).

CNHP is the state's primary comprehensive biological diversity data center, gathering information and field observations to help develop state-wide conservation priorities. After operating in Colorado for 14 years, the Program was relocated from the State Division of Parks and Outdoor Recreation to the University of Colorado Museum in 1992, and more recently to the College of Natural Resources at Colorado State University.

The multi-disciplinary team of scientists and information managers at CNHP gathers comprehensive information on the rare, threatened, and endangered species and significant plant communities of Colorado. Life history, status, and locational data are incorporated into a continually updated data system. Sources include published and unpublished literature, museum and herbaria labels, and field surveys conducted by knowledgeable naturalists, experts, agency personnel, and our own staff of botanists, ecologists, and zoologists. Information management staff carefully plot the data on 1:24,000 scale U.S.G.S. maps and enter it into the Biological and Conservation Data System. This locational information is incorporated into a GIS system (Arcview and Arcinfo). The Element Occurrence database can be accessed from a variety of angles, including taxonomic group, global and state rarity rank, federal and state legal status, source, observation date, county, quadrangle map, watershed, management area, township, range, and section, precision, and conservation unit.

CNHP is part of an international network of conservation data centers that use the Biological and Conservation Data System developed by The Nature Conservancy. CNHP has effective relationships with several state and federal agencies, including the Colorado Natural Areas Program, Colorado Department of Natural Resources and the Colorado Division of Wildlife, the U.S. Environmental Protection Agency, and the U.S. Forest Service. Numerous local governments and private entities also work closely with CNHP. Use of the data by many different individuals and organizations, including Great Outdoors Colorado, encourages a proactive approach to development and conservation thereby reducing the potential for conflict. Information collected by the Natural Heritage Programs around the globe provides a means to protect species before the need for legal endangerment status arises.

Concentrating on site-specific data for each element of natural diversity enables us to evaluate the significance of each location to the conservation of natural biological diversity in Colorado and in the nation. By using species imperilment ranks and quality ratings for each location, priorities can be established for the protection of the most sensitive or imperiled potential conservation areas. A continually updated locational database and priority-setting system such as that maintained by CNHP provides an effective, proactive land-planning tool.

# **Appendix 3- The Natural Heritage Ranking System**

Information is gathered by CNHP on Colorado's plants, animals, and plant communities. Each of these species and plant communities is considered an **element of natural diversity**, or simply an **element**. Each element is assigned a rank that indicates its relative degree of imperilment on a five-point scale (e.g., 1 = extremely rare/imperiled, 5 = abundant/secure). The primary criterion for ranking elements is the number of occurrences, i.e., the number of known distinct localities or populations. This factor is weighted more heavily because an element found in one place is more imperiled than something found in twenty-one places. Also of importance are the size of the geographic range, the number of individuals, trends in both population and distribution, identifiable threats, and the number of already protected occurrences.

Element imperilment ranks are assigned both in terms of the element's degree of imperilment within Colorado (its State or S-rank) and the element's imperilment over its entire range (its Global or G-rank). Taken together, these two ranks give an instant picture of the degree of imperilment of an element. For example, the lynx, which is thought to be secure in northern North America but is known from less than 5 current locations in Colorado, is ranked G5S1. The Rocky Mountain columbine, which is known only from Colorado, from about 30 locations, is ranked a G3S3. Further, a tiger beetle that is only known from one location in the world at the Great Sand Dunes National Monument is ranked G1S1. CNHP actively collects, maps, and electronically processes specific

occurrence information for plants considered extremely imperiled to vulnerable (S1 - S3). Those with a ranking of S3S4 are "watchlisted," meaning that specific occurrence data are collected and periodically analyzed to determine whether more active tracking is warranted. A complete description of each of the Natural Heritage ranks is provided in Table 1.

#### Table 1: Definition of Colorado Natural Heritage Imperilment Ranks

Global imperilment ranks are based on the range-wide status of a species. State imperilment ranks are based on the status of a species in an individual state. State and Global ranks are denoted, respectively, with an "S" or a "G" followed by a character. **These ranks should not be interpreted as legal designations.** 

**G/S1** Critically imperiled globally/state because of rarity (5 or fewer occurrences in the world/state; or very few remaining individuals), or because of some factor of its biology making it especially vulnerable to extinction.

**G/S2** Imperiled globally/state because of rarity (6 to 20 occurrences), or because of other factors demonstrably making it very vulnerable to extinction throughout its range.

G/S3 Vulnerable through its range or found locally in a restricted range (21 to 100 occurrences).

**G/S4** Apparently secure globally/state, though it might be quite rare in parts of its range, especially at the periphery.

**G/S5** Demonstrably secure globally, though it may be quite rare in parts of its range, especially at the periphery.

**GX** Presumed extinct.

**G#?** Indicates uncertainty about an assigned global rank.

**G/SU** Unable to assign rank due to lack of available information.

**GQ** Indicates uncertainty about taxonomic status.

**G/SH** Historically known, but not verified for an extended period, usually.

**G#T#** Trinomial rank (T) is used for subspecies or varieties. These taxa are ranked on the same criteria as G1-G5.

**SR** Reported to occur in the state, but unverified.

S? Unranked. Some evidence that species may be imperiled, but awaiting formal rarity ranking.

Notes: Where two numbers appear in a state or global rank (e.g., S2S3), the actual rank of the element falls between the two numbers.

#### **Element Occurrence Ranking**

Actual locations of elements, whether they be single organisms, populations, or plant communities, are referred to as **element occurrences**. The element occurrence is considered the most fundamental unit of conservation interest and is at the heart of the Natural Heritage Methodology. In order to prioritize element occurrences for a given species, an element occurrence rank (EO-Rank) is assigned according to their ecological quality whenever sufficient information is available. This ranking system is designed to indicate which occurrences are the healthiest and ecologically the most viable, thus focusing conservation efforts where they will be most successful. The EO-Rank is based on 3 factors:

**Size** – a quantitative measure of the area and/or abundance of an occurrence such as area of occupancy, population abundance, population density, or population fluctuation.

**Condition** – an integrated measure of the quality of biotic and abiotic factors, structures, and processes within the occurrence, and the degree to which they affect the continued existence of the occurrence. Components may include reproduction and health, development/maturity for communities, ecological processes, species composition and structure, and abiotic, physical or chemical factors.

**Landscape Context** – an integrated measure of the quality of biotic and abiotic factors, and processes surrounding the occurrence, and the degree to which they affect the continued existence of the occurrence. Components may include landscape structure and extent, genetic connectivity, and condition of the surrounding landscape.

Each of these factors is rated on a scale of A through D, with A representing an excellent grade and D representing a poor grade. These grades are then averaged to determine an appropriate EO-Rank for the occurrence. If there is insufficient information available to rank an element occurrence, an EO-Rank of E is assigned. Possible EO-Ranks and their appropriate definitions are as follows:

- A The occurrence is relatively large, pristine, defensible, and viable.
- **B** The occurrence is small but in good condition, or large but removed from its natural condition and/or not viable and defensible.
- The occurrence is small, in poor condition, and possibly of questionable viability.
- **D** The occurrence does not merit conservation efforts because it is too degraded or not viable.
- H Historically known, but not verified for an extended period of time.
- **X** Extirpated.
- **E** Extant. The occurrence does not contain enough information to rank using the above ranks.
- **F** The occurrence was not relocated; failed to find.

# **Appendix 4- Potential Conservation Areas**

In order to successfully protect populations or occurrences, it is helpful to delineate Potential Conservation Areas. These PCAs focus on capturing the ecological processes that are necessary to support the continued existence of a particular element occurrence of natural heritage significance. Potential Conservation Areas may include a single occurrence of a rare element or a suite of rare element occurrences or significant features.

The goal of the process is to identify a land area that can provide the habitat and ecological processes upon which a particular element occurrence, or suite of element occurrences, depends for their continued existence. The best available knowledge of each species' life history is used in conjunction with information about topographic, geomorphic, and hydrologic features, vegetative cover, as well as current and potential land uses. **The proposed boundary does not automatically recommend exclusion of all activity.** It is hypothesized that some activities will prove degrading to the element or the process on which they depend, while others will not. Specific activities or land use changes proposed within or adjacent to the preliminary conservation planning boundary should be carefully considered and evaluated for their consequences to the element on which the conservation unit is based.

## Potential Conservation Planning Boundaries

Once the presence of rare or imperiled species or significant plant communities has been confirmed, the first step towards their protection is the delineation of a **preliminary** conservation planning boundary. In general, the potential conservation area boundary is our best estimate of the primary area supporting the long-term survival of targeted species and plant communities. In developing such boundaries, CNHP staff considered a number of factors that include, but are not limited to:

- the extent of current and potential habitat for the elements present, considering the ecological processes necessary to maintain or improve existing conditions;
- species movement and migration corridors;
- maintenance of surface water quality within the PCA and the surrounding watershed;
- maintenance of the hydrologic integrity of the groundwater;
- land intended to buffer the PCA against future changes in the use of surrounding lands;
- exclusion or control of invasive exotic species;
- land necessary for management or monitoring activities.

As the label "conservation planning" indicates, the boundaries presented here are for planning purposes. They delineate ecologically sensitive areas where it is recommended that land-use practices be carefully planned and managed to ensure that they are compatible with protection goals for natural heritage resources and sensitive species. Please note that these boundaries are based primarily on our understanding of the ecological systems and thorough analyses of the human context and potential stresses were not conducted. All land within the conservation planning boundary should be considered an integral part of a complex economic, social, and ecological landscape that requires wise land-use planning at all levels.

#### **Off-Site Considerations**

Furthermore, it is often the case that all relevant ecological processes cannot be contained within a PCA of reasonable size. Taken to the extreme, the threat of ozone depletion could expand every PCA to include the whole globe. The boundaries illustrated in this report signify the immediate, and therefore most important, area in need of protection. Continued landscape level conservation efforts are needed. This will involve county-wide efforts as well as coordination and cooperation with private landowners, neighboring land planners, and state and federal agencies.

#### Ranking of Potential Conservation Areas

One of the strongest ways that CNHP uses element and element occurrence ranks is to assess the overall biodiversity significance of a PCA, which may include one or many element occurrences. Based on these ranks, each PCA is assigned a **biodiversity** (or B-) **rank**:

- **B1** Outstanding Significance: only location known for an element or an excellent occurrence of a G1 species.
- **B2** <u>Very High Significance</u>: one of the best examples of a community type, good occurrence of a G1 species, or excellent occurrence of a G2 or G3 species.
- **B3** High Significance: excellent example of any community type, good occurrence of a G3 species, or a large concentration of good occurrences of state-rare species.
- **B4** <u>Moderate or Regional Significance</u>: good example of a community type, excellent or good occurrence of state-rare species.
- **B5** <u>General or State-wide Biodiversity Significance</u>: good or marginal occurrence of a community type, S1, or S2 species.

If an element occurrence is unranked due to a lack of information the element occurrence rank is considered a C rank. Similarly, if an element is a GU or G? it is treated as a G4.

## **Protection Urgency Ranks**

Protection urgency ranks (P-ranks) refer to the time frame in which conservation protection should occur. In most cases, this rank refers to the need for a major change of protective status (e.g., agency special area designations or ownership). The urgency for protection rating reflects the need to take legal, political, or other administrative measures to alleviate threats that are related to land ownership or designation. The following codes are used to indicate the rating which best describes the urgency to **protect** the area:

- **P1** Immediately threatened by severely destructive forces, within 1 year of rank date; protect now or never!
- **P2** Threat expected within 5 years.
- **P3** Definable threat but not in the next 5 years.
- **P4** No threat known for foreseeable future.
- P5 Land protection complete or adequate reasons exists not to protect the PCA; do not act on this PCA.

A protection action involves increasing the current level of legal protection accorded one or more tracts within a PCA. It may also include activities such as educational or public relations campaigns or collaborative planning efforts with public or private entities to minimize adverse impacts to element occurrences in an area. It does not include management actions. Threats that may require a protection action are as follows:

- 1) Anthropogenic forces that threaten the existence of one or more element occurrences at a PCA; e.g., development that would destroy, degrade or seriously compromise the long-term viability of an element occurrence and timber, range, recreational, or hydrologic management that is incompatible with an element occurrence's existence;
- 2) The inability to undertake a management action in the absence of a protection action; e.g., obtaining a management agreement;
- 3) In extraordinary circumstances, a prospective change in ownership or management that will make future protection actions more difficult.

## **Management Urgency Ranks**

Management urgency ranks (M-ranks) indicate the time frame in which a change in management of the element or PCA should occur. This rank refers to the need for management in contrast to protection (e.g., increased fire frequency, decreased herbivory, weed control, etc.). The urgency for management rating focuses on land use management or land stewardship action required to maintain element occurrences at the potential conservation area.

A management action may include biological management (prescribed burning, removal of exotics, mowing, etc.) or human use management (building barriers, rerouting trails, patrolling for collectors, hunters, or trespassers, etc.). Management action does not include legal, political, or administrative measures taken to protect a potential conservation area. The following codes are used to indicate the action needed to be taken at the area:

- M1 Management action required immediately or element occurrences could be lost or irretrievably degraded within one year.
- M2 New management action will be needed within 5 years to prevent the loss of element occurrences.
- M3 New management action will be needed within 5 years to maintain current quality of element occurrences.
- **M4** Although not currently threatened, management may be needed in the future to maintain the current quality of element occurrences.
- M5 No serious management needs known or anticipated at the PCA.

# **Appendix 5- Element Occurrence Records**