ASSESSMENT OF EXPERIMENTAL OUTPLANTINGS OF THE ENDANGERED VENTURA MARSH MILKVETCH (ASTRAGALUS PYCNOSTACHYUS VAR. LANOSISSIMUS)



Prepared by:

Mary E. Meyer South Coast Region California Department of Fish and Game 4949 Viewridge Avenue San Diego, California 92123

November 30, 2007

For the California Department of Fish and Game and United States Fish and Wildlife Service Section 6 Contract E-2-P-22

| Tab | Table of Contents | | |
|-----|--|----|--|
| I | Executive Summary | 1 | |
| II | Project Overview and Current Status | 3 | |
| | Current Population Status | 6 | |
| III | Biology of Ventura Marsh Milkvetch | 8 | |
| IV | Approach and Methodology | 19 | |
| | 2004 Plantings | 20 | |
| | Propagation | 20 | |
| | Site Selection | 22 | |
| | Installation | 23 | |
| | Maintenance | 31 | |
| | Monitoring the 2002 and 2004 Plantings | 33 | |
| V | Habitat Characteristics | 33 | |
| | General Habitat at the Wild Site | 37 | |
| | General Habitat at the Outplanting Sites | 37 | |
| | South Ormond Beach | 37 | |
| | Mandalay State Beach | 39 | |
| | McGrath State Beach | 40 | |
| | Carpinteria Salt Marsh Reserve | 42 | |
| | Coal Oil Point Reserve | 44 | |
| VI | Climate, Hydrology and Soils | 50 | |
| | Climate and Rainfall Patterns | 50 | |
| | Hydrology | 56 | |
| | Flooding and Inundation | 56 | |
| | Perched Water Tables | 58 | |
| | South Ormond Beach | 59 | |
| | Wild Site | 60 | |
| | Mandalay State Beach | 61 | |
| | McGrath State Beach | 61 | |
| | Carpinteria Salt Marsh Reserve | 63 | |
| | Coal Oil Point Reserve | 63 | |
| | Coal Oil Point Reserve - Lagoon | 63 | |
| | Coal Oil Point Reserve - Pond | 64 | |
| | Soils | 64 | |
| VII | Monitoring Results and Discussion | 67 | |

| | Original 2002 Outplants | 67 | | |
|------|--|-----|--|--|
| | Survivorship | 67 | | |
| | Growth and Flowering | 70 | | |
| | Flower and Fruit Production | 75 | | |
| | Seed Production at Key Sites | 78 | | |
| | 2004 Supplemental Plantings | 80 | | |
| | Survivorship | 80 | | |
| | Growth and Flowering | 84 | | |
| | Wild Site in 2006 | 89 | | |
| | Natural Recruitment | 92 | | |
| | 2003 Observations | 99 | | |
| | 2004 Observations | 100 | | |
| | 2005 Observations | 101 | | |
| | 2006 Observations | 103 | | |
| | Comparison of First Year Natural Recruits | 105 | | |
| | Seed Dormancy Observations | 116 | | |
| | Seed Dispersal Observations | 109 | | |
| VIII | Milkvetch Health and Diseases | | | |
| | Sudden Onset Decline in Full Flower on Whole Milkvetch Patch | 113 | | |
| | Sudden Onset Decline in Full Flower on Single Individuals | 115 | | |
| | Miniaturization | 117 | | |
| | Spots and Weak Chlorophyll | 117 | | |
| | Tissue Senescence and Blackening | 118 | | |
| IX | Herbivory | | | |
| | Gastropod Herbivory | 119 | | |
| | Snails | 120 | | |
| | Snail Management | 120 | | |
| | Slugs | 121 | | |
| | Small Mammal Herbivory | 121 | | |
| | Gophers | 122 | | |
| | Rabbits | 123 | | |
| | Herbivory Symptoms Attributed to Rabbits | 124 | | |
| | Rabbit Herbivory Effects on Vegetation at Planting Sites | 125 | | |
| | Vegetative Structure Favoring Rabbit Herbivory | 126 | | |
| | Seasonal Variations in Rabbit Herbivory and Milkvetch Response | 127 | | |
| | Rabbit Herbivory at the Wild Site | 128 | | |

| | Direct Seeding Trials | 160 | |
|----|---|-------------------|--|
| | Outplanting dates | 160 | |
| | Seed germination | 160 | |
| | Suggested Revisions to Methods and Protocols | | |
| XI | Recommendations for Future Modifications | | |
| | Associated Vegetation at Poorly Performing Dry Sites | 157 | |
| | Juvenile Establishment | 157 | |
| | General Trends in Milkvetch and Vegetative Cover at Key Sites | 155 | |
| | Seasonal Variation in Vegetative Cover | 154 | |
| | General Vegetation Trends across the Study Period | 153 | |
| | Non-Native Weeds | 144 | |
| | Native and Naturalized Species | | |
| x | Associated Vegetation | 144 | |
| | Skunks | 143 | |
| | Moles | 143 | |
| | Other Animal Direct and Indirect Effects on Milkvetch | 143 | |
| | Other Organisms Found under Isopod Traps | 141 | |
| | Management Response | 141 | |
| | Management Recommendations for Argentine Ant Control Isopod Herbivory | 140 | |
| | Other Argentine Ant Effects on Milkvetch | 139 | |
| | Aphids, Argentine Ants and Milkvetch | <u>138</u> 139 | |
| | Seed Beetles | 137 | |
| | Damage to Milkvetch | 136 | |
| | Moth and Butterfly Caterpillars | 135 | |
| | Arthropod Herbivory | 135 | |
| | Disruption of Vole Runways | 135 | |
| | Duration of Caging and Exclosures | 135 | |
| | Overgrowth of Vegetation in Cages and Exclosures | 134 | |
| | Large Exclosures | 134 | |
| | Individual Caging of Milkvetch | 133 | |
| | Chemical Herbivory Deterrents | 133 | |
| | Small Mammal Herbivory- Management Strategies | 133 | |
| | Milkvetch Palatability and Selenium | 130 | |
| | Ground Squirrels | 130 | |
| | Meadow Voles | 129 | |

| | Herbivory Exclosures | 160 | | | |
|------|---|------|--|--|--|
| | Planting Configuration | 160 | | | |
| | Ongoing Management is Critical | 161 | | | |
| | Site Selection Criteria | | | | |
| | Other Potential Outplanting Situations | 165 | | | |
| XII | Conclusion | 167 | | | |
| | Acknowledgements | 170 | | | |
| XIII | Literature Cited | | | | |
| | | | | | |
| Pho | tographs | Page | | | |
| 1 | Ventura marsh milkvetch in full flower at MSB-4 in the summer of 2004 | 2 | | | |
| 2 | Ventura marsh milkvetch fruits in various stages of maturity | 9 | | | |
| 3 | Exposed root crown on planted individual in early winter, prior to reshooting | 10 | | | |
| 4a | Infructescences retained on previous year's stem, April 4, 2004, MSB-4 | 11 | | | |
| 4b | Ground littered with pods and infructescences | 12 | | | |
| 5 | | | | | |
| 6 | MSB-4 original plot area in summer of 2005 following flooding | 15 | | | |
| 7a | Ventura marsh milkvetch fruits in various stages of maturity | | | | |
| 7b | Grey and orange woolly bear caterpillar on milkvetch plant | | | | |
| 8 | MAND-1 plot in June 2003. | | | | |
| 9 | Scientific aid Anne McLeod examines a single VMMV surviving at CSMR-2, in summer 2003 | | | | |
| 10 | Naturally recruited juvenile in May 2003 | 19 | | | |
| 11 | | | | | |
| 12 | | | | | |
| 13a | Wild site at North Shore, a group of juveniles in Colony "A" in June 2003 | 36 | | | |
| 13b | Several adult plants in full flower in "Colony B" area, in 2003 | 36 | | | |
| 14a | South Ormond planting site, wetland transition zone | 38 | | | |
| 14b | | | | | |
| 15 | Dense masses of naturally recruited juveniles in early summer at MSB-5, following | 42 | | | |
| | flooding of the plot earlier that winter in 2005 | | | | |
| 16 | Center of the CSMR-5 plot in May 2003, looking south | | | | |
| 17 | Center of CSMR-Plot 5, in summer of 2004, looking north | | | | |
| 18 | COPR Lagoon, August 9, 2004. Looking south | | | | |

| 19 | COPR-Pond in August 2004 | 47 | | |
|-------|--|----------|--|--|
| 20 | COPR-Pond in August 2005 | | | |
| 21 | COPR-Pond. August, 2005 | | | |
| 22 | | | | |
| 23 | | | | |
| 24 | | | | |
| 25 | MSB-4, August 2004, looking northeast | 54 55 | | |
| 26 | Sudden loss of soil moisture or water table at COPR-Pond, August 2004 | 114 | | |
| 27 | Moisture stress showing stages of color loss as vigor declines | 118 | | |
| 28 | Miniaturization on right, normal growth on left, CMSR-5, 2003 | 116 | | |
| 29 | Miniaturization on reshooting CSMR-5 plant, 2004 | 117 | | |
| 30 | Severely herbivorized area at CSMR 5C | 83 | | |
| 31 | Sudden decline of mature individual in full flower, CSMR-5, 2003 | 115 | | |
| 32 | Young juvenile milkvetch in twiggy litter | 92 | | |
| 33 | Milkvetch and arroyo willow juveniles are protected amongst stems emerging from | 93 | | |
| | below ground rhizomes of Mexican or Baltic rush | | | |
| 34 | Juvenile at MSB-3 is being overtopped by goldenrod and western ragweed | 93 | | |
| 35 | COPR-Lagoon in May 2007 | 98 | | |
| 36 | Masses of naturally recruited juveniles at CSMR-5C in mid-June, 2006 | | | |
| 37 | Abundant milkvetch juveniles amongst competitors in March 2005 on previously | 102 | | |
| | inundated ground in the central MSB-3 plot | | | |
| 38 | Milkvetch seed magnified 30 times, viewed through digital camera and eyepiece of | 108 | | |
| | compound microscope | | | |
| | | | | |
| | | | | |
| Aeria | al Photographs | Page | | |
| 1 | Mandalay State Beach and North Shore wild site, October 23, 1979. | 34 | | |
| 2 | South Ormond Beach planting site. Plot behind cypress trees. | 37 | | |
| 3 | Coal Oil Point Reserve-Pond introduction site | 45 | | |
| | | | | |
| | | | | |
| Maps | 6 | Page | | |
| 1 | Vicinity | 24 | | |
| 2 | Ventura/Oxnard Area | 25 | | |
| 3 | Carpinteria Salt Marsh Reserve | 26 | | |

| 4 | Coal Oil Point Reserve | 27 | |
|-------|--|-------|--|
| | | | |
| Figu | 7 6 5 | Page | |
| | | | |
| 1 | Annual rainfall by water year at various sites | 51,52 | |
| 2 | Depth to water table at various sites in centimeters | 59 | |
| 3 | Survivorship of 2002 outplants at Carpinteria Salt Marsh Reserve | 67 | |
| 4 | Survivorship of 2002 outplants at the McGrath State Beach sites | 68 | |
| 5 | Mean height of longest shoot in original 2002 outplants | 74 | |
| 6 | Mean number of flowering shoots in the original 2002 outplants | 74 | |
| 7 | Mean number of inflorescences on randomly selected stem | 74 | |
| 8 | Percent survival of 2004 outplants at more favorable planting sites | 80 | |
| 9 | Number of milkvetch surviving at more favorable planting sites | 81 | |
| 10 | 2004 outplants- mean height of tallest shoot | 87 | |
| 11 | 2004 outplants- mean number of flowering shoots | 87 | |
| 12 | 2004 outplants- mean number of inflorescences on randomly selected stem | 88 | |
| 13 | Mean number of shoots produced on first year plants, 10-11 months old, at key | 89 | |
| | sites; comparing sunny and shady locations with milkvetch planted in tree shelters | | |
| 14 | Number of naturally recruited juveniles documented at various sites during the | 97 | |
| | August monitoring sessions | | |
| 15 | Height of juveniles in early May 2003 at the McGrath State Beach and Carpinteria Salt Marsh Reserve sites | 100 | |
| 16 | Mean height of naturally recruited first year flowering and non-flowering cohorts at | 106 | |
| | key sites | | |
| 17 | Total vegetation cover at key sites | 156 | |
| 18 | Total milkvetch cover at key sites | 156 | |
| | | | |
| | | | |
| Table | es | Page | |
| 1 | Overall status of Ventura marsh milkvetch at all sites in August 2006 | 7 | |
| 2a | eed production at key sites in 2003 | | |
| 2b | Seed production at key sites in 2004 | 78 | |
| 3 | 2004 Outplants- Planting dates, number planted and percent survival through summer 2006 | 23 | |
| 4 | Trees and Shrubs Closely Associated with Ventura Marsh Milkvetch Growing Sites | 146 | |

| | Harbonesus Diante Classic Associated with Martune March Millaustab Crowing | 4 4 7 | | |
|------|---|-------|--|--|
| 5 | Herbaceous Plants Closely Associated with Ventura Marsh Milkvetch Growing | 147 | | |
| | Sites | 151 | | |
| 6 | Invasive Weeds Associated with Ventura Marsh Milkvetch Growing Sites | | | |
| 7 | Plant Species Associated with Poorly Performing Dry Sites | 159 | | |
| 8 | 2002 Outplants- Survivorship at experimental introduction sites through August | 68 | | |
| | 2006 | | | |
| 9 | 2002 Outplants- Growth and flowering in their second growing season, August | 72 | | |
| | 2003 | | | |
| 10 | 2002 Outplants- Growth and Flowering 2004-2006 | 73 | | |
| 11a | Flower and fruit production at key sites in 2003 | 76 | | |
| 11b | Flower and fruit production at key sites in 2004 | 76 | | |
| 12 | Theoretical estimate of seed production in selected plants. 2002 estimate based | 79 | | |
| | on data from Soza et al. 2003 | | | |
| 13 | 2004 Outplants- Survivorship, growth and flowering in 2004 | 85 | | |
| 14 | 2004 Outplants- Survivorship, growth and flowering in 2005 and 2006 | 86 | | |
| 15 | Monitoring status of wild population of Ventura marsh milkvetch in August 2006 | 91 | | |
| 16 | Naturally Recruited Cohorts- Survivorship and flowering of 2003, 2004 cohorts | 95 | | |
| 17 | Naturally Recruited Cohorts- Survivorship and flowering in 2005 cohorts | 96 | | |
| 18 | Naturally Recruited Cohorts- Survivorship and flowering in 2005 cohorts | 97 | | |
| 19 | Status and flowering in naturally recruited cohorts- 2006 | 104 | | |
| 20 | Distance of 2003 juveniles from the closest parent in centimeters | 110 | | |
| | | | | |
| Арре | ndix | | | |
| | Appendix A. Maternal lines that produced seed | 175 | | |
| | | | | |

I. EXECUTIVE SUMMARY

This report describes a multi-year effort from 2003-2006 to establish experimental outplantings of the state and federally endangered Ventura marsh milkvetch (*Astragalus pycnostachyus* var. *lanosissimus*) at coastal locations in Ventura and Santa Barbara Counties in California. Ventura marsh milkvetch (VMMV) is a short lived robust perennial herb in the pea (Fabaceae) family which was historically collected from coastal locations ranging from the City of Ventura to northern Orange County. It was presumed to be extinct until its rediscovery in 1997, when 66 reproductive plants and 307 associated juveniles were observed growing on imported fill capping a closed oil waste dump site in backdunes near Oxnard. Efforts to establish additional populations of this endangered plant are critical to furthering its recovery but have been hampered by limited information on habitat preferences at historic collection sites where VMMV were once known to occur and now appear to be extirpated.

To further identify potentially suitable habitat and better understand its biological requirements, this project introduced nursery-raised juvenile milkvetch plants into a variety of locations and observed the response of the plants over the study period. Planting areas were located in either coastal dune or coastal estuary systems. Most planting sites were associated with wetland margins or were otherwise proximate to a perched water table.

Three hundred nursery-raised juvenile milkvetch were installed in 2002 at ten microsites at McGrath State Beach and Carpinteria Salt Marsh Reserve by our contractor, Rancho Santa Ana Botanic Garden. This report describes overall survivorship, growth, flowering and fruit production in these individuals starting in their second summer, 2003. VMMV have persisted and produced naturally recruited individuals at four of the original ten 2002 introduction sites. At three of these sites near McGrath Lake, the planting areas were flooded in 2005, and planted and naturally recruited individuals did not survive the inundation. The introduced populations recovered following dry-down when new milkvetch emerged from seed produced by the founder plants, and at two sites, milkvetch now grow in new locations from flood-dispersed seed. The new locations represent the outermost, drier margins of the wetland transition zone on the east banks of McGrath Lake. Microsites in dry low dunes and in high saltmarsh proved unsuitable and milkvetch did not persist at these locations.



Photo 1. Ventura marsh milkvetch in full flower at MSB-4 in the summer of 2004.

An additional 227 plants were installed at four coastal sites in April 2004. Plants did not establish at South Ormond Beach, as microsites in high salt marsh proved unsuitable, hypersaline conditions occurred in summer, and high gopher activity on the more upland portion removed plants in late summer/fall. The Coal Oil Point Reserve-Pond 2004 plantings largely failed due to extreme dry conditions in summer 2004 and loss of the local water table. This site also experiences periodic inundation during wet winters which has drowned established plants. Additional plantings at the Carpinteria Salt Marsh Reserve and Coal Oil Point Reserve Lagoon site were relatively successful and a significant spike in natural recruitment occurred at both sites in 2006. Factors influencing survival and growth of planted individuals include the distance to a perched water table, salinity, availability of low competition growing sites and vegetation gaps, and herbivory by small mammals like rabbits and non-native snails. Emergence of natural recruits from seed produced by introduced plants has been documented at most locations. At more favorable sites, some naturally recruited plants have achieved sufficient growth to flower and produce seed in their first year, which has not been documented at the "wild" site in Oxnard. At many of the introduction sites, milkvetch exhibited greater overall size, vigor and reproductive output than is typically seen at the wild site, where underground oil contamination hampers plant growth and moisture stress is common, particularly in years of low rainfall.

This report describes the general habitat characteristics, weather patterns, hydrology and soils at various introduction sites, summarizes the results of annual monitoring efforts, and includes observations on natural recruitment, seed dormancy and dispersal. Herbivory by native small mammals, isopods, non-native snails and others is described and management strategies employed to address herbivory are described. We recommend modifications to site selection criteria to better locate areas suitable for introduction. We describe additional potential outplanting situations that could be explored and suggest some revisions to the methods and protocols used to date to initiate introductions. Further investigation into factors which may influence observed differences in milkvetch palatability and herbivory levels between different sites is recommended. Most introduction sites require some level of active management and further work to increase population size at successful test outplanting locations will be necessary to further recovery of VMMV.

II. PROJECT OVERVIEW AND CURRENT STATUS

This report summarizes a multi-year effort from 2002-2006 to examine survivorship, reproduction and mortality in experimental outplantings of the state and federally endangered Ventura marsh milkvetch (VMMV, or milkvetch) at coastal locations in Ventura and Santa Barbara Counties. The Ventura marsh milkvetch (*Astragalus pycnostachyus* var. *lanosissimus*) is a short-lived perennial member of the pea family (Fabaceae). Its existence was documented in a handful of collections made in the late 1800's through the early 1930's from coastal areas in Los Angeles, Ventura and Orange Counties. Dr. Rupert Barnaby believed it was likely extinct at the time he described it as a unique variety in 1964. A few years later, the last known living Ventura marsh milkvetch plant was collected in 1967 from a roadside ditch across from the entrance to McGrath State Beach near the Santa Clara River in Ventura. The milkvetch was not seen again until August 1997, when a population was

discovered growing on imported fill capping a closed oil waste dump site located on private land in backdune habitat near West 5th Street and Harbor Boulevard, about 2.5 miles south of the 1967 sighting near McGrath State Beach. The new population in 1997 consisted of 374 plants growing in an area under an acre, 65 of which had flowered and produced fruit, while the remainder was non-flowering juveniles (Ikeda and Meyer, 2000).

Shortly after the exciting discovery of an extant population in 1997, the California Department of Fish and Game (CDFG) and United States Fish and Wildlife Service (USFWS) worked cooperatively with the developer who planned to clean up the belowground oil contamination at the oil waste site and develop residential housing in a project called North Shore at Mandalay Bay. The wildlife agencies convened a team of local scientific experts who have met periodically to discuss conservation of the milkvetch. The South Coast Region of the CDFG developed an informal Conservation Strategy for VMMV, which identified a series of steps to obtain information needed to better understand VMMV's biological and ecological requirements with the goal of eventually establishing new populations and furthering recovery within suitable habitat in its historic range.

The primary challenge facing recovery of Ventura marsh milkvetch is our poor understanding of its ecosystem and habitat requirements. It has been collected on only two occasions since 1911, and very little habitat information was documented from historic collection sites. The "wild" site discovered in 1997 has been entirely modified by human activity. Aerial photography shows that in the vicinity of where VMMV now grows, no vegetation was extant in 1983 following closure of the oil waste dump (see **Aerial Photo 1**, from 1979). Vegetation gradually recovered across the site over the next decades and the milkvetch area now supports a mix of native shrubs typically found in wetland and upland habitats. However, the combination of imported fill and below-ground oil wastes has created growing conditions that do not readily correspond with those found in more natural coastal habitats. VMMV's habitat requirements are therefore unclear. In the absence of good historic habitat information, our approach involved introducing juvenile, nursery-grown milkvetch into different types of coastal wetland transitional habitat areas and observing its response in an attempt to better understand its niche.

A series of efforts were undertaken since 1997 to increase our knowledge of the Ventura marsh milkvetch's horticultural, life history, and habitat requirements. These are summarized briefly below:

• Phase I. 1996-2001. Collection of seed from the wild population and storage for long

term conservation at Rancho Santa Ana (RSABG) and Santa Barbara Botanic Gardens (SBBG). Seeds stored by maternal line. Establishment of seed germination protocols and propagation of plants in controlled environments at RSABG. This effort is largely summarized in Soza, et al. (2003).

- Phase II. 2000-2001. Dr. Dieter Wilken and Trisha Wardlaw from the SBBG undertook a life history and ecological habitat assessment for the population at the wild site, evaluated the habitat of the closest relative, the Northern brine milkvetch (*Astragalus pycnostachyus* var. *pycnostachyus*), examined potentially suitable introduction sites in coastal Ventura and Santa Barbara Counties, and made general recommendations for experimental introductions. This effort is summarized in Wilken and Wardlaw (2001).
- Phase III. 2001-2003. Three hundred twenty two milkvetch were propagated from known maternal lines and installed by RSABG in spring of 2002 at McGrath State Beach (MSB) in Ventura County and Carpinteria Salt Marsh Reserve (CSMR) in Santa Barbara County. The principal investigators were Valerie Soza, Michael Wall and Dylan Hannon. Enclaves of around 30 plants were installed in five differing microsites at each location. Survivorship, reproduction and mortality were examined at the various sites, and the results through February 2003 are summarized in Soza, et al. (2003).
- Phase IV. 2003-2007. The CDFG took over direct management and monitoring of the original 2002 outplants in March of 2003. We contracted with RSABG to propagate an additional 150 milkvetch from known maternal lines. These were installed in spring 2004 in the vicinity of a successful plot at the CSMR-5 and at eight other planting plots at four additional sites including Ormond Beach near Pt. Mugu Naval Air Station south of the City of Oxnard (Aerial Photo 2); Mandalay State Beach, very close to the wild site (Aerial Photo 1); and the Coal Oil Point Reserve (COPR) near Devereux Slough adjoining the campus at UC Santa Barbara in Goleta (Aerial Photo 3).

This report summarizes the results of the Phase IV project and describes work starting with the introduction of supplemental plants in spring of 2004. We have tracked survivorship, reproduction and mortality for the original outplants installed in 2002, the supplemental plants installed in 2004, and naturally recruited individuals at all sites. By planting milkvetch in a variety of locations and habitat conditions, we have learned a great deal about the factors influencing survivorship and natural recruitment and have improved our understanding of what constitutes suitable habitat for this variety and how it interacts with other plants and animals in its environment.

CURRENT POPULATION STATUS

Table 1 summarizes the current status of reproductive adults and juveniles at the wild site and at eight introduction sites. Tallies of individuals naturally recruited from seed produced by the introduced plants are shown in bold. The wild site supported 60 reproductive adults and 44 juveniles in August 2006. At the introduction sites, the majority of reproductive adult plants seen in 2006 (N= 148) are naturally recruited individuals which emerged in spring 2005 from the seedbank at three of the five McGrath State Beach plots following repeated inundation and flooding of the planting locations during the extremely wet winter of 2005. This seed was produced by the original outplants installed in 2002 which prolifically flowered and produced fruit during their second growing season in 2003, and to a lesser extent in 2004. The CSMR-5C and COPR-Lagoon sites exhibited a large increase in juvenile survival and establishment in 2006 from seed produced primarily by supplemental plants installed at the sites in 2004. The remaining sites have modest to low numbers of adults and exhibit low levels of natural recruitment leading to older age classes.

General decline in the 2002 outplants over the course of four years was expected, as milkvetch are believed to live only about 3-4 years (Wilken and Wardlaw, 2001). Natural recruitment has been sufficient to maintain modest-sized milkvetch populations at four of the ten original 2002 outplanting plots. The MSB-3 plot has exhibited an increasing population trend from naturally recruited plants and in 2006, it supported three times the number of adult plants and twice the amount of occupied habitat (165 sq meters) compared with the original outplanting (48 sq meters). The MSB-4 plot has exhibited a stable trend and similar amount of occupied habitat (50 sq meters) as the original 2002 plantings. A spatial shift occurred following the 2005 floods at both plots, and flood-recruited populations now occupy new areas adjacent to the original planting sites. The 2004 planting sites have had mixed results, exacerbated, in part, by extreme weather conditions in the initial installation year (2004, rainfall 75% of normal) and 2005 (very wet). For locations near Ventura, for example, water year 04/05 was the fourth wettest year on record with over twice normal rainfall (VCWPD, 2007).

Our observations over four growing seasons suggest the primary factors affecting survivorship, reproductive output and recruitment at various planting sites include: depth to a perched or near-surface water table, variation in rainfall resulting in episodes of extreme drought or extreme flooding, salinity, competition with associated plant species, and herbivory by non-native snails, small mammals like rabbits, isopods and others. In the following sections, we summarize the observations and data collected from March 2003 through the fall of 2006.

| SITE | REPRODUCTIVE ADULTS | YEAR RECRUITED* | NON- FLOWERING JUVENILES | 2006 NON- FLOWERING JUVENILES | 2006 JUVENILES FLOWERING |
|---------------------------------|------------------------|--------------------|--------------------------------|--|--------------------------------|
| WILD SITE | 60 | | 48 | | |
| MSB-3 E (NORTH) | 76 | 2005 | | 26 | 1 |
| MSB-3 | 29 | 2005 | 10 | 1 | |
| MSB-4 E (SOUTH) | 29 | 2005 | 1 | | |
| MSB-5 | 14 | 2005 | 25 | | |
| CSMR-5 | 3 | 2002 | | 30 | |
| | 1 | 2004 | | | |
| | 1 | 2005 | | | |
| CSMR-5B | 1 | 2004 | | 25 | |
| CSMR-5C | 11 | 2004 | | 232+/- | 114 +/- |
| | 1 | 2005 | | | |
| COPR LAGOON | 13 | 2004 | | 338+/- | 167 +/- |
| | 1 | 2005 | | | |
| COPR POND | 1 | 2004 | | 3 | |
| | 1 | 2005 | | | |
| MAND-1 | 5 | 2003 | | | |
| | 3 | 2005 | 16 | | |
| MAND-1A | 1 | 2004 | | | |
| MAND-1B | 8 | 2004 | 2 | | |
| TOTAL AT EXPERIMENTAL SITES: | 199 | | 54 | 655 +/- | 282 +/- |

| Table 1. | Overall status of Ventura marsh milkvetch at all sites in August 2006. Natural recruits in |
|----------|--|
| | bold. Sites: MSB - McGrath State Beach; CSMR - Carpinteria Salt Marsh Reserve; COPR - |
| | Coal Oil Point Reserve; MAND – Mandalay State Beach. |

* Year adults were planted or naturally recruited CSMR 5C and COPR-Lagoon 2006 recruits include counted and estimated individuals. Flowering estimated 33%. Non-flowering juveniles at all introduction sites are naturally recruited from 2005.

III. BIOLOGY OF VENTURA MARSH MILVKETCH

The Ventura marsh milkvetch (Astragalus pycnostachyus var. lanosissimus) is a short-lived perennial herb in the Fabaceae (Pea family) which produces stout leafy annual growth stems from a near-surface root crown. The variety was described by Barnaby (1964) from herbarium specimens, all of which were collections from the late 1880's and early 1900s. The variety lanosissimus has densely white-lanate (woolly) herbage, almost silvery when young (Barnaby, 1964). The underlying foliage is a muted sage green color. Barnaby describes the stems as hollow, but this is not the case in plants observed during this study. Barnaby describes the stems as measuring four to nine decimeters (40-90 cm) in height. In this study, the largest stem produced was 227 cm (7.5 feet), and the mean height of the longest stems produced at favorable, mesic growing locations in 2003 ranged from 100-170 Ventura marsh milkvetch has compound leaves with 27-39 leaflets (Barnaby, 1964) cm. and lower stipules that are fused around the stems of the plant, forming a sheath (Hickman, 1993). Flower color is reported as greenish-white or cream (Barnaby, 1964); ochroleucous (yellowish white) (Munz, 1974). In living specimens, both the calyx tube and flower are a very pale butter yellow with a hint of green (Photo 5). Young buds exhibit a kiss of pink on the banner. Inflorescences are dense spikelike racemes supporting backwardly imbricated flowers, 7-10 millimeters long, with a keel measuring 7-9 mm (Barnaby, 1964). Fruits are small (8-11 mm), deflexed and very slightly inflated with an incurved persistent style forming a curved beak (Photo 2). Fruits produce 8-12 ovules, although we have rarely seen more than six mature seeds. The fruits are reported as very tardily dehiscent which is consistent with our observations (Barnaby, 1964). Some pods and relatively intact infructescences are commonly retained on the annual growth stems into the next growing season (Photo 4a).



Photo 2. Ventura marsh milkvetch fruits and flowers.

The stems are reported as erect (Munz, 1974; Barnaby, 1964). This is generally the case, but robust plants growing in mesic locations in full sunlight produce stems which, by summer, spread laterally from their weight and sometimes lie on the ground, exposing the center of the plant's base. In full sunlight, the stout stems are a dark intense maroon color while younger, newer growth stems are green. This pigment appears to be a response to sunlight- stems in shaded locations lack the intense maroon color and appear greener. A stem lying beneath other plants will exhibit the maroon color along only that portion of the stem exposed to sunlight. The stems are generally stout and firmly attached to the root crown. On larger individuals with good vigor, side branches form at a 45 degree angle, producing a broad, flat fan-like branch that could be described as shaped like an inverted chevron. These lateral side branches tend to snap more easily than the primary stems.

Snapped or otherwise partially broken branches sometimes are able to remain alive while only connected to the plant by a small amount of vascular tissue.

Annual growth stems are produced each year from a superficial or shallowly-buried root crown. This root crown is sometimes visible on planted individuals and appears as a swollen, enlarged woody base that supports meristematic tissue across its surface (**Photo 3**). New shoots are produced on the surface of the root crown, and enlarge over time. On older planted individuals with an exposed root crown, one can sometimes see that the new shoots of the year tend to form on the outer periphery of the root crown while the older central portion exhibits decay.



Photo 3. Exposed root crown on planted individual in early winter, prior to reshooting. The stems are still physiologically active and support both green and maroon pigments.

Milkvetch typically start to produce new annual growth stems in mid January in most years. Sometimes, they still have green growth tips and upper leaves on the previous year's stems as new annual growth shoots are produced. In 2004, we observed that plants subjected to severe herbivory as they attempted to reshoot had some prior year annual growth stems that remained alive and these stems produced new shoots at the nodes, with leaves and flowers that next summer. Live growth stems still retain epithelial pigment and green vascular tissue, while truly dead stems become brown then fade to grey with age

(Photo 3).

Some members of the genus *Astragalus* are known nitrogen fixers. Parker (2006) examined the root system of a single VMMV growing at the Coal Oil Point nursery site and found no evidence of root nodules indicative of associated nitrogen fixers. Impact Sciences (1998) indicated no nodules were observed on the roots of an excavated individual.

Ventura marsh milkvetch produces abundant inflorescences and infructescences (**Photo 2; 4b**). Flowering typically starts in late June, with peak flowering in late July to early August. At sites where moisture is not limiting, flowering continues into October. Meanwhile, fruits ripen more or less continuously, and the majority is ripe by November. When ripe, loose hardened seeds can be heard rattling inside the slightly inflated dry pods when the stem is sharply tapped.



Photo 4a. Infructescences retained on previous year's stem, April 4, 2004, MSB-4



Photo 4b. Ground littered with pods and infructescences. Young horseweed (*Conyza canadensis*, bright green) is co-occurring at MSB-4.

Wilken and Wardlaw (2001) examined seed set in bagged and unbagged inflorescences. Their observations suggest Ventura marsh milkvetch is self-compatible and perhaps partly autogamous. Open-pollinated flowers produced 2.8 seeds per fruit (1.9 mature and 0.9 empty) while bagged flowers produced 2.0 seeds per fruit (1.7 mature and 0.3 empty). Empty ovules were reported as damaged by seed weevils. In this study, mean number of mature seeds per fruit produced in 2003 and 2004 at introduced sites ranged from 2.93-4.45 while the wild population in 2003 produced the lowest number (2.71) (**Table 2a, 2b**).

Wilken and Wardlaw (2001) observed few insect pollinators during their observations of the wild population in 2000. We commonly observed insect activity at most introduction sites. The most common visitor has been tentatively identified as *Bombus vosnenenskii* (pale yellow band on abdominal segment number 2 and yellow on the top of the head and face)(Hogue, 1993) (**Photo 5a**). A few individuals can be observed over many hours foraging through a patch. At MSB-5 during a summer monitoring session, several of these bumble bees spent the day foraging between milkvetch and adjacent sand bar willow (*Salix exigua*).



Photo 5a. Bumble bee visiting Ventura Marsh milkvetch inflorescence.

Female carpenter bees (probably *Xylocopa* sp.) also visit milkvetch, especially at the McGrath Lake sites where they are also attracted to Hooker's evening primrose (*Oenothera elata* ssp. *hookeri*) which produces large yellow flowers (**Photo 6**). Marine blue (*Leptotes marina*) (**Photo 5b**) and common hairstreak (*Strymon melinus*) butterflies visit milkvetch flowers. Honey bees (*Apis mellifera*) are often observed visiting milkvetch at the CSMR which is adjacent to residential areas, but are less frequently seen at other locations. Small native bees and wasps are infrequently seen. Consumption of developing pods and ovules by various caterpillars and damage to seed by seed weevils has been documented (**Photos 7a,7b**).



Photo 5b: Leptotes marina



Photo 6. MSB-4 original plot area in summer of 2005 following flooding. Rebar corresponds with the location of previously-introduced 2002 milkvetch, which all died from inundation. The central plot area now exhibits dense, continuous thatch produced by expansion of herbaceous rhizomatous perennials. Hooker's evening primrose (yellow flowers) is abundant. Vegetation gaps are no longer present and western ragweed, which previously dominated this central area prior to milkvetch introduction, has been displaced by the expanding obligate wetland plants. Seedlings which emerged in this area following flooding could not keep pace with growth of competing vegetation, experienced unmanageable levels of snail damage, and failed to establish.



Photo 7a. Ventura marsh milkvetch fruits in various stages of maturity. Developing pods are turgid, green and purple (in sunlight). Hardened, ripe pods (right) have contracted slightly in size and the ventral suture is now apparent. Common hairstreak caterpillar is consuming ovules (arrow). Holes through the pod valves are visible on several fruits.



Photo 7b. Grey and orange woolly bear caterpillar on milkvetch plant.

Barnaby (1964) reports this variety is a perennial. We now know from observation over the last ten years since the plant's rediscovery that individuals live between three-five years. One planted individual in this study is currently five years, six months old and vigorously reshooting as of May 2006 (**Photo 8**). Based upon observations over the course of this study, first year naturally recruited juveniles growing in favorable locations, estimated to be at least five months old in early August, can become reproductive if they reach sufficient size. The production of multiple shoots on first year plants and reaching a height of at least 40 cm (1.5 feet) is generally an indication that the individual may mature sufficiently to flower and produce fruit in its first year. In contrast, plants at the wild site do not flower in their first year, and only 50% of the plants flowered in their second year (Wilken and Wardlaw, 2001). Compacted soils, shallow rooting depth above the oil contamination, and loss of soil moisture in late summer likely contribute to the slower development and lower vigor seen at the wild site compared with some introduction areas.

Milkvetch typically achieve their maximum size and vigor in their second growing season at favorable locations where moisture is not limiting (**Photo 9**). Plant vigor usually declines gradually in subsequent years as plants age. Milkvetch produce considerable litter in areas where experimental populations have been introduced. The ground in winter can be covered in dropped leaves, fallen infructescences and pods, while old stems and twigs are abundant (**Photo 4a, 4b**).



Photo 9. Scientific aid Anne McLeod examines a single VMMV surviving at CSMR-2, in summer 2003. Adjacent rebar corresponds with locations where milkvetch were planted in 2002 and failed to establish, probably due to excessive salinity. This is a typical size mature adult in maximum vigor in its second growing season.

Ventura marsh milkvetch appears capable of producing new plants from seed on an annual basis without noticeable disturbance triggers. Seed produced at the original introduction sites in fall 2002 resulted in some seedlings observed as early as February, 2003 (Soza et al., 2003) (**Photo 10**). Seedlings are observed after episodes of heavy rainfall and in water year 2004/2005, carpets of seedlings germinated starting as early as two weeks after heavy rainfall in mid-October 2004 with new cohorts appearing periodically into May. *Astragalus* is known to produce some seed with prolonged dormancy of over 40 years and the presence of a seed bank is likely (Ikeda and Meyer, 2000). The resurrection of Ventura marsh milkvetch at the wild site provides indirect proof of a dormant seedbank-since no living vegetation was present in the area where milkvetch now grow at the time the oil waste site was closed in 1982, it appears likely the population established from dormant seed brought in from some other location or somewhere nearby. We discuss seed dormancy and dispersal mechanisms in more detail in a later section of this report.



Photo 10. Naturally recruited juvenile in May 2003, first observed by RSABG in February of 2003, in the central portion of MSB-3. This individual has already produced at least three shoots, remains short and compact yet is producing large leaves. It successfully matured and flowered in the plot later that summer. Note the adjacent western ragweed, a common milkvetch associate, and rejected leaves and tips on the ground, associated with rabbit herbivory. By August of 2003, herbivores had entirely removed most of the western ragweed co-occurring in what became a very dense milkvetch patch.

IV. APPROACH AND METHODOLOGY

Our approach for establishment of experimental outplantings of Ventura marsh milkvetch was to introduce nursery-grown juvenile plants into different habitats in order to observe their survivorship, growth, flowering and reproduction, thereby increasing our understanding of habitat preferences and tolerances for this species. The CDFG's VMMV conservation strategy included the goal of establishing multiple colonies at each of five protected sites along the Ventura and southern Santa Barbara County coast. The 2002 and 2004 milkvetch installations represent test outplantings aimed to providing an initial

indication of the suitability of the various sites to support one or more viable milkvetch populations. We anticipated the certain planting sites would be more successful than others. The planting effort described here represents the first steps toward examining habitat preferences and further work to increase the size of populations and occupied habitat will be needed at favorable locations to further recovery.

We initiated work to identify potential introduction sites by contracting with the SBBG. Through that effort, Wilken and Wardlaw (2001) identified several potential introduction locations and prepared a relative ranking system based upon five criteria they judged important to the survival of milkvetch. The five criteria focused on coastal sites with native shrub cover under 75%; low cover of herbaceous species and exotic weeds; close proximity to a fresh or brackish water table; relatively compact, stable sandy substrates; and presence of adjacent buffer habitat supporting insect pollinators. As a result of this analysis, the top three ranked introduction sites were located near McGrath Lake/backdunes at McGrath State Beach, the upper margins of the Carpinteria Salt Marsh, and in backdunes at the south end of Mandalay State Beach. An area near Calleguas Creek at Point Mugu Naval Air Station ranked reasonably well, but the fifth location, Emma Woods State Beach, appeared to lack appropriate planting sites and therefore was ranked last.

Based upon these recommendations, the CDFG contracted with RSABG to initiate experimental introductions at the McGrath State Beach and Carpinteria Salt Marsh locations in 2001. We obtained authorization to initiate this effort from the management authority at each site- the California Department of Parks and Recreation and the University of California Natural Reserve. Five plots were selected at each location and planted with container-grown juvenile plants introduced in April 2002.

Both the Wilken and Wardlaw (2001) and Soza, et al. (2003) reports should be reviewed in their entirety as they serve as the background for the current effort described here. The latter report also includes important information on the initial 2002 outplanting sites at MSB and CSMR.

2004 PLANTINGS

Propagation

The CDFG contracted with RSABG, in Claremont, California, to produce 150 supplemental Ventura Marsh milkvetch plants for outplanting, intended for delivery in early

January, 2004. Procedures for seed germination and nursery conditions under which milkvetch were raised are generally as described in Soza, et al., 2003. The original source of all seeds was the North Shore wild population and largely stem from seed collections made in 1997, 1998 and 1999. Collections also included seed collected from pods produced in 1996, which were still retained on the old annual growth stems in the fall of 1997. The maternal lines of these original seed collections have been tracked throughout the various introduction projects.

In 1998, the USFWS contracted with RSABG to produce a set of milkvetch plants under nursery conditions for the purposes of bulking, or increasing, the number of available seeds we anticipated needing for experimental introductions. Soza, et al. (2003) describes this effort in detail. These nursery-raised plants exhibited a modest amount of flowering and fruiting in July 2000 (at an age of about 1.5 years). It was subsequently determined that seed set was very low. The health and vigor of the nursery-grown plants was initially strong, but gradually waned, attributed potentially to root stress in pots (Soza, et al. 2003).

In January 2001, we decided to transfer the remaining mother plants from RSABG to an outdoor in-situ nursery site on the immediate coast, at the Coal Oil Point Reserve (COPR) in Goleta, southern Santa Barbara County. Dr. Cristina Sandoval, director of the COPR, oversaw this installation and maintained the nursery site for several years. Over 6000 seeds were obtained that fall from 16 plants representing 12 maternal lines. The COPR nursery site was also the location where about 20 VMMV previously propagated by the Sunburst Plant Disease Clinic in Turlock (at the request of the North Shore developers) were relocated on March 11, 2000. The maternal lines of these plants are unknown, but they lived for several years and produced seed which has been stored for experimental work.

For this supplemental planting effort, the source of seeds used were primarily from Coal Oil Point-2001 regenerated seed (11 maternal lines); RSABG 2000 regenerated seed (2 maternal lines) and 3 maternal lines taken directly from the 1997 wild site collection. Ventura marsh milkvetch seeds were germinated on August 8 and September 5, 2003. Four hundred twenty eight seeds were germinated from 16 known maternal lines. Two hundred thirty two (54%) were transferred to 2 inch pots in late September to mid-October 2003. Milkvetch were transferred to tree tube containers in early to mid-January 2004. The tree tube containers were 25 cm (10 inches) long by 6 cm (2.5 inches) wide, and slightly tapered toward the bottom, with longitudinal grooves that encourage downward root growth. Nine died after transfer to the containers. Two hundred twenty three milkvetch plants were

delivered in good condition the last week of March 2004.

Site Selection

The initial strategy for installing supplemental plants was aimed at increasing the number of maternal lines and total plant numbers at the most successful, original 2002 outplanting sites. Our assumption was that certain planting plots would perform better than others, and to further recovery, follow-up plantings to increase the number of individuals and maternal lines would therefore be desirable. However, because four of the five CSMR plots performed poorly, and because the Department of Parks and Recreation requested that additional plants not be installed at the McGrath Lake area, we needed to locate additional planting sites to receive the 223 supplemental milkvetch.

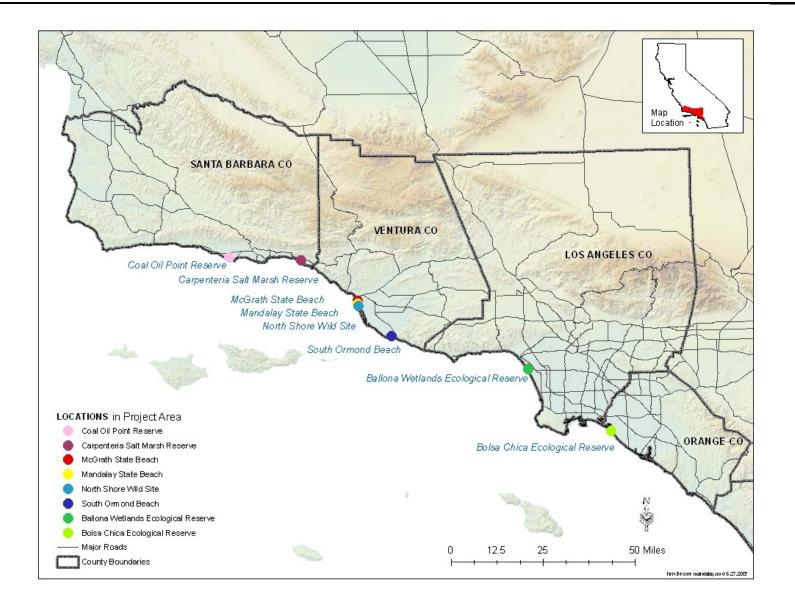
The two critical factors influencing our decision for selecting additional introduction sites were the lack of protected coastal habitats in the local area and support from landowners/managers to allow the introduction of an endangered species into the area. Both Wayne Ferren, Jr., Director of the CSMR and Dr. Cristina Sandoval, Director of the COPR were supportive of our efforts to conserve VMMV and were amenable to introducing milkvetch into appropriate areas on the reserves. Given the shortage of coastal wetlands in Ventura County, we decided with the input of other local experts that VMMV introduction into coastal wetlands in southern Santa Barbara County was acceptable although we have no historic records of it having been collected there.

All 2004 planting sites were located on the near coast in Ventura and/or Santa Barbara Counties (**Map 1**). We selected localized planting locations adjacent or near coastal wetlands associated with dune systems or coastal estuaries and lagoons (**Map 2**). The specific sites chosen for plantings were sometimes placed in or near locations where we had previously experimented with small scale planting trials in 2003 using surplus plants and/or Coal Oil Point-nursery bulked seed, and had a favorable response from planted milkvetch. Two receiver sites were chosen at the COPR at Goleta, just north of Santa Barbara, called the "Lagoon" and "Pond" sites (**Map 3**). A small planting area about twenty meters south of the Lagoon site was also used, called "Jaumea". Additional receiver sites were selected at Mandalay State Beach in the vicinity of an informal planting site called MAND-1 (**Map 4**). This general area was evaluated and included in Wardlaw and Wilken's 2000 report. A third site was located at south Ormond Beach on lands owned by the California Coastal Conservancy (**Aerial Photo 2**).

Installation

The 2004 outplantings were installed at the various sites from late March through mid-May. Plants awaiting installation were held temporarily out-of-doors in Matilija Canyon near Ojai, about fifteen miles inland from the coast, and placed under the shade of large sycamore trees and were watered every few days. Trays of tree tubes were kept on a table about 1 meter above the ground to reduce the chances of pests such as slugs or isopods becoming established in or beneath the pots. **Table 3** shows the planting dates for various sites and the number of individuals installed at each site.

A mix of various maternal lines was installed at each site. Appendix A lists the maternal lines represented at each study site which produced seed during at least one season following installation. All plants were installed by Mary Meyer and/or Anne Macleod, with the exception of the COPR- Lagoon where we received assistance from Valentine Jervisu from RSABG; and CSMR-5 B and C where Bob Sorrel, a volunteer, assisted.







Map 4. Coal Oil Point Reserve



| SITE | PLANTING DATE 2004 | NUMBER PLANTED | ALIVE SUMMER 2004 | PERCENT SURVIVAL THRU SUMMER 2004 | ALIVE SUMMER 2005 | PERCENT SURVIVAL THRU SUMMER 2005 | ALIVE SUMMER 2006 | PERCENT SURVIVAL THRU SUMMER 2006 |
|-------------|--------------------------|-------------------|-------------------------|---|-------------------------|---|-------------------------|---|
| | | | | | | | | |
| Ormond | 26-Mar | 40 | 7 | 17.5 | 0 | 0 | 0 | 0 |
| MAND-1A | 6-Apr | 10 | 4 | 40 | 3 | 30 | 1 | 10 |
| MAND-1B | 6-Apr | 10 | 4 | 40 | 0 | 0 | 0 | 0 |
| CSMR 5B | 7-Apr | 15 | 14 | 93 | 2 | 13 | 1 | 6 |
| CSMR 5C | 7-Apr | 25 | 25 | 100 | 20 | 80 | 11 | 44 |
| COPR-LAGOON | 12-Apr | 54 | 49 | 90.7 | 31 | 57 | 13 | 24 |
| COPR-JAUMEA | 12-Apr | 6 | 6 | 100 | 0 | 0 | 0 | 0 |
| MAND-1C | 26-Apr | 15 | 8 | 53 | 7 | 47 | 7 | 47 |
| COPR-POND A | 6-May | 10 | * | * | 0 | 0 | 0 | 0 |
| COPR-POND C | 6-May | 5 | * | * | 0 | 0 | 0 | 0 |
| COPR-POND D | 6-May | 8 | * | * | 1 | 13 | 1 | 13 |
| COPR-POND B | 14-May | 17 | * | * | 0 | 0 | 0 | 0 |
| TOTAL | | 215 | 117 | 54% | 64 | 30% | 34 | 16% |

 Table 3. 2004 Outplants- Planting dates, number planted and percent survival through summer 2006.

* Plants in severe decline due to abrupt drop in water table

Plant installation was relatively straightforward and required two people. Holes were dug by hand with a spade. The holes were just big enough to accept the milkvetch root mass. Soil from the hole was placed on a tarp or newspaper and the milkvetch were carefully lifted from their tree tubes by slightly bending the sides to loosen, then inverting the tube while holding the plant and soil mass in one hand (**Photo 11**). Milkvetch were placed in the planting hole, and the excess native soil returned and tamped down around the newly installed plant. Typically, one person held the tree tube soil and root mass together while the second person placed the fill soil back into the hole. This method helped prevent root breakage, as the milkvetch roots were still fairly delicate during the installation period, particularly shortly after delivery in March. Placement of soil onto a tarp or newspaper reduced disturbance to adjacent vegetation and helped ensure enough soil to return to the planting holes. Care was taken to position the milkvetch bases even with ground level. This placement resulted in the root crowns sometimes developing at the soil surface (see **Photo 3**) rather than just below the ground surface (see **Photo 12**). All plants were given about ½ gallon of freshwater following installation.



Photo 11. Inverted planting tube showing young roots which have formed on nursery-raised juvenile. Newspaper was in the bottom of the tube to reduce soil loss out of the drain holes.



Photo 12. Naturally-recruited plant with a root crown just below the surface. Severe herbivory on new annual growth shoots forming on the near-surface root crown has occurred. The root crown continues to attempt to produce new stems (nibbled green shoots). Damage likely caused by rabbits, although other herbivores may also contribute. Epithelium on the previous year's growth stems has been stripped off, probably by sow or pill bugs.

Below-ground gopher exclusion cages were generally not used, with the exception of CSMR- 5B and 5C. At this location, ½ inch diameter hardware cloth was used to make a cone that was placed in the ground, and milkvetch were installed in the cone. The cone was about 38 cm (15 inches) deep and 25 cm (10 inches) across at the top. The tip was folded down to close it and the side wired closed. A decision was made to use hardware cloth rather than chicken wire, because the chicken wire cages previously installed had broken down rapidly because of soil salinity. The cone shape was relatively easy to make, but was not ideal and considerable time was spent making sure no air pockets were left between the outside of the cone and the surrounding planting hole. The small diameter of the holes in the hardware cloth was also problematic- as milkvetch grew in size and girth, some roots were observed near the surface girdled by the hardware cloth. Despite this problem, plant growth did not appear to be hampered by the cages.

Generally, little or no site preparation occurred prior to installation. The exception was the vicinity of CSMR-5, where considerable weeding had previously occurred. During subsequent visits, however, weeding and occasionally thinning of competing native vegetation occurred at most introduction sites.

Across the various planting sites, milkvetch were typically installed about one to two

meters apart and scattered across available areas lacking shrub cover, exhibiting lower herb cover with some bare ground and usually beyond the drip lines of established willows or coyote brush. At the COPR- Lagoon, we installed plants in enclaves of three, each about 0.75 meters apart, and each enclave was then separated by a few meters.

Maintenance

The spring 2004 supplemental plants were hand watered periodically following installation. Potable water lacking chlorine (typically, water was either from Matilija Creek near Ojai or from a groundwater well in Matilija Canyon) and was hand carried to planting sites in partially filled 5 gallon buckets, then poured slowly around the base of the plant from a 1 gallon plastic bottle. Sites were re-visited within one to two weeks of installation, and an additional ½ gallon of fresh water was applied during these visits. Following initial planting and watering, the new plants were generally watered three to six times during the spring and summer growing period, with no supplemental irrigation provided after September 2004. Watering interval ranged from three to four weeks. CSMR-5B and 5C were last watered on July 5. There has been no further irrigation at any of the planting sites.

Maintenance activities for the 2002 and 2004 plantings included selective removal or partial removal of non-native weeds, occasional thinning of native competitors, occasional trimming of branches of woody neighbors, removal of ice plant near planting areas, hand removal of non-native snails, localized periodic snail baiting using *Deadline*, and installation of temporary wire herbivory exclusion cages. In spring 2004, we also applied several herbivory deterrents that appeared ineffective. Maintenance activities specific to herbivory are described in more detail in a later section of this report.

MONITORING THE 2002 AND 2004 PLANTINGS

All milkvetch planting areas were periodically visited, typically about once a month, and observations and notes taken on their vigor, condition, reproductive status, the presence of seedlings/juveniles of the year and management needs. Planted individuals were given a unique identifying number that included the maternal line in order to allow tracking survival by line at each site. Naturally recruited juveniles were generally tagged and numbered, sometimes during May monitoring visits, or later over the summer as they were observed. Unique identifiers were used noting the recruitment year (i.e. 03-27 for a 2003 recruit). Where very dense and abundant juvenile recruitment occurred, we were not always able to mark all the plants due to the time involved and difficulties separating and identifying different

individuals.

Generally, numbers of seedlings/juveniles of the year were either counted with an absolute number, or roughly estimated when growing in dense masses. We usually census seedlings/juveniles of the year sometime in May of each spring. Prior to May, seedlings/juveniles can disappear quickly, often due to cropping by herbivores, and efforts to mark or count them are time consuming. In 2003, we recorded information on the location and size of juveniles at the MSB and CSMR-5 plots in early May. We measured the distance of each seedling/juvenile from the root crown of the nearest potential parent plant, in centimeters. We measured the height of juveniles from the base to the tip of the longest leaf. We recorded the micro-topographic position of juveniles, categorized as either depressions, slopes, mounds or flat ground. In 2004, we selected a haphazard set of naturally recruited plants at the MSB-4 site, and marked 27 plants scattered throughout the planting area in order to track growth and survival. The remaining juveniles were marked only with pin flags. In 2005, large numbers of juveniles were present at the MSB sites following dry down of previously inundated areas. We therefore marked a haphazardly selected set of thirty plants at each site from throughout the plots and followed those over the next summer.

Each summer during peak flowering, generally in late July through early August, a formal monitoring assessment was conducted for planted and naturally recruited reproductive plants. For each plant monitored, we recorded the total height of the longest stem in centimeters (from base to apical meristem). We counted the total number of vegetative and reproductive shoots. For flowering individuals, we then randomly selected a stem and counted all the inflorescences on that stem. To randomly select the stem and reduce bias, we visually focused on the base of the plants while ignoring the tops of the stems, picked a starting stem and counting direction, and then used a random number generated by the calculator to come up with a branch to sample. If the chosen branch was vegetative, the next branch was selected. Occasionally, in dense patches were milkvetch were large and stems and individuals were overlapping (generally, the MSB locations) we sometimes could not reach the selected stem without risking breakage, and had to discard that selection and pick another random number/stem. So, our sampling may have occasionally been biased against sampling buried stems. In 2003, we were unable to reach eight plants in the center of MSB-3 and 1 plant in MSB-5 because the plants were so large and co-mingled.

We obtained estimates of flower, fruit and seed production for the original outplants at various planting sites in 2003 and 2004. We randomly chose ten individuals from each planting site and haphazardly selected one inflorescence from each individual during the main

summer monitoring session. We counted the total number of flowers on each inflorescence. In early November, we again randomly chose ten individuals from each site and haphazardly selected one infructescence from each sampled plant for analysis. We counted the total number of pods on each infructescence, total number of pods containing seed and total number of damaged pods (pods with a caterpillar or weevil hole). We then removed the seed from the pods and tallied the total number of seeds produced on that infructescence. We counted all observable seeds that exhibited weevil damage. We kept separate tallies for seed which had a black, thickened looking seed coat. We also pulled a single sample of 100 seeds from each site and reported the 100 seed weight in grams. We did not obtain a mean for this measurement.

We made sketch maps for each planting plot that allowed us to track physical locations within the plot. The sketch maps were sometimes used in the field to record information or delimit the edge of shrub cover and extent of patches of associated vegetation. During the August monitoring visit, we generally assessed the associated vegetation in the vicinity of the immediate area where milkvetch were planted or growing. We typically estimated cover for the dominant, most important species in the plot and listed the species growing at the time of the visit. We also took baseline photographs of the overall planting area during the August monitoring visit.

V. HABITAT CHARACTERISTICS

The general location of the wild-North Shore population and outplanting sites in coastal areas of Santa Barbara and Ventura Counties is shown on **Map 1. Map 2, 3**, and **4** show the general location of introduction sites. Sites ranged in elevation from 4-11 feet msl. Specific elevations were difficult to obtain for some locations where detailed topographic maps were not available. All planting sites were relatively flat to slightly sloping. Plant species associated with the wild site and various outplanting sites are listed in **Tables 4, 5**, and **6** and are discussed in more detail in the Associated Species section later in this report.

GENERAL HABITAT AT THE WILD SITE

The North Shore wild site is located immediately east of Harbor Boulevard north of Oxnard and south of Ventura (**Map 2**). The 91 acre area of backdunes was a permitted oil field waste disposal facility which operated from 1954 through 1981 and the site has been

vacant since October 1982 (CRWQCB, 2000). Several large basins were used to spread oil wastes (**Aerial Photo 1**). Clean material was used to cap, or cover, the surface area where oil wastes had been spread. Upon closure, vegetation gradually reestablished at the site to varying degrees. In August, 1997, Kate Symonds of the USFWS and Morgan Wehtje with CDFG observed an unusual plant which was subsequently identified by botanist Tim Thomas of the USFWS as the presumed extinct Ventura marsh milkvetch. The milkvetch area is at an elevation of about 30 feet msl and is relatively flat but slopes slightly to the northwest (Impact Sciences, 2005).



Aerial Photo 1. Mandalay State Beach and North Shore wild site, October 23, 1979. Red trianglevicinity of MAND-1 planted in 2003. Turquoise triangle- vicinity of MAND-1B, planted in 2004. Yellow arrow corresponds with the general location of the wild North Shore population. Note the series of basins where oil wastes are being deposited in background, and lack of vegetation in and around the location where milkvetch now grow.

The low basins where oil was concentrated were vegetated by a mix of upland and facultative wetland shrubs that developed over the decades following closure. The milkvetch population established in the southeast corner of the western basin, and abutted the staging area where oil waste materials were brought in and spread into the larger adjoining basin. The habitat area abuts a berm of higher ground which was used for vehicle access and staging operations. Vegetation over most of the western basin was still very sparse and patchy until recent remediation activities, initiated in fall of 2006, removed the vegetation there. The southeast corner where VMMV occur continues to support high cover of shrubs and has more developed shrub-dominated vegetation than other nearby locations.

Wilken and Wardlaw (2001) provide a general overview of the site and conditions there. Dominant shrubs include coyote brush (*Baccharis pilularis*) and mulefat (*B. salicifolia*). Scattered deer weed (*Lotus scoparius*), occasional laurel sumac (*Malosma laurina*), California sagebrush (*Artemisia californica*) and arroyo willow (*Salix lasiolepis*) also occur. Few native herbs occupy the area and the primary annual grass present is rabbit foot grass (*Polypogon monspeliensis*). Several problematic weeds are present, including tocolote (*Centaurea melitensis*) and white sweet clover (*Melilotus alba*). Patches of bare ground occur, sometimes exhibiting a white fine texture that is likely remnant bentonite, which was reportedly use to cap the oil wastes along with a mix of imported fill. Heavy litter occurs in portions of the milkvetch habitat with the highest shrub cover. Wilken and Wardlaw (2001) estimated about 90% cover of vegetation at the site in 2000. Native dune vegetation occurs southeast of the milkvetch habitat area and was modified by remediation in 2006.

Two "colonies" of milkvetch were originally discovered in 1997, termed Colony "A" and Colony "B". The two colonies are separated by about 8 meters (26 feet). Colony A has declined in recent years and only four milkvetch juveniles were growing there in 2006. It has very low shrub cover and extensive areas of compacted, fine soils. Colony B is associated with areas of higher shrub cover, more ground litter, is better protected from drying Santa Ana winds and likely benefits from fog drip associated with adjacent vegetative cover (**Photo 13a**, **13b**). A third colony, Colony "C", consisted of a single individual observed in 1997 and growing about 15 meters (50 feet) due west of Colony B. In 1998, this individual had died and several juveniles were present, but they subsequently disappeared and milkvetch have not been seen there since. The Colony C area is within the current 1.66 acre milkvetch preserve area where oil remediation is prohibited.



Photo 13a. Wild site at North Shore, a group of juveniles in Colony "A" in June 2003. White compacted soil, probably bentonite, caps portions of the underlying contaminated soil. Pink pin flags mark juveniles.



Photo 13b. Several adult plants in full flower in "Colony B" area, in 2003. The habitat is largely shrubdominated and includes mulefat and California sagebrush. A dead coyote brush skeleton is in view. Pink pin flags mark milkvetch juveniles.

GENERAL HABITATS AT THE OUTPLANTING SITES

South Ormond Beach: Ormond Beach in southern Oxnard is just north of the Point Mugu Naval Air Station (**Map 1**). This area is on the immediate coast, southeast of the nearby Reliant electric generating plant, and had recently come under public ownership, making it accessible for milkvetch introductions. Long term restoration plans are in preparation by the California Coastal Conservancy. General habitats present include coastal dunes and non-tidal coastal salt marsh. The area selected for planting was about 500 feet southwest of the Reliant electric plant and was a location where upcoming restoration activities were not anticipated.

The 2004 planting site was a transitional area of slightly higher ground between dunes and the wetlands supporting salt marsh and mud/salt flats at an elevation of approximately 4 msl, and abutted a narrow access roadway separating the wetlands from the dunes (a large fence separates the two habitat types here) (**Aerial Photo 2**).



Aerial Photo 2. South Ormond Beach planting site. Plot behind cypress trees. Photo taken October 2006, California Coastline Project.

Vegetation in the planting area on the highest ground was a narrow patch of coyote brush, a phraeatophytic perennial, mixed with spiny rush (*Juncus leopoldii* ssp. *acutus*), a robust obligate wetland perennial (**Photo 14a**). The mid-zone of the planting area was dominated by herbaceous vegetation, including a mix of western ragweed (*Ambrosia psilostachya*), salt grass (*Distichlis spicata*) and *Parapholis incurva*. The lowest, wettest area supported brackish salt marsh plants such as jaumea (*Jaumea carnosa*), alkali weed (*Cressa truxillensis*), and brass buttons (*Cotula coronopifolia*), with occasional pickleweed

(Anthrocnemum subterminale) (Photo 14b).



Photo 14a, b. South Ormond planting site, wetland transition zone. Below, upper salt marsh zone. Pin flags correspond to locations where milkvetch were planted. Milkvetch failed to establish in the salt marsh portion and losses to gophers were severe in the transitional zone as water tables dried down over the summer months. This location failed to establish.



Mandalay State Beach: Supplemental 2004 plantings were installed on state-owned land managed by the Ventura County Parks Department at Mandalay State Beach (**Map 2**). Wilken and Wardlaw (2001) evaluated a general area here roughly three acres in size. We call the initial test planting location here, MAND-1. We had previously installed 20 surplus milkvetch plants in a localized area here on February 23, 2003. These plants were left over from the RSABG original 2002 outplanting endeavor and were delivered in good condition in one gallon pots. They were therefore about 15 months old when installed. These plants were watered once on planting day and required little or no management attention. Surplus milkvetch installed here grew fairly well and therefore, the general area appeared promising for the addition of more milkvetch plants (**Photo 8**). The Mandalay supplemental planting sites are about 400 meters (1/4 mile) west of the North Shore wild population (**Map 2**, **Aerial Photo 1**).



Photo 8. MAND-1 plot in June 2003. Surplus plants were installed here in late January 2003. This site is more distant to the perched water table than other planting locations. Successful recruitment occurred only in the very wet 2005. The area is subject to intense herbivory, yet milkvetch are rarely damaged. Arroyo willow and coyote brush occupy the dune swale here, and may protect the planting site from drying Santa Ana winds. The oldest planted milkvetch of known age is located in the far back of this plot (arrow) and overtopped by growth of arroyo willow.

The general Mandalay area behind the primary foredunes supports a deflation plain with low backdunes and shallow dune swales. The swales are typically either dominated by herbaceous facultative wetland plants or occupied by mature bands of dense arroyo willow. Patches of phraeatophytic coyote brush are common. This general area had been recently restored- mats of invasive iceplant and large *Myoporum laetum* were sprayed with herbicides and left in place to break down, and several acres of dune scrub were restored nearby. The planting sites in the vicinity of MAND-1 are at an elevation of about 9 feet msl. The MAND-1 plot abutted dense arroyo willow on its north side, with scattered coyote brush, but otherwise supported very low cover of herbaceous plants, alot of litter and organic matter and the plot is open to the adjacent low dunes (**Photo 8**). Sparse herbaceous vegetation included western ragweed, a mix of Mexican and Baltic rush (*Juncus balticus* and *J. mexicanus*), horsetail (*Equisetum laevigatum*), slender carex (*Carex praegracilis*) and tansy mustard (*Descurainia pinnata*). Weeds such as summer mustard (*Hirchfeldia incana*) also occur here.

Two other informal planting trials using surplus plants also occurred at the Mandalay State Beach. These sites are called MAND-2 and MAND-3 (**Map 2**). They were located on the far north end of Mandalay State Beach and were not in the area evaluated by Wilken and Wardlaw (2001). Elevation in the vicinity of MAND-2 and -3 is around 10-11 feet msl. Thirty surplus plants were installed here on February 23, 2003 at two sites. Both sites were very shallow dune swales, with low levels of herbaceous vegetation and few herbaceous wetland indicator species. Surplus milkvetch at these sites exhibited low vigor and the sites appeared too dry, so supplemental plants were not installed at these locations in 2004.

Three additional planting areas were therefore selected for installation in 2004 and were located in the vicinity of MAND-1 near West 5th Street. MAND- 1A was a small herbdominated opening inside a patch of mature arroyo willow, about three meters north of the original MAND-1. The other two areas (1B and 1C) were 30-60 meters west of the MAND-1 in open areas abutting dense arroyo willow and coyote brush. We avoided planting in the only nearby area where surface standing water occurs in wetter years, due to concern the area was vulnerable to episodes of prolonged inundation.

McGrath State Beach: A general area east of McGrath Lake at McGrath State Beach (MSB) was evaluated and described in Wilken and Wardlaw, (2001). Five microsites were selected for milkvetch installation there by RSABG and are described in detail in Soza, et al. (2003). Two sites were located in low dunes east of the lake (MSB-1 and MSB-2). MSB-1 supported sparse dune vegetation while MSB-2 was more disturbed, flat, and exhibited high cover of

annual grasses. MSB-3, 4 and 5 were located closer to the shore of the lake in wetland transitional habitats (**Map 2**). Lake shore vegetation transitions from tall emergent tules to patchy mature arroyo willows and dense areas of herb-dominated and scrub/shrub-dominated wetland vegetation. The lake margin wetland vegetation transitions into drier upland dune vegetation and low dune swales with higher plant cover. The low swales support some herbaceous wetland vegetation, especially in wetter years.

In 2002 when milkvetch were originally installed, the planting area at MSB-3 was dominated by patchy western ragweed and adjacent to taller, dense mugwort (*Artemisia douglasiana*) and western goldenrod (*Euthamia occidentalis*) patches. Several coyote brush were present on the south end, which then transitioned into dense impenetrable scrub-shrub wetland vegetation including poison oak (*Toxicodendron diversilobum*). MSB-4 in 2002 was also located in a patch of western ragweed with scattered coyote brush and pockets of open ground. The southeast plot edge abutted a topographically lower area supporting salt grass, slender carex, and Mexican and Baltic rush. With increased moisture conditions over the study period, this vegetation expanded into the milkvetch area and has eliminated open ground (see **Photo 6**). MSB-5 is located adjacent to a large arroyo willow and a thicket of sand bar willow (*Salix exigua*) and is very close to the lakeshore. These willows have overtopped the planting area since 2002 and little light reaches the milkvetch beneath (**Photo 15**). Generally, increased rainfall and mesic trends in the last several years have resulted in a substantial increase in vegetative cover in these wetland margins areas and unvegetated open areas are currently scarce or absent within the original 2002 planting sites.



Photo 15. Dense masses of naturally recruited juveniles in early summer at MSB-5, following flooding of the plot earlier that winter in 2005. This band of juveniles was sufficiently exposed to sunlight, allowing some of them to mature and flower in their first summer. Recruits that emerged behind this group and were overtopped by sandbar and arroyo willow did not establish. Vegetation gaps and areas with more open growing conditions are largely no longer present in the vicinity of this plot and milkvetch here are in decline.

Carpinteria Salt Marsh Reserve: Five microsites were selected for experimental introductions by RSABG in 2002 at the CSMR, following general recommendations in Wilken and Wardlaw (2001). A detailed description of these sites is included in Soza, et al. (2003). Four sites were affiliated with the upper fringes of the high salt marsh and topographically above low areas supporting salt flats and pickleweed salt marsh. Supplemental 2004 plants were installed immediately west and east of CSMR-5, the most successful CSMR planting site where the original 2002 outplants were doing well (Map 3). The general planting area is a transitional area adjacent to the outer fringes of the salt marsh on deltaic deposits left by past flooding of nearby Santa Monica Creek, which is now a managed flood control channel. The planting area abuts a narrow band of dense covote brush on the north side and opens out onto the larger marsh with finger meadows dominated by Italian ryegrass (Lolium mulitflorum) located just above low areas of salt flats and pickleweed salt marsh (Photo 16). Invasive poison hemlock (Conium maculatum) dominates the planting site and adjoining dense coyote brush stand. Italian thistle (Carduus pycnocephalus), and castor bean (Ricinis communis) are also a serious problem here and take over unless weeded out. When the invasives are kept under effective control, western goldenrod expands into the planting area (Photo 17).



Photo 16. Center of the CSMR-5 plot in May 2003, looking south. Active salt marsh in background, dense coyote brush stands to the left and largely out of view. Planted milkvetch are prolifically reshooting going into their second summer. Note that the central plot area has very low cover of associated vegetation and is being kept open by rabbit herbivory. A band of taller, robust wild radish in full flower (background) has screened off the milkvetch plot, creating a safe site for rabbit herbivory. Adult milkvetch are also providing increased shrub cover in this previously more open site. Orange pin flags correspond to locations of juveniles which came up from seed produced by the plants in 2003. None of these juveniles survived.



Photo 17. Center of CSMR-Plot 5, in summer of 2004, looking north. Planted milkvetch are now in their third summer, and plants throughout the plot exhibit different levels of vigor. Both plants in the foreground still retain annual growth stems from the prior year but have produced less vigorous reshooting compared with larger plants in the background. Note the lack of bare ground and presence of dry annual grass litter, suggesting less herbivory has occurred in this central plot area than in the year prior. A bright green patch of western goldenrod is visible in the left background, having expanded into areas previously dominated by poison hemlock which we weeded out.

The new 2004 supplemental planting plots, CSMR-5B and CSMR-5C, abutted the original CSMR-5 planting area and it is now a contiguous area (**Map 3**). CSMR-5B was toward the north and west of the original plot and close to the disturbed embankment and dirt access roadway. CSMR-5C was southeast and adjacent to the CSMR-5 and abutted the coyote brush stand.

Coal Oil Point Reserve: Two 2004 planting sites were selected at the COPR in Goleta (**Map 4**). Both sites had previously been locations where surplus milkvetch plants had been planted in winter of 2003 and were growing well. Two small-scale direct seeding plots were established at these sites in winter 2003 and milkvetch seedlings had successfully emerged and matured here (**Photo 18, 19**). The COPR-Lagoon is a transitional area of slightly higher ground adjacent to Devereux Slough and below upland, coastal prairie and is at an elevation

of around 16 msl. The COPR-Lagoon had previously been the location of several very large non-native *Melaleuca* trees which were removed in 2002. Some subsequent restoration of native herbaceous plants and ongoing weeding was also occurring here.



Aerial Photo 3. Coal Oil Point Reserve-Pond introduction site. Original 2003 planting area is shown in red. The pink dot is the general location of supplemental planting area "D" and plant 46-16, which is the only plant to survive the dry down in 2004 and floods of 2005. The turquoise dot is the location of one robust naturally recruited adult which emerged following flooding in 2005. Juveniles continue to appear in and around the original planting area from the seed bank. Devereux Slough is visible in the background. Photo taken October 2006, California Coastline Project.

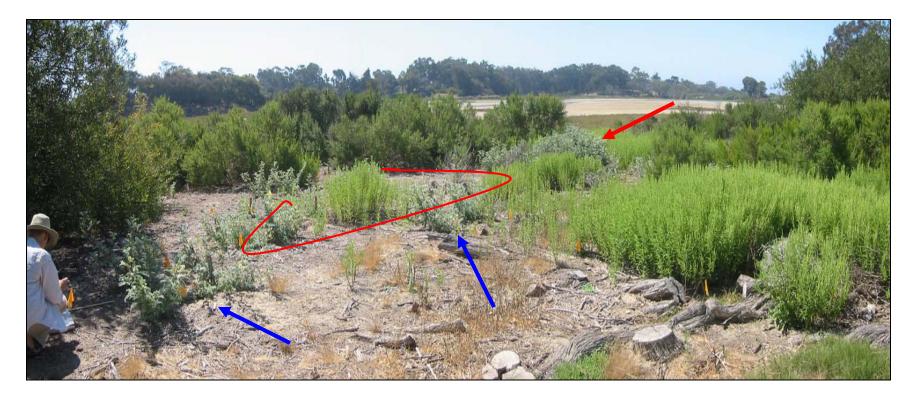


Photo 18. COPR Lagoon, August 9, 2004. Looking south, Devereux Slough in background (low water levels). Stumps of *Melaleuca* are visible here and there. As of 2007, western ragweed has overtaken most of the open ground in this picture. Blue arrows point to 2004 supplemental plants which are starting to flower. Red arrow points to 2003 direct seeding plot. Area in red corresponds to the general location where a dense patch of naturally recruited milkvetch established in 2006.

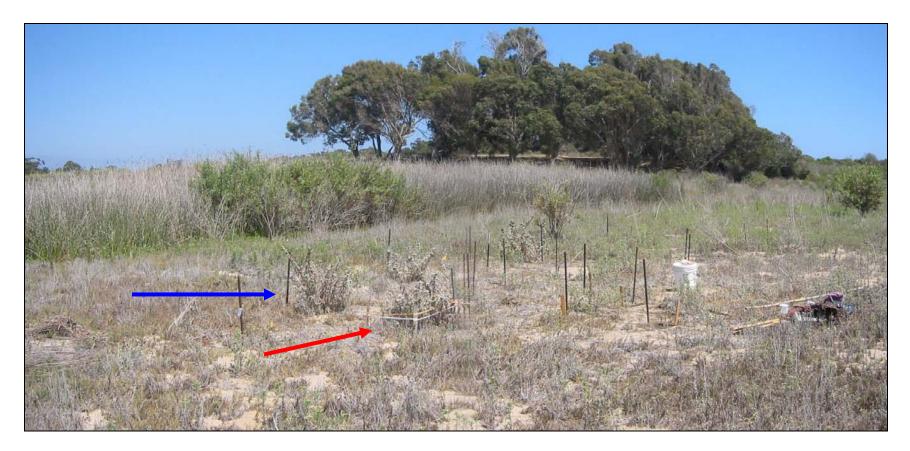


Photo 19. COPR-Pond in August 2004. Red arrow points to the location of a successful 2003 direct seeding plot where several milkvetch established and are flowering. Blue arrow points to one of five surviving milkvetch installed in January 2003. Stakes and rebar mark the locations of 2004 supplemental plants installed in May and correspond with COPR-Pond A. Conditions are very dry, tules around pond are dry, and the perched water table has apparently dropped or dried out. Note sparse cover of herbs and extensive gopher turbation. Additional milkvetch were planted in May 2004 to the west of this location but only one established.

The COPR-Lagoon planting area in 2003 supported scattered patches of coyote brush and several adjacent large arroyo willows. The central area was sparsely-vegetated with patches of herbaceous plants, primarily western ragweed and annual grasses such as rabbit foot grass, bromes, and slender fescue (*Vulpia myuros*) (**Photo 18**). Adjacent low spots supported localized patches of jaumea and salt grass. An additional planting area was also chosen, located about 20 meters south of the Lagoon plot, in a dense patch of jaumea, on the outer fringes of the lagoon and adjacent to arroyo willow and coyote brush. This site was called COPR-Jaumea.

The other COPR site is referred to as the "Pond" site (Map 4). The planting area was an herb-dominated meadow/dune hollow located inland from the foredunes and adjacent to a 1-2 acre pond at an elevation of around 13 feet msl (Aerial Photo 3). The dune hollow is surrounded by adjacent coyote brush stands which transition into robust dunes supporting dune scrub habitat. The dune hollow had previously supported numerous individuals of non-native pampas grass (Cortaderia sp.), which were removed by COPR management in 2002. The dune hollow supports a diverse mix of annual and perennial herbs such as rabbit foot grass, cudweed (Gnaphalium luteo-album), patchy salt grass, western ragweed, and slender carex (Photo 20, 21, 22). Coyote brush and other shrubs are located around the outer margins of the dune hollow and are concentrated at or beyond the edge of an area of dune hollow which floods when the pond expands in wetter years. A small oil seep occurs here in the herbaceous dune hollow. This may be a natural oil seep. Some oil seepage was within inches of previously installed 2003 milkvetch surplus plants. The seep was less evident at the time milkvetch were installed- one plant became immersed in oil following planting and died. Supplemental 2004 milkvetch were installed in an area labeled COPR-A, which was in the vicinity of the 2003 informal planting and seeding trials in the herbaceous dune hollow several meters south of the margin of the pond. COPR-B, C and D were located 20-40 meters west of the COPR-A location, and were in areas with scattered to patchy coyote brush, western ragweed and other herbs and grasses.



Photo 20. COPR-Pond in August 2005. This area was submerged for a prolonged period in winter and spring due to floods and supports an herb-dominated dune hollow with dense cover of annuals and biennials. Orange pinflags are adjacent to milkvetch that emerged following drydown from seed previously produced in the plots by the 2003 introductions. No bioturbation is visible.



Photo 21. COPR-Pond in August, 2005. Naturally recruited juveniles have kept pace with adjacent growth of rabbit foot grass, cudweed and salt grass.



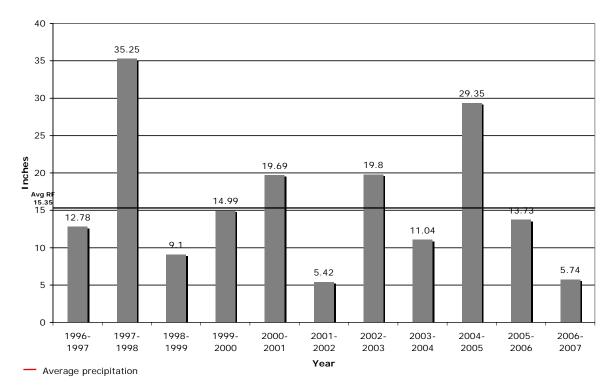
Photo 22. August 2006 natural recruits at COPR-Pond in high herb cover with 3-5% bare ground/light litter. Milkvetch juvenile growth is typically able to keep pace with growth of adjacent vegetation here and some juveniles have successfully matured and flowered in their first year at the COPR-Pond.

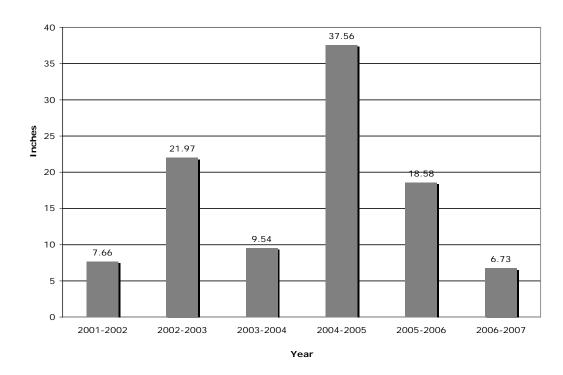
VI. CLIMATE, HYDROLOGY AND SOILS

Climate and Rainfall Patterns

Rainfall across the study period stimulates emergence of milkvetch from seed and affects the general growth of all vegetation, including milkvetch, its competitors and weeds. Rainfall affects both soil moisture and localized perched water tables. **Figure 1** shows the variation in annual rainfall across the study period for the McGrath, Carpinteria and Goleta area. Rainfall data was obtained from various sources and weather stations. Rainfall in the McGrath area came primarily from the Ventura Port Harbor District Station (VCWPD, 2006). Rainfall for the Goleta area came from the Weather Underground (2006) for the KCAGOLET1 station (near the airport). Rainfall for Carpinteria came from Station 208, Carpinteria Fire Station (SBCFCD, 2007).

McGrath-Ventura/Oxnard Annual Rainfall

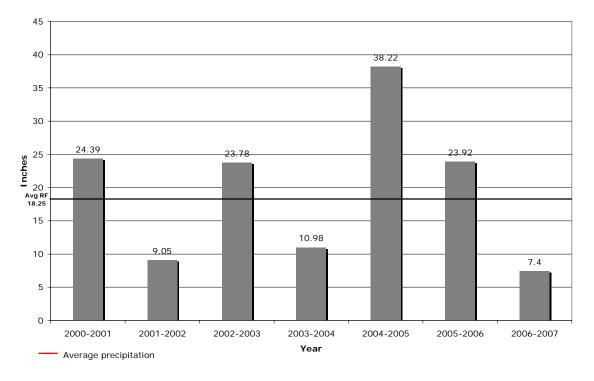




Carpinteria Rainfall Chart

Figure 1. Annual rainfall by water year at various sites (continues next page)

COPR-Goleta Area Annual Rainfall



Average rainfall ranged from a low of 15.35 inches in the Ventura/Oxnard area in Ventura County to a high 18.25 inches for the Goleta/COPR area. Generally, the Santa Barbara coast receives several inches more rainfall than the Ventura area. Recent rainfall patterns show a large variation in overall rainfall between years and both the wild and introduced milkvetch populations have been subjected to extremes. Water year 2004/2005 was the fourth wettest year in the City of Ventura since records began in 1873 (VCWPD, 2007). Substantial rainfall began in mid-October 2004 when almost four inches of rain fell-this represents an unusual amount of heavy rain in fall, and resulted in the earliest emergence of milkvetch seedlings ever observed. Flooding and inundation of planting plots occurred by mid-January following heavy rain at planting sites adjacent to depressional wetlands including South Ormond, McGrath 3-5 and the COPR-Pond (**Photo 23, 24, 25**).

Both the 2002 and 2004 installation years when milkvetch were outplanted in late spring were dry years. The summer of 2007 will likely be one of the driest years milkvetch has experienced since its discovery in 1997. Unofficial rainfall totals indicate water year 2006/2007 is tied for third place as the third driest year on record for the city of Ventura (VCWPD,2007).



Photo 23. MSB-3 in January, 2005. McGrath Lake has overtopped its banks. The original 2002 milkvetch planting area is entirely submerged due to high rainfall and located behind the red line. The prior-year's annual growth stems are still supporting some fruits that are above-water. Following dry-down, seedlings emerged throughout the flooded area and established best along the front edge/drier outer margins of the wetland transition zone.



Photo 24. MSB-3E (north plot extension) in August 2006. Two year old natural recruits in full flower (blue line) and corresponding to previously flooded ground in Photo 23. The original MSB-3 planting area has experienced a substantial increase in wetland margin vegetation and vegetation gaps no longer exist. Arroyo willows in the background are much larger.

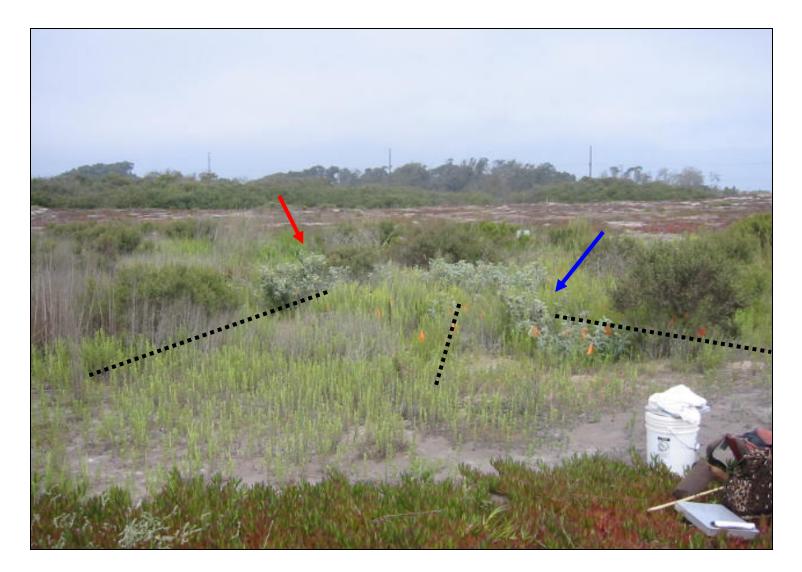


Photo 25. MSB-4, August 2004, looking northeast. The original 2002 plot is in the mid-ground. The red arrow is pointing to a 2002 planted milkvetch. Blue arrow and orange pin flags correspond with 2004 naturally recruited juveniles, some of which are in flower. This entire area was inundated in 2005. Black dotted lines traverse sparsely vegetated western ragweed and more open ground where milkvetch subsequently emerged in 2005 following flooding and now corresponds to MSB-4E.

Summer fog may play a role in influencing survival of adult and juvenile milkvetch by moderating temperatures and increasing humidity. Fog drip through condensation also contributes moisture to these coastal sites, potentially benefiting plant growth. Information on the number and distribution of foggy days was incomplete or unavailable for most study sites. The most complete information was from the Goleta area for 2002-2004. The general pattern shows that the number of foggy days per month ranged from a low of one day to a high of 10 days. Summer and fall of 2004 had far fewer foggy days compared with 2002 and 2003. There were few foggy days in June and July during the three years reported. Based upon our familiarity with the regional area, Goleta is usually less foggy in summer than the Carpinteria and McGrath/Ventura areas. Episodes of Santa Ana wind conditions create hotter temperatures and low humidity conditions, particularly in late summer and fall months.

Ventura marsh milkvetch grows best on the immediate coast where high humidity and cool summer temperatures occur. VMMV raised in Claremont in an "open screen house" exhibited low vigor and stress, low seed set and low seed weight (Soza, et al. 2003). VMMV propagated for the North Shore developers by the Sunburst Plant Disease Clinic in the San Joaquin Valley near Turlock required controlled weather conditions in an artificial environator and almost died when misting and temperature control equipment failed during hot weather.

Hydrology

Flooding and Inundation: Several milkvetch outplanting locations experienced prolonged periods of inundation during episodes of extremely high rainfall and flooding during water year 2004/2005. Locations where milkvetch had been planted adjacent to depressional wetlands such as McGrath Lake, COPR Pond, and South Ormond Beach experienced inundation for a period of a couple of weeks to several months when the surrounding area received a large influx of runoff from localized sheet flow and direct precipitation. No adult or juvenile milkvetch survived in areas that were inundated with the exception of one plant which survived at the COPR-Pond D and appeared to be on the outer edges of inundation and may have been inundated for only a very short time.

Our first experience with inundation occurred at the COPR nursery site, where milkvetch propagated by the RSABG had been transferred to a sandy dune swale at the Reserve in early January 2001. A period of heavy rain occurred and milkvetch failed to survive in locations where they were inundated for about ten days.

At the MSB planting sites in 2005, inundation occurred on two occasions. Heavy rains led to flooding around January 12, and the water had receded by January 25 (**Photo 23**). All planted and naturally recruited milkvetch and carpets of seedlings that had germinated in December and early January drowned. New carpets of seedlings were observed following dry down. Inundation of the MSB sites occurred again around February 20 and all water had receded by March 15.

Flooding also occurred at the South Ormond Beach and COPR Pond sites in winter 2005. On January 25, 2005, the South Ormond area was a lake, and the entire larger wetland basin in the surrounding area was under water including the planting area. Few seeds had been produced by the surviving plants installed in 2004, and therefore, milkvetch did not recover at this location. On February 4, 2005, the COPR Pond site was visited and the entire dune hollow where milkvetch had been planted was completely under water. On March 24, it was still under water. We did not revisit the site until August 10, when about forty juveniles were located in the vicinity of the original 2003 plants where seed had been produced. This observation suggests that milkvetch seed can survive prolonged inundation and still sprout following dry-down.

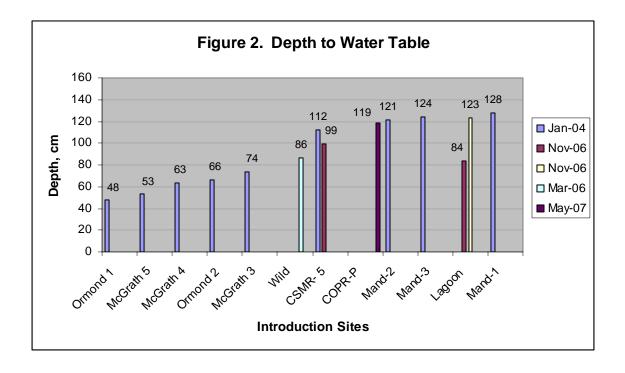
Planting sites affiliated with coastal lagoons and estuaries, such as COPR-Lagoon and CSMR-5 did not experience inundation during these events as far as we could determine. The Lagoon site exhibited signs of surface sheet flow moving across the site toward the slough, but was not flooded with standing water. Both the COPR-Lagoon and CSMR-5 sites were described as very soft and spongy following heavy winter and early spring rainfall. It is likely the water table was higher during this period, but it was not measured. Previously planted milkvetch seemed to survive reasonably well at these locations despite the abnormally wet conditions observed during that timeframe.

It should be noted that the McGrath State Beach area where milkvetch were installed in 2002 also experienced severe flooding in water year 97/98 and based upon reports at the time, a larger area was flooded than in 04/05. At COPR-Pond, the original planting area was also flooded in 2003 and 2006. We do not know the duration of these events. In 2003, five of the eight plants installed in January 2003 apparently survived a period of inundation. In 2006, the COPR-Pond dune hollow was again flooded for a period of time and inundated individuals did not survive. The prior years' rainfall appears to influence whether the pond will inundate the adjacent dune hollow and how long the inundation may persist. These observations indicate the COPR-Pond is one of the most widely fluctuating planting sites hydrologically and it is vulnerable to both desiccation and flooding more frequently than the other sites.

Perched Water Tables: The presence of a near-surface perched water table appears to be an important factor affecting overall milkvetch vigor, size and reproductive output. Most outplanting locations have some type of high water table or perched water table within 150 cm of the ground surface. We typically observe an area moistened by capillary action above the area of saturation, and it is likely that plant roots must reach this moist zone, especially in drier years. Where the below-ground water table is not persistent and/or vulnerable to dry down, vigorous milkvetch can abruptly senesce or even die. At locations where moisture becomes abruptly limiting, milkvetch can stop producing flowers, pods stop developing and seeds fail to mature.

In the following section, we summarize available information and observations related to water tables associated with planting sites. The MSB area has some of the best information regarding hydrology. Information from other sites is limited. In 2003, graduate student Sophie Parker from UCSB submitted a research proposal to examine soil moisture, water table fluctuations and salinity at the MSB, CSMR and COPR planting sites. Only a limited amount of information was obtained from primarily the COPR-Lagoon site in 2004 and is reported in Parker (2006).

We measured the depth to ground water at most planting sites on at least one occasion and report the results in **Figure 2.** A hand-held auger with a 2 inch diameter sand bucket was used to reach the water table below. The depths reported represent locations where free water emerged from the augered material. We were unable to reach a water table at the MSB-1 and MSB-2 sites, because the dry sandy soils collapsed and would not allow us to auger deep enough to find the water table. All 2004 samples were taken by CDFG between January 5 and February 6, 2004; the wild site was sampled by Larry Lodwick of Impact Sciences on March 21, 2006. Lagoon samples with differing values were about three meters apart, suggesting that below-ground water depth may vary spatially.



South Ormond Beach: The general area is located south of Oxnard on the immediate coast in a remnant salt marsh/coastal dune system. Historically, the wetlands at South Ormond Beach were probably connected to the west arm of Mugu Lagoon and received muted tidal flow, but this is no longer the case. Levees and dikes have cut off the historic hydrologic tidal regime. Periodic freshwater inflow occurs here, mostly from winter storms and spring runoff, and the site probably receives most of its water as runoff from inland sources and from the site's high water table (Jones and Stokes, 1994). The general area where milkvetch was installed is behind the foredunes and adjacent to salt marsh and mud flats, and was mapped as coastal grassland on maps prepared in 1855 and 1857, although salt marsh and mudflats were present at that time further to the southeast and west (Jones and Stokes, 1994).

Jones and Stokes (1994) report a shallow, permanent groundwater table throughout most of this area and depth to the permanent water table varies from 17 to 54 inches (43-137 cm) depending upon topographic location. The water table forms where percolation is impeded by a layer of clay subsoil. We located the water table at two locations in the Ormond planting site in January 2004. Depth to saturation at the Ormond-1 location was the closest to the surface topography of any location we have sampled- this location corresponds with high salt marsh vegetation dominated by *Jaumea*, and milkvetch did not thrive in this location, likely due to the onset of hypersalinity in late spring/early summer. The other

Ormond-2 sample was located on higher ground near coyote brush and spiny -rush at 66 cm, that location was similar to the depth sampled at the MSB 3 and MSB- 4 locations. A second sample measured on July 16, 2004 found the soil moist at 50 cm in the high marsh/Jaumea zone and moist soil at 62 cm on higher ground near coyote brush. We did not reach a saturated water table with the hand held auger on that day, indicating it had dropped below 130 cm. These limited samples and observations of plant vigor suggest the water table likely did drop in the local area over the summer months. It should be noted that the adjacent mud/salt flats which were flooded in winter were dry by early summer in 2004.

Wild Site: The North Shore wild site is just east of Harbor Boulevard between Oxnard and Ventura. Due to the presence of oil contamination and remediation plans, the water table in this area has been examined across the site. Unfortunately, fluctuations in free water held near the surface above the oil contamination in the vicinity of growing milkvetch is not well understood and has not been studied in detail. Milkvetch grow on the outer margins of a shallow basin where oil wastes were spread. The topography in the area adjacent to the 1.66 acre milkvetch exclosure is currently being modified by remediation activities leading to subsequent residential development. The North Shore site abuts the Edison Canal to the immediate east, which is connected to the ocean through the Channel Islands Harbor. The man-made Edison Canal exhibits tidal fluctuations and is reported to serve as a "sink", exposing and intercepting the perched water table present on the adjacent Oxnard Plain (URS, 2005).

The perched water table on the North Shore site is reported at a depth of 18 to 29 feet below ground surface (CRWQCB, 2000). Excavation of a single Ventura marsh milkvetch in 1997 found all its primary roots to be located above the oil waste deposit about 10-20 inches below the Colony "A" habitat area (Impact Sciences, 1998). This information suggests milkvetch are not utilizing moisture from the perched aquifer as it is too deep, but instead, are accessing near-surface moisture above the oil waste layer.

Soil moisture within the root zone of milkvetch at the wild site appears to be generated by annual rainfall held near the soil surface by the oil below. As a result, low areas at the North Shore site support facultative wetland indicators such as mulefat (*Baccharis salicifolia*) and arroyo willow (*Salix lasiolepis*). Phraeatophytes like coyote brush and poison oak also occur here. The overall stature of shrubs at the wild site suggests the shrubs are sometimes stunted, cannot achieve the height and vigor typical of normal growing conditions in clean soil, and are often short-lived, resulting in a lot of skeletons of shrubs that

have died (**Photo 13b**). There is generally more shrub cover at the North Shore site compared with the late 1990's when the plants were first discovered as the site gradually recovered following the 1982 closure.

Our observations of milkvetch growth over the years at the North Shore site suggests that water within the root zone can become limiting during summer and fall in drier years. It is common to observe milkvetch in full flower at the wild site suddenly producing stunted flowers, pods stop developing, and leaves become senescent (similar to **Photo 26, Photo 27**). A shallow monitoring well was installed at the site by Impact Sciences in March 2006. The water table was measured at 86 cm on March 21, 2006 and no oil was encountered (Larry Lodwick, pers. comm). In early spring 2007, Larry Lodwick reported no detectable free water in the well. Water year 2006/2007 is reported to be the fourth driest year in historic record for Ventura. This suggests that growing conditions at the wild site will be very difficult for milkvetch this summer- seed production will likely be low and milkvetch will be vulnerable to increased mortality.

Mandalay State Beach: The general area is downslope and immediately west of the North Shore wild site, on the west side of Harbor Boulevard. We have not been able to locate reported information on the water table in this area. It is possible that water table depth in this area is also affected by the interception of the perched water table by the Edison Canal, located east of the site.

We measured the depth to water table in January 2004 at MAND-1, MAND-2 and MAND-3. Depth ranged from 121-128 cm. This indicates the Mandalay planting sites are further from the perched water table than the CSMR and MSB 3, 4, and 5 sites. The Mandalay sites are therefore one of the driest locations, relative to a perched water table, where milkvetch were planted. Milkvetch planted in January 2003 at the MAND- 2 and 3 locations exhibited low vigor and low rates of flowering. While nursery-raised plants with an established root system have survived and persisted reasonably well at the MAND-1 location, recruitment of juveniles from the seed bank was only observed during the extremely wet 2005 growing season, suggesting these depths to saturation are marginal for milkvetch and normally exceed the tolerances for seedling establishment.

McGrath State Beach: The Oxnard Plain abuts the Ventura County coastline and supports a perched water table. In the vicinity of McGrath State Beach and west of Harbor Boulevard, a contiguous near surface water table has been documented (ESA, 2003). Groundwater is

perched above a dense subsurface clay layer. This clay layer causes any water from surface sources such as rainfall or agricultural runoff to remain perched near the surface. The clay layer at MSB ranges in elevation from 3 to 8 feet msl (ESA, 2003). McGrath Lake represents an elevational low point where the near-surface groundwater is exposed as surface water as it flows through the area. This water flows generally from the southeast to the northwest. The pH in the lake water was reported as between 8.24 and 9.39, indicating brackish conditions (ESA, 2003).

Larry Lodwick of Impact Sciences recently reported measurements of pH and temperature taken on April 19, 2007 from a shallow monitoring well installed at MSB-4 and from McGrath Lake itself. The pH of McGrath Lake was 8.34, while the pH in the shallow monitoring well at MSB-4 was 8.22. McGrath Lake was 21.4 degrees Centigrade while the water in the shallow monitoring well was 20 degrees C.

Studies conducted by Impact Sciences to support a wetland restoration effort on a 28 acre private land parcel adjacent to the MSB planting sites provide additional information on the perched water table. Samples taken approximately monthly from several wells on the parcel from July through December 2004 found that water levels varied by less than one foot (Impact Sciences, 2004). The well located closest to MSB-5 at a surface elevation of 7.85 feet, fluctuated from between 1.38 feet (42 cm) and 1.85 feet (60 cm) during the five month sampling period. Review of historic data from the nearby Mandalay Generating Station found the maximum fluctuation to be about two feet (IWR, 2004). Water levels observed at the Mandalay Generating Station location are reported as at or near the lowest levels observed since sampling began in 1996, and they conclude the fall 2004 levels are likely representative of the lower range of possible depths (i.e. conditions reported were representative of the regional drought, and during normal or wetter climatic periods, the depths to water on the McGrath parcel may be shallower) (IWR, 2004). Annual rainfall in water year 06/07 is about ½ that reported for 03/04, and therefore, the perched water table this coming summer may exceed the low range reported for 2004.

We measured the depth to water table at MSB-5 in January 2004 at 53 cm, which is within the range of readings reported by Impact Sciences. The elevation of the planting area at MSB-5 is around 7 feet msl. MSB-3 and 4 are also at an elevation of around 7-8 feet msl. Surface water elevations in the adjacent McGrath Lake normally are around 3-4 feet msl (URS, 2005).

This information taken together suggests a relatively stable perched aquifer is located across the general MSB area, including the adjacent 28 acre private parcel. We have not observed declines in vigor of established milkvetch adults at MSB 3, 4 and 5 that we would attribute to a sudden drop or change in the water table at this location. Milkvetch exhibit high vigor and abundant flowering at the MSB sites, suggesting that milkvetch can generally tolerate the fluctuations, pH levels and water temperatures reported here. A one to two foot drop in water table could have a bigger effect on survivorship of juveniles of the year, who are less deeply rooted.

Carpinteria Salt Marsh Reserve: The CSMR supports estuarine emergent wetlands including tidal and non-tidal wetlands with limited transitional and upland habitat. The CSMR-5 planting site is located in the northeast corner of Basin II, west of Santa Monica Creek. The deltaic alluvium deposited there by past flooding associated with the stream supports some degraded wetland-upland transitional habitat, generally at an elevation above 4.9 feet msl.

The CSMR Management Plan (no date) indicates the presence of a shallow groundwater body, held near the surface by impermeable beds that prevent the downward movement of rain, irrigation and stream water in the deeper groundwater-bearing beds. We measured the depth to water table at the CSMR-5 planting location on two occasions-January 2004 and again in November 2006. The depth was similar on both dates (112 cm and 99 cm, respectively) and is about 25 cm (10 inches) deeper than the MSB-3 location.

Coal Point Reserve: The COPR supports a complex of habitats including Devereux Slough and Lagoon, coastal dunes, coastal prairie and remnant scrub. The information we summarize here is largely taken from the COPR Management Plan, UCSC (2004).

<u>COPR-Lagoon</u>: The planting area at the Lagoon site is on the northwest margin of the slough in a transitional area between the wetlands and coastal prairie. The planting area is adjacent and below a paved roadway and experiences surface sheet flow in localized areas during rainfall events. The area was not inundated during the very wet winter of 2005. The COPR-Jaumea plot is about 20 meters to the south along the west lagoon margin.

Devereux Lagoon and Slough are strongly influenced by freshwater runoff and have only occasional tidal circulation. When freshwater flows are sufficient, the sand berm at the mouth of the estuary is breached and the slough can empty rapidly. It then experiences tidal fluctuation for a period of days or weeks. Therefore, salinity and inundation regimes can vary within a period of a few days. When the lagoon mouth is closed, the lagoon and slough can become full of water, even in years of low rainfall like 06/07. It is likely that the perched water table beneath the planting site is influenced by water levels in the adjoining lagoon, but this has not been quantified.

At the Lagoon planting site, Parker (2006) installed a drive-point piezometer on June 10, 2004 in a low swale on the edge of the Lagoon planting site and failed to reach saturation. The maximum depth of the piezometer was 150 cm. Parker (2006) concluded that at times of year when soils are moist and the water table is higher, freshwater input from precipitation and lateral flow through the soil profile likely comprise the majority of soil moisture for milkvetch and she indicated that high salinity is unlikely to be a problem for milkvetch plants.

In November 2006, we augered two holes about three meters apart in the central area of the Lagoon planting site, and we reached saturation at 84 cm and 123 cm. This limited information suggests there is variability within the local planting area which may be sufficient to influence milkvetch growth in localized areas.

<u>COPR-Pond</u>: The COPR-Pond where milkvetch were planted is located west of the slough, behind the foredunes and below upland coastal prairie (**Aerial Photo 3**). The planting area could be described as a shallow dune hollow abutting and contiguous with a pond that lies on the north end. The pond is a depression about 1-2 acres in size which appears on topographic maps and aerials dating back into the early 1920's. It is labeled "oil sump" on USGS topographic 1:24,000 scale maps. The pond itself is immediately above a suspected fault projected to extend northwest from the known Coal Oil Point Fault and may be a sag pond.

There is no information in the COPR Management Plan on subsurface hydrology and therefore we do not know if there is a perched water table in this general area or not. It appears likely that the pond and vicinity receive some subsurface flows from the surrounding upland terraces and surface runoff from ephemeral drainages that converge in the pond area.

The pond itself and the adjacent dune hollow exhibit large fluctuations in water levels that appear to be largely influenced by rainfall and drought. A series of oblique aerial photos of the pond area available from the California Coastal Records project can be viewed online at <u>www.californiacoastline.org</u>. Aerial photos from 1972-2006 show that water levels in the pond fluctuate. None of the photos at this web site capture the extent of extreme inundation of the larger dune hollow as it occurred in winter 2005 and to a lesser extent in 2006.

The normal high water pond margin is vegetated with tall tules. During periods of high winter rainfall such as occurred in water year 05/06, the adjacent dune hollow becomes inundated and the area is submerged for prolonged periods as the pond expands. In very dry years, the pond can dry up entirely in summer. This appeared to occur in summer of water year 2001/2002 based upon aerial photos taken for the California Coastal Records Project. In water year 03/04, which received about twice the rainfall as 01/02, water in the pond dropped, but some water was still present. That summer, in 2004, adult established milkvetch in full bloom abruptly ceased flowering, pods failed to form and leaves dropped. Associated vegetation such as western ragweed also appeared stressed. The 2004 supplemental milkvetch largely did not survive this dry down.

Soils

Soil chemistry has been previously described for certain study sites. In 1998, Impact Sciences (1998) sampled soils at the wild site in occupied and nearby unoccupied habitat. In 2001, Wilken and Wardlaw reported on some soil properties from samples taken at the wild site (labeled- Oxnard) in occupied and unoccupied habitat; from occupied sites of the Northern brine milkvetch at locations in San Mateo County (Pescadero, San Gregonio, and Pomponio locations); and for some potential introduction areas which included the Carpinteria, McGrath, and Mandalay sites. The latter samples were taken in the general area of the CSMR 1, 2 and 5 locations; the MSB-4 and 5; and MAND-1 vicinity. Two samples were also taken from the Coal Oil Point Reserve nursery site. This location does not correspond to either the Lagoon or Pond planting locations, and was located in a dry sandy dune swale in the foredunes NW of the preserve manager's residence.

Soil texture was reported based upon sieve samples and reflects the proportion of large to small soil particles (Wilken and Wardlaw, 2001). Large particles between 1-2 mm in size generally correspond with sand while small soil particles under 1mm in size correspond with silt and clay. Four of the five Carpinteria samples had between 30% and 61% fine soil particles, while four of the five MSB samples had only 1% small soil particles. The wild (Oxnard) site, Mandalay and COPR sites all showed low levels of small soil particles-generally between 3-16%. Samples from the upper 2-3 cm at the wild site ranged between

11-14% which may reflect the presence of more fine clay particles in the surface soil placed to "cap" the waste site. It was reported that the site was capped with bentonite at closure (Impact Sciences, 1998) (**Photo 13a**). We note there is considerable surface variation at the wild site with regard to surface soil texture, with some areas appearing very compacted with an almost white appearance versus other areas that look more typical of sandy coastal dune soils with some organic matter and light litter. Milkvetch grow at the wild site in both conditions- although plants seem to take longer to mature on compacted, light colored fine soils and the most robust plants in the Colony "B" area are associated with locations that exhibit less surface clay and compaction.

The high percentage of small soil particles at the CSMR-5 site is consistent with our observation that the site has a high clay content compared to all the other introduction sites. Soza et al. (2003) describes the general soil texture at CSMR-5 location as sandy loam, whereas we would describe the area as a clay loam. Soils at CSMR-5, and the adjacent 5B and 5C plots, become very hard and compact in summer. It is often difficult or impossible to remove metal pin flags once the soils dry out, and plastic plant markers cannot be pushed into the dry soils in summer. The site forms localized areas with periodic long cracks in the soil, creating a gap several centimeters wide and deep. These cracks sometimes end at the base of planted milkvetch, and may introduce air near the root crown, pests and pathogens. We sometimes observe juveniles in more compacted areas of the CSMR-5 site desiccating in summer, unable to root sufficiently deep in the hardening dry surface soils.

Soza et al. (2003) reports on soil samples taken from the original 2002 introduction sites at the CSMR and MSB locations. Their information indicates that CSMR sites 1, 2 and 4 where milkvetch did not thrive, were saline, between 20-38.6 mmhos/cm. CSMR-3 was very saline (111 mmhos/cm) and had compact clay soil. This site exhibited the lowest level of survival of all the initial CSMR planting sites (CSMR-1 losses were due to gophers). By November of 2002, 48 percent of the milkvetch installed at CSMR-3 had died and only 2 of 16 remaining plants were reproductive. By February 2003, only 3% were still alive. This information suggests that the level of salinity at the CSMR-3 plot may have exceeded milkvetch tolerance. Twenty two of the thirty one milkvetch planted at CSMR-4 in 2002, which was located the furthest out into the salt marsh, survived until sometime in mid-July of 2003, when white surface crusts started to form, indicating the onset of hypersaline soil conditions. By early August, they had all died. Salinity at CSMR-5 where milkvetch have grown reasonably well is around 6.15. This is more in line with the lower salinities of the McGrath samples, ranging from 0.43-3.22 mmhos/cm; and wild site samples, ranging from

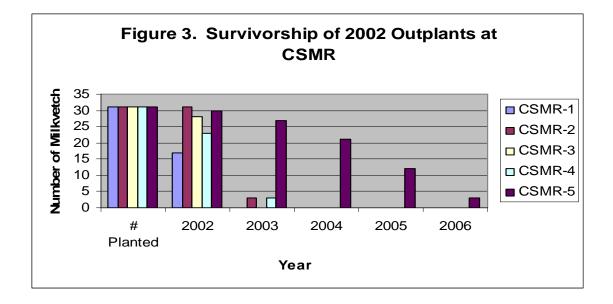
0.7-4.1 mmhos/cm. No soil samples have been taken at the South Ormond, COPR-Lagoon and COPR-Pond locations. Soil texture at these three sites appeared to be generally sandy and coarse.

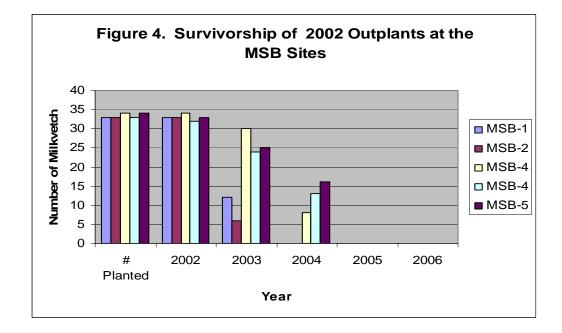
VII. MONITORING RESULTS and DISCUSSION

ORIGINAL 2002 OUTPLANTS

Survivorship

Figure 3 and **4** show the overall survivorship of the original 2002 outplants from 2002 through 2006. **Table 8** summarizes the number of live plants across the study period as observed during each summer monitoring session. By August 2003, in their second growing season, around half of the original outplants had died. The bulk of these failed plants were located at poorly performing sites indicating growing conditions were largely unsuitable. At McGrath State Beach, MSB-1 and MSB-2 located in drier low dune areas distant from the wetland margins of McGrath Lake failed to thrive although many produced new shoots in 2003. By August, the majority was in poor condition and most of the leaves had senesced leaving only green shoot tips indicating severe moisture stress (**Photo 28**).





| Table 8. 2002 Outplants- Survivorship at experimental introduction sites through |
|--|
| August 2006. |

| SITE | # PLANTS INSTALLED APR 2002 | LIVE PLANTS FEB 2003 | LIVE PLANTS AUG 2003 | LIVE PLANTS AUG 2004 | LIVE PLANTS AUG 2005 | LIVE PLANTS AUG 2006 |
|-------------|-----------------------------------|-------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| CSMR-1 | 31 | 5 | 0 | 0 | 0 | 0 |
| CSMR-2 | 31 | 13 | 3 | 0 | 0 | 0 |
| CSMR-3 | 31 | 1 | 0 | 0 | 0 | 0 |
| CSMR-4 | 31 | 22 | 2 | 0 | 0 | 0 |
| CSMR-5 | 31 | 27 | 27 | 21 | 12 | 3 |
| CSMR TOTAL | 155 | 68 | 32 | 21 | 12 | 3 |
| MSB-1 | 33 | 31 | 12 | 0 | 0 | 0 |
| MSB-2 | 33 | 26 | 6 | 0 | 0 | 0 |
| MSB-3 | 34 | 33 | 30 | 8 | 0 | 0 |
| MSB-4 | 33 | 27 | 24 | 13 | 0 | 0 |
| MSB-5 | 34 | 30 | 25 | 16 | 0 | 0 |
| MSB TOTAL | 167 | 147 | 97 | 37 | 0 | 0 |
| GRAND TOTAL | 322 | 215 | 129 | 58 | 12 | 3 |

At the Carpinteria Salt Marsh, the CSMR-1 group was largely eliminated by gopher damage in their first summer (2002). The CSMR-2, 3, and 4 largely failed by August 2003, and were located on the fringes of the high salt marsh. These locations exhibited high salinity and in early summer of 2003, white salt deposits started to form indicating the onset of hypersaline conditions. The CSMR-4 plants abruptly died shortly thereafter. Three plants survived and grew reasonably well in the CSMR-2 site. These were located at the far north end of the planting plot at the extreme edge of the high marsh-salinity was probably not as high in this localized area (Photo 9). The remaining locations at MSB-3, 4, and 5 and CSMR-5 all survived reasonably well through the 2003 growing season. A few plants were lost at the McGrath sites, primarily from herbivory. Small mammal herbivory became severe at the McGrath sites in winter-early spring of 2004. Many plants at MSB-3 and MSB-4 failed to produce annual growth stems as a result of severe cropping of new shoots attempting to form on the root crown (Photo 12). Herbivory was most severe at the MSB-3 location, and the only plants which survived were five individuals that had been caged and three individuals on the exposed north edge of the plot where herbivores were apparently reluctant to browse. The MSB-5 location had less severe herbivory on the new shoots and therefore exhibited better survival. Mortality at MSB-5 was concentrated on the southeast edge of the planting plot, adjacent to moist lakeshore habitat which harbored high levels of snails that proved difficult to control.

At CSMR-2, an eruption of gopher activity occurred in May 2004, the entire area was churned up and the three CSMR-2 plants died. Some gopher and mole activity was also observed in the CSMR-5 planting area in summer 2004. Eight plants exhibited lower vigor and odd growth that seemed to correspond with air pockets tied to gopher and mole activity in the vicinity of the plant bases. The heavy clay soils and formation of periodic cracks in the heavy soil also appeared to contribute to the problem of air pockets by the milkvetch root crowns. Four plants exhibited a mix of normal shooting on one side and abnormal shooting on the other side. Symptoms of abnormal growth included short stems that produced miniaturized leaves with reduced elongation between leaflets (**Photo 28**; **29**). Two plants which exhibited these symptoms were in good condition the next summer, but the remainder either died or was in poor condition by summer 2005.

The remaining 2002 outplants at the MSB sites failed to survive flooding and prolonged inundation that occurred in the extremely wet winter of 2005. Had these sites not flooded, some of these plants would have likely survived into their fourth summer growing season. At CSMR-5, which was not inundated, around 40 percent were still alive in August

2005. It should be noted that these plants were 5-6 months old when they were installed in April 2002. Therefore the 12 plants surviving at the CSMR-5 location were around three years, ten months old in August 2005. Three of these plants reshooted in spring 2006, survived through their last summer, and therefore lived for around five years before failing to reshoot in winter 2007.

The oldest living planted milkvetch of known age was planted at the MAND-1 site in winter 2003 and was a surplus plant left over from the original RSABG propagation. This plant is therefore five years, five months old as of May 2007, and is currently exhibiting surprisingly good vigor and numerous reshoots. Wilken and Wardlaw (2001) estimated longevity of VMMV at the wild site to be at least three years, and generally between three and four years. The results here suggest the potential for greater longevity at more favorable growing sites compared with the wild site.

Growth and Flowering

Tables 9 and 10 summarize the overall growth and flowering documented during the late July- early August annual summer monitoring sessions. Figure 5 shows the mean height of the longest shoots produced on the 2002 outplants. Figure 6 shows the mean number of flowering shoots, and Figure 7 shows the mean number of inflorescences on a randomly selected branch, for each site. Inflorescences were not sampled in 2005. The dry, poorly performing MSB-1 and 2 plots produced short plants with fewer shoots which did not flower and were in severe decline by early August 2003. In contrast, plants at the MSB 3-5 locations located on the outer fringes of the wetland margin adjacent to McGrath Lake achieved outstanding growth, abundant shooting and prolific flowering in 2003, their second summer. At MSB-3, the plants were so large and co-mingled that we were unable to reach the center of the plot and therefore could not monitor 7 of the 30 plants. Plants at MSB-4 were more spread out. We were only unable to monitor 1 of the 25 plants at MSB-5.

The MSB-4 plants in 2003 achieved about 2/3 the height compared to the MSB-3 and 5 plots, produced fewer shoots and about 50% fewer inflorescences. The CSMR-5 plants were taller than the MSB-4 plants and produced similar numbers of flowering shoots but the mean number of inflorescences was twice that produced by the MSB-4 plot and similar to that produced at the MSB-3 and 5 plots. The longest annual growth stem measured over the entire five year period of this study was 227 cm (7.5 feet) in the MSB-5 plot in August 2003. At the time we were monitoring these plots, we had no idea that milkvetch could achieve the amount of growth, biomass and prolific flowering seen at the MSB and CSMR-5 plots.

Substantial decline in milkvetch growth and flowering was observed in summer of 2004 in their third growing season. Severe herbivory at the MSB-3 and 4 sites resulted in the production of larger numbers of shoots stimulated by repeated cropping of shoots attempting to emerge from the root crown. Half as many shoots were produced at the MSB-5 site where small mammal herbivory was far less severe. Despite producing more overall shoots, fewer shoots flowered at the herbivorized sites. The CSMR-5 plants, which were largely not herbivorized by small mammals, produced more flowering shoots compared with the MSB 3 and 4 sites.

| SITE | TOTAL INSTALLED | TOTAL SURVIVING | PERCENT FLOWERING | MEAN HEIGHT (CM) | STD. DEV. | MEAN # SHOOTS/ PLANT | STD. DEV. | MEAN # FLOWERING SHOOTS/ PLANT | STD. DEV. | MEAN # INFL. PER RANDOM STEM | STD DEV |
|--------|--------------------|--------------------|----------------------|------------------------|--------------|----------------------------|--------------|---|--------------|---------------------------------------|------------|
| | | | | | | | | | | | |
| CSMR-2 | 31 | 3 | 100% | 84.7 | 42.5 | 7.3 | 6.8 | 6.0 | 5.3 | 62.0 | 49.8 |
| CSMR-5 | 31 | 27 | 100% | 136.7 | 15.7 | 13.9 | 6.3 | 12.9 | 6.0 | 51.6 | 28.3 |
| | | | | | | | | | | | |
| MSB-1 | 33 | 14 | 0 | 25.6 | 8 | 10.0 | 4.9 | 0.0 | 0.0 | 0.0 | 0.0 |
| MSB-2 | 33 | 6 | 0 | 41.5 | 9.1 | 10.8 | 3.5 | 0.0 | 0.0 | 0.0 | 0.0 |
| MSB-3 | 34 | 30 | 100% | 164.1 | 21.4 | 19.7 | 7.0 | 18.0 | 7.8 | 57.5 | 56. |
| MSB-4 | 33 | 24 | 92% | 99.3 | 33.4 | 14.3 | 10.2 | 12.1 | 10.4 | 25.6 | 41. |
| MSB-5 | 34 | 25 | 96% | 170.6 | 44.7 | 17.7 | 10.0 | 17.6 | 9.4 | 55.1 | 57. |

Table 9. 2002 Outplants- Growth and flowering in their second growing season, August 2003.

TOTAL: 229 129

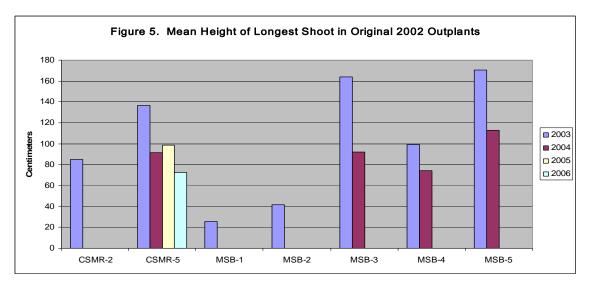
| SITE | TOTAL INSTALLED | TOTAL SURVIVING AUG 2004 | PERCENT FLOWERING | MEAN HEIGHT (CM) | STD. DEV. | MEAN # SHOOTS/ PLANT | STD. DEV. | MEAN # FLOWERING SHOOTS/ PLANT | STD. DEV. | MEAN # INFL. PER RANDOM STEM | STD. DEV. |
|--------|--------------------|--------------------------------|----------------------|------------------------|--------------|----------------------------|--------------|---|--------------|---------------------------------------|--------------|
| CSMR-5 | 31 | 21 | 86% | 91.75 | 31 | 44.4 | 27.6 | 32.1 | 25 | 14.2 | 12.6 |
| MSB-3 | 34 | 8 | 100% | 92 | 9.3 | 45.5 | 18.2 | 17 | 7.9 | 8 | 6.5 |
| MSB-4 | 33 | 13 | 85% | 74.2 | 30.3 | 40.2 | 21.2 | 23.9 | 12.1 | 13.9 | 12.7 |
| MSB-5 | 34 | 16 | 75% | 112.6 | 49 | 23.7 | 16.6 | 16.8 | 15.6 | 17.7 | 14.9 |
| TOTAL: | 132 | 58 | | | | | | | | | |

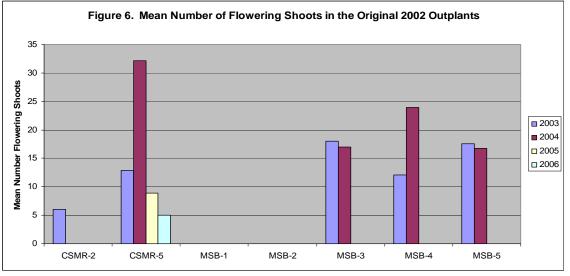
 Table 10. 2002 Outplants- Growth and Flowering 2004-2006.

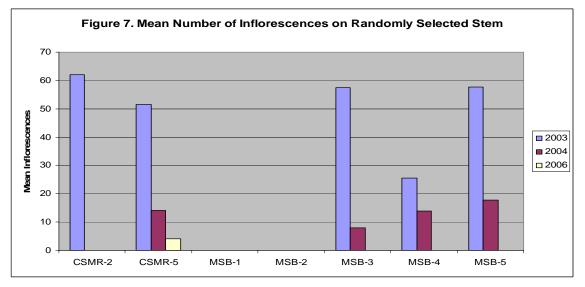
| | | AUG 2005 | | | | | | | | | |
|--------|----|----------|-----|------|------|------|-----|-----|---------------|--------------|------|
| CSMR-5 | 31 | 12 | 92% | 98.4 | 37.7 | 11.1 | 6.7 | 8.9 | 5.7 | N/A* | N/A* |
| TOTAL: | 31 | 12 | | | | | | × | * N/A= data r | ot collected | |

| | | AUG 2006 | | | | | | | | | |
|---------|----|----------|------|-------|----|-----|-----|---|-----|---|---|
| | | | | | | | | | | | |
| 00000 5 | 04 | 0 | 070/ | 70.07 | 10 | | 0.4 | - | 4.0 | | 0 |
| CSMR-5 | 31 | 3 | 67% | 72.67 | 43 | 6.3 | 3.1 | 5 | 4.2 | 4 | 0 |
| | | | | | | | | | | | |
| | | | | | | | | | | | |

TOTAL: 31 3







Flooding in 2005 eliminated all the remaining original planted milkvetch at the MSB sites. Twelve plants survived at CSMR-5 where flooding did not occur, and were similar in overall height to the previous year, but produced substantially fewer shoots indicating vigor was declining. In their last summer, the remaining three individuals were again shorter, with few shoots and only 2 produced flowers.

Flower and Fruit Production

We collected information on the numbers of flowers, fruits and seeds produced by the original outplants and the MAND-1 location, in both 2003 and 2004. We also obtained similar data for the wild site in 2003. Fruit production appeared very low at the wild site in 2004 and at the MSB 3 and 5 plots, so data was not collected. Data was obtained by collecting ten inflorescences and ten infructescences randomly from the plots (N= 5 for MAND-1). Flowers were not collected at the wild site.

Tables 11a and 11b show the mean number of flowers per inflorescence, mean number of pods per infructescence, mean number of pods per infructescence producing seed, and percent damaged pods (primarily caterpillar damage) for 2003 and 2004. **Tables 2a and 2b** show the total mean number of seeds per infructescence, total mature seeds, and mean number of seeds per pod. We also report the percent of seed that exhibited weevil exit holes and percent of the seed that had a black and rough--appearing seed coat (abbreviated BT). We also obtained the 100 seed weight in grams for a single sample pulled from all the seed obtained from sampling infructescences at each site.

| SITE | MEAN # FLOWERS PER INFL | STD. DEV. | MEAN # PODS PER INFR | STD. DEV. | MEAN # PODS WITH SEED | STD. DEV. | PERCENT PODS PRODUCING SEED | PERCENT DAMAGED PODS * |
|--------|-------------------------------|--------------|----------------------------|--------------|--------------------------|--------------|--------------------------------------|------------------------------|
| WILD | N/A | N/A | 36.9 | 8.647 | 25 | 13.24 | 0.68 | 0.1056 |
| MSB-3 | 37.9 | 8.06 | 39.1 | 5.587 | 34.8 | 6.71 | 0.89 | 0.031 |
| MSB-4 | 31.5 | 8.25 | 34.6 | 6.93 | 31.2 | 7.07 | 0.902 | 0.075 |
| MSB-5 | 38 | 3.8 | 38.8 | 7.22 | 34.2 | 8.07 | 0.881 | 0.08 |
| CSMR-5 | 36.2 | 4 | 35.5 | 7.34 | 31.8 | 7.18 | 0.896 | 0.099 |
| MAND-1 | 39.2 | 2.17 | 37.3 | 5.62 | 21.3 | 14.24 | 0.571 | 0.367 |

Table 11a. Flower and fruit production at key sites in 2003. N= 10 (N=5 for MAND-1)

Table 11b. Flower and fruit production at key sites in 2004. N=10 (N=5 for MAND-1)

| SITE | MEAN # FLOWERS PER INFL | STD. DEV. | MEAN # PODS PER INFR | STD. DEV. | MEAN # PODS PER INFR WITH SEED | STD. DEV. | PERCENT PODS PRODUCING SEED | PERCENT DAMAGED PODS* |
|--------|-------------------------------|--------------|----------------------------|--------------|---|--------------|--------------------------------------|-----------------------------|
| MSB-3 | 39.5 | 5 | N/A | | | | | |
| MSB-4 | 37.6 | 9.05 | 32.8 | 6.16 | 25.6 | 5.58 | 0.78 | 0.088 |
| MSB-5 | 36.8 | 8.12 | N/A | | | | | |
| CSMR-5 | 34.8 | 11.25 | 44.6 | 5.66 | 42.4 | 6.31 | 0.95 | 0.092 |
| MAND-1 | 47.8 | 5.02 | 43.7 | 6.58 | 38.5 | 8.75 | 0.88 | 0.126 |

* damaged pods exhibit holes made by caterpillars or weevils

In 2003, all sites produced fairly similar numbers of flowers per inflorescence and pods per infructescence. Around 90% of the pods produced seeds at the MSB and CSMR sites. In contrast, only 57% of the MAND-1 pods and 68% of the wild site pods produced seed and the standard deviation was higher suggesting more variability at these locations compared to the other groups. Pod damage, primarily from caterpillars, ranged from 3-11 % at the MSB, CSMR and wild sites, while MAND-1 experienced 37% damage, indicating a higher level of caterpillar activity here. Some of the caterpillars seen in milkvetch plots are also seen on nearby mustard plants. The MAND-1 location has more summer mustard (*Hirschfeldia incana*) than the other sampled sites which might account for the increased caterpillar herbivory seen here.

Seed production in 2003 was substantially lower at the wild and MAND-1 sites compared with the MSB and CSMR locations. Mean number of seeds per pod ranged from a low of 2.7 for the wild site to a high of 4.45 for MSB-4. We observed very low levels of weevil-damaged seed at all sites. The proportion of seed with a rough, black seed coat ranged from 3-14%. The MSB-3 and 5 plants had the lowest 100 seed weight, while the wild, MAND-1 and MSB-4 produced heavier seed. In their third growing season, the MSB and CSMR sites in 2004 produced similar numbers of flowers compared with 2003. MSB-4 produced fewer fruits in 2004 than CSMR-5 and MAND-1. At MSB-4, only 78% of the pods produced seed. Mean number of seeds per pod ranged from 2.93 to 3.55.

Comparison with data reported for the first year plants in Soza, et al. (2003) is interesting. The McGrath sites produced a mean of 13.7 mature fruits per infructescence in 2002 while our second year plants in 2003 produced more than twice as many (31-35). Mean number of mature seeds per fruit and mean seed weight were slightly higher in the first year plants. Our second year plants produced more flowers per inflorescence and similar numbers of pods per infructescence, whereas on the first year plants, one third fewer fruits were produced (20.3) compared with flowers (30.1). The latter result may relate to lower visitation by insect pollinators. Wilken and Wardlaw (2001) reported one third fewer total seeds produced per fruit in bagged inflorescences which excluded pollinators compared with open pollinated inflorescences. The young plants in their first year in 2002 were still inside tree shelters, some flowers were located on stems emerging from the top of the shelters but other flowers were inside the shelters. Low biomass and confinement within the tree shelters may have reduced visibility and access by pollinators, which could have resulted in fewer flowers being visited and open-pollinated and possibly reducing seed set as a result.

| SITE | TOTAL # SEEDS/INFR | STD. DEV. | TOTAL # MATURE SEED/INFR* | STD. DEV. | PERCENT BT *** | PERCENT SEED WITH EXIT HOLES * | MEAN # SEEDS/POD | 100 SEED WEIGHT (g) |
|--------|-----------------------|--------------|---------------------------------|--------------|-------------------|--------------------------------------|---------------------|------------------------|
| Wild | 68.3 | 36.95 | 63.7 | 34.44 | 0.059 | 0.00088 | 2.71 | 0.309 |
| MSB-3 | 136.9 | 37 | 121.8 | 38.9 | 0.097 | 0.0003 | 3.92 | 0.263 |
| MSB-4 | 139.1 | 52.3 | 121.3 | 55.34 | 0.123 | 0.0001 | 4.45 | 0.308 |
| MSB-5 | 109.3 | 45.8 | 104.3 | 46.7 | 0.138 | 0.0007 | 3.17 | 0.268 |
| CSMR-5 | 115.8 | 41.13 | 113 | 41.13 | 0.024 | 0.024 | 3.84 | 0.297 |
| MAND-1 | 68 | 40.14 | 65.8 | 42.88 | 0.032 | 0.0003 | 3.02 | 0.306 |
| | | | | | | | | |

Table 2a. Seed production at key sites in 2003. N= 10 (N=5 for MAND-1)

| Table 2b. Seed | production at key | y sites in 2004. | N= 10 (| (N=5 for MAND-1) |
|----------------|-------------------|------------------|---------|------------------|
| | | | | |

| SITE | TOTAL # SEEDS/INFR | STD. DEV. | TOTAL # MATURE SEED/INFR* | STD. DEV. | PERCENT BT*** | PERCENT SEED WITH EXIT HOLES** | MEAN # SEEDS/POD | 100 SEED WEIGHT (g) |
|--------|-----------------------|--------------|---------------------------------|--------------|------------------|--------------------------------------|---------------------|---------------------------|
| MSB-4 | 99.9 | 37.44 | 97.9 | 37.52 | 0.125 | 0.02 | 3.82 | 0.271 |
| CSMR-5 | 151.9 | 32.16 | 150.7 | 32.69 | 0.075 | 0.00079 | 3.55 | 0.309 |
| MAND-1 | 115.3 | 35.17 | 112.7 | 35.01 | 0.067 | 0.022 | 2.93 | 0.282 |

* mature seed includes sound seed plus BT seed minus seed with exit holes ** exit holes are made by bruchid beetle larvae which consumed the developing ovule *** seed with a rough, black seed coat

Seed Production at Key Sites

We calculated the theoretical estimated seed production for 2002, 2003 and 2004 and report these data in Table 12. The 2002 estimate is based upon the data reported in Soza et al. (2003). We used mean values from the monitoring data as follows: (# reproductive plants) X (# flowering shoots) X (# inflorescences per random branch) X (# pods with seed) X (# seeds per pod).

| SITE | 2002 | 2003 | 2004 |
|--------|--------|-------|---------|
| MSB-3 | 5,914 | 4.2 M | n/a |
| MSB-4 | 10,570 | 1.03M | 422,337 |
| MSB-5 | 5,941 | 2.63M | n/a |
| CSMR-5 | 5,665 | 2.19M | 1.44 M |

Table 12. Theoretical estimate of seed production at selected sites. 2002 estimate based on data from Soza et al. 2003

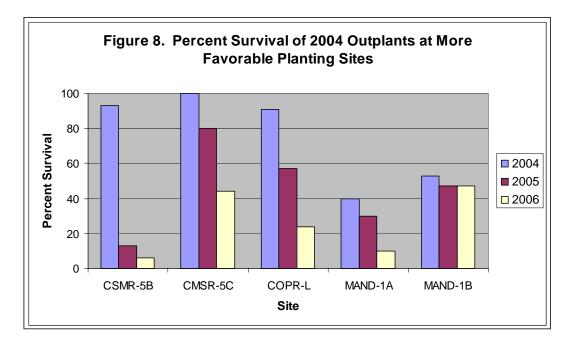
The first year plants produced modest levels of seed within the plots. Since plants at the wild site had never been observed to flower in their first year, this result was impressive and suggested better vigor at the introduction sites and more rapid maturation of plants. By their second year, the successful MSB and CSMR plots potentially produced millions of seeds. The greatest amount of seed production occurred at the MSB-3 location and was the combined result of more surviving plants with abundant flowering shoots and prolific flowering and fruiting. The next summer, in 2004 and plants in their third growing season, MSB-4 produced about half the amount of seed as the previous year. At CSMR-5, seed production in 2004 was about 65% of that seen in the previous year.

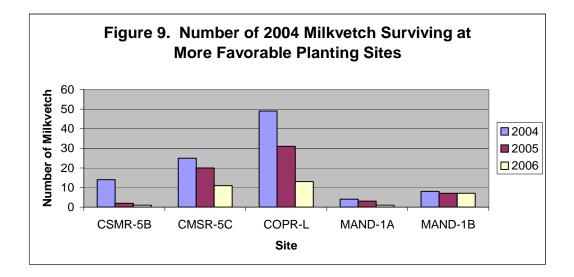
These data represent the theoretical seed output utilizing summer (typically early August) flowering data. Less seed may actually have produced in these plots due to herbivory in fall on stems that contained developing fruits and herbivory on pods, often by snails, which is not accounted for here. These results therefore assumed that the mean number of influctescences produced on a random stem would be the same as the mean number of inflorescences. In reality, this is unlikely to be the case, and should these types of data be recorded again, marking and resampling of the random stems in fall would provide a more accurate overall estimate and could help better quantify losses from late summer/fall herbivory or other causes. On the other hand, the mean number of inflorescences was sampled the last week of July into the second week of August. Flowers typically continue to be produced at sites where moisture is not limiting through August and into September, indicating more flowers, and potentially more seeds, may have been produced than we report here.

2004 SUPPLEMENTAL OUTPLANTS

Survivorship

Table 3 summarizes the number of live plants and percent survival across the study period as observed during each summer monitoring session at each site. **Figure 8** shows the percent survival of 2004 outplants at more favorable growing sites across the study period. **Figure 9** shows the number of 2004 milkvetch surviving at the various planting sites through the summer 2006 monitoring session. Within the first few months of planting, 90-100 percent of the plants persisted at the CSMR-5B, 5C, COPR-Lagoon and COPR-Jaumea sites. At the Mandalay sites, 40-53% of the milkvetch were still alive in August, 2004. Losses occurred primarily from small mammal herbivory (attributed to rabbits), gopher activity (MAND-1C) and unknown factors. Rabbit herbivory abruptly increased at the CSMR-5B plot in late summer while the CSMR-5C plot was largely unherbivorized.





The South Ormond site experienced failure of plants installed in the high salt marsh when hypersaline conditions occurred in mid summer. Gopher activity, not observed when the plants were installed, resulted in loss of 14 more plants at the South Ormond site. We hypothesize that gophers may have been present in an adjacent small area of higher ground (**Photo 14a**), and they may have expanded activity out into the planting area as the perched water table dropped in summer.

Plants installed at the COPR-Pond failed to establish due to an abrupt drop in the water table which occurred in late July 2004. Five previously established adults planted in 2003 were in full flower when water levels dropped or disappeared, and the plants exhibited severe moisture stress and started to decline and die. Associated herbaceous vegetation in the general area also appeared stressed from lack of water.

In the wet winter of 2005, the South Ormond planting site was inundated for a prolonged period and all the remaining plants drowned. The COPR- Pond area was also inundated, and the planting areas were all largely under water for weeks. One plant, plant 46-16 located at COPR-Pond plot D, had been reported as dead in 2004, but apparently this plant in fact had recovered and survived the inundation, probably because it was on the extreme edge of the flooded area. Plants at the COPR-Jaumea plot did not reshoot in spring, 2005, possibly due to higher water levels associated with the very wet conditions.

At COPR-Lagoon, high rainfall in 2005 resulted in surface sheet flow across localized areas of the planting site and plants failed to reshoot at locations where sheet flow was the most concentrated (i.e. localized shallow swales). These low areas were more likely to have experienced some submergence, as well, although we never directly observed standing water. Wet conditions lead to significant expansion of associated vegetation and about ½ the planting area became densely vegetated with western ragweed, salt grass and ungrazed rabbit foots grass. Snail control was problematic in these densely vegetated moist areas and contributed to failure of milkvetch. Five milkvetch experienced sudden leaf drop during peak blooming in August of 2005, and four of them failed to reshoot in 2006. Two of these plants were growing adjacent to one another and we hypothesize that some type of damage occurred to the root system resulting in the observed symptoms and subsequent failure to reshoot.

Only two milkvetch survived at the CSMR 5B plot in 2005 due to prior severe rabbit herbivory and gopher activity near plants. Three plants at CSMR-5C which had exhibited abnormal, compact growth in 2004 failed to reshoot in 2005. Four additional plants exhibited sudden leaf drop while in full flower during the August 2005 monitoring visit and these four plants did not recover (example, see **Photo 31**). This was the same symptom as we saw at the COPR-Lagoon. All four plants were growing near each other and we assume something affected their below-ground root system. The root crowns were firmly attached indicating that the problem was probably not caused by gophers which typically eat the root all the way up to just below the root crown. This same symptom (sudden leaf drop and decline while in full bloom) was observed on several naturally recruited plants at MSB 3E and 4E in 2006 and these plants included several of the largest and most vigorous individuals.

The majority of planting sites exhibited additional mortality in their third growing season, 2006. MAND-1B exhibited a stable trend and no additional deaths occurred. Most of the remaining plants were at the COPR-Lagoon and CSMR-5C sites. At COPR-Lagoon, 2006 deaths were concentrated in locations where rhizomatous perennials such as salt grass, jaumea or western ragweed had expanded. There was evidence of meadow voles (severe cropping and runways) in areas with rabbit foot grass and salt grass, and milkvetch did not reshoot in those areas. Most of the Lagoon site currently exhibits high cover of herbaceous associates and only limited areas are relatively open. At CSMR 5-C in 2006, failed plants were primarily those affected by sudden leaf drop the previous summer and rabbit herbivory.

Overall, the supplemental 2004 outplants were subjected to extreme weather conditions including severe drought in their first summer, 2004, and extreme flooding and rainfall in 2005. Supplemental milkvetch failed to establish at locations such as South Ormond and COPR-Pond due to inundation in winter 2005. Competition from growth of associated herbaceous vegetation was a problem in 2005, especially at the MSB, COPR-Lagoon and CSMR 5-C locations (**Photo 30**). Snail control was ineffective at locations with dense, herbaceous vegetation and moist conditions. Herbivory by rabbits was severe at certain planting locations and limited to localized areas at other locations. Sudden leaf drop and dieback affected plants in localized areas at both the CSMR-5C and COPR-Lagoon sites.



Photo 30. Severely herbivorized area at CSMR 5C adjacent to cage full of lush growth, May 2005. Wild rye-grass dominated meadow in background extends out into salt marsh and is largely not herbivorized. Most annual grasses and many forbs have been cleared by herbivores leaving open ground. Overgrowth within cages engulfed seedlings and adults where herbivores were excluded, despite repeated efforts to thin and cut back vegetation inside the cages. Note a narrow band of unherbivorized maturing Italian thistle which was subsequently removed. Beneath was unherbivorized western ragweed, protected from herbivores by the tough thistle leaves and spines.

Growth and Flowering

Tables 13 and 14 summarize the overall growth and flowering documented during the late July-early August annual summer monitoring sessions. Figures 10, 11 and 12 show the mean height of the tallest shoot, mean number of flowering shoots and mean number of inflorescences on a randomly selected stem for various sites. We did not obtain data on the number of flowers produced in 2005.

In their first summer, both COPR-Lagoon and CSMR-5C produced vigorous plants with multiple shoots and abundant flowering. The Lagoon group produced the most vigorous plants, with a mean of six shoots per plant, most of which were flowering shoots, and 75% of the plants flowered. COPR-Lagoon and CSMR-5B produced 3 times the mean number of inflorescences on a random branch compared with the CSMR-5C group. CSMR-5C produced 1-10 inflorescences per stem while the Lagoon ranged from 1 to 87 inflorescences per random stem. Fourteen plants at the Lagoon produced more than 10 flowers per flowering branch. The CSMR-5B and MAND-1B plots exhibited relatively good growth and abundant flowers.

The South Ormond group produced the shortest plants and only 29% flowered. The MAND 1A and 1C plots, which were growing in relatively shady areas near large arroyo willows, produced few shoots and modest flowering, as did the COPR-Jaumea group.

| SITE | NUMBER PLANTED | ALIVE SUMMER 2004 | MEAN HEIGHT (CM) | STD. DEV. | MEAN # SHOOTS | STD. DEV. | MEAN # FLOWERING SHOOTS | STD. DEV. | MEAN # PER RANDOM STEM | STD. DEV. | PERCENT FLOWERING |
|-------------|-------------------|-------------------------|------------------------|--------------|------------------|--------------|-------------------------------|--------------|------------------------------|--------------|----------------------|
| Ormond | 40 | 7 | 27.3 | 18.5 | 4.6 | 2.8 | 1 | 0 | 2.5 | 0.71 | 29 |
| MAND-1A | 10 | 4 | 59 | 11.6 | 1.5 | 0.6 | 1.25 | 0.5 | 3.25 | 1 | 100 |
| MAND-1C | 10 | 4 | 46 | 17.5 | 1 | 0 | 1 | 0 | 3 | 0 | 25 |
| CSMR 5B | 15 | 14 | 63 | 34.6 | 5.9 | 2.9 | 2 | 2.4 | 14 | 11.5 | 43 |
| CSMR 5C | 25 | 25 | 42 | 14.8 | 5.4 | 3 | 2.4 | 1.7 | 4.2 | 2.5 | 80 |
| COPR-LAGOON | 54 | 49 | 59 | 22.8 | 6.1 | 4.3 | 6.4 | 4.4 | 14.9 | 18.1 | 75 |
| COPR-JAUMEA | 6 | 6 | 58 | 13.1 | 2.8 | 1.5 | 1.8 | 1.3 | 3.8 | 2.6 | 83 |
| MAND-1B | 15 | 8 | 44.3 | 20.6 | 3.5 | 1.2 | 2.6 | 1.5 | 10.6 | 6.1 | 63 |
| COPR-POND A | 10 | * | ** | | ** | | ** | | ** | | |
| COPR-POND C | 5 | * | ** | | ** | | ** | | ** | | |
| COPR-POND D | 8 | * | ** | | ** | | ** | | ** | | |
| COPR-POND B | 17 | * | ** | | ** | | ** | | ** | | |
| | | | | | | | | | | | |

 Table 13. 2004 Outplants- Survivorship, growth and flowering in 2004.

TOTAL 215 117 * Plants in severe decline due to abrupt drop in water table

** No data collected

| | NUMBER | ALIVE MID | MEAN | | | | MEAN # | | MEAN # | | |
|-------------|---------|-----------|--------|-------|--------|------|------------|------|-----------|------|-----------|
| | PLANTED | SUMMER | HEIGHT | STD. | MEAN # | STD. | VEGETATIVE | STD. | FLOWERING | STD. | PERCENT |
| SITE | 2004 | 2005 | (CM) | DEV. | SHOOTS | DEV. | SHOOTS | DEV. | SHOOTS | DEV. | FLOWERING |
| | | | | | | | | | | | |
| MAND-1A | 10 | 3 | 106.3 | 15.8 | 7 | 1.5 | 3.3 | 2.08 | 3.3 | 3.2 | 100% |
| MAND-1B | 15 | 7 | 108.9 | 36.26 | 14.9 | 6.5 | 4.3 | 3.7 | 11.1 | 7.3 | 100% |
| CSMR 5B | 15 | 2 | 146 | 12.7 | 18.5 | 4.9 | 2 | 0 | 17.5 | 6.4 | 100% |
| CSMR 5C | 25 | 20 | 121.8 | 28.2 | 13.9 | 6.9 | 4.1 | 3.7 | 11.6 | 6.6 | 95% |
| COPR-LAGOON | 54 | 31 | 120.5 | 29.1 | 17.6 | 9.3 | 5 | 6.1 | 14.7 | 9.1 | 97% |
| COPR-POND D | 8 | 1* | ** | | ** | | ** | | ** | | 100% |
| | | | | | | | | | | | |
| TOTAL: | 127 | 64 | | | | | | | | | |

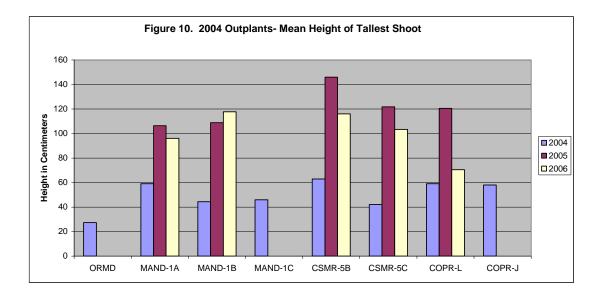
 Table 14. 2004 Outplants- Survivorship, growth and flowering in 2005 and 2006.

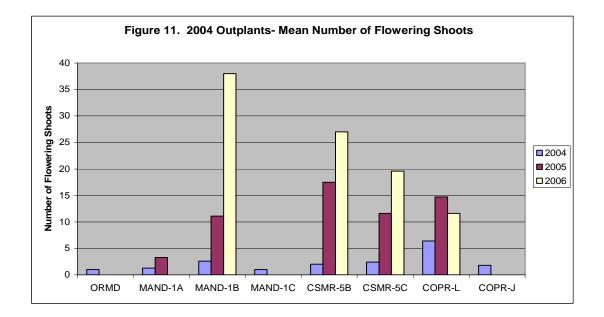
| | 2006 | | | | | | | | | |
|----|----------------------|--|--|--|--|---|---|--|--|---|
| 10 | 1 | 96 | N/A | 20 | N/A | 0 | | 0 | | 0% |
| 15 | 7 | 117.7 | 39.7 | 41.7 | 23.6 | 38 | 23.5 | 8.9 | 8.8 | 100% |
| 15 | 1 | 116 | N/A | 35 | N/A | 27 | N/A | 18 | N/A | 100% |
| 25 | 11 | 103.4 | 26 | 20.8 | 10.7 | 19.6 | 10.6 | 26.5 | 20.1 | 100% |
| 54 | 13 | 70.4 | 33.1 | 28.4 | 26.1 | 11.6 | 12.2 | 13.6 | 11.2 | 77% |
| 8 | 1* | ** | | ** | | ** | | | | 100% |
| | 15 15 25 54 | 10 1 15 7 15 1 25 11 54 13 | 10 1 96 15 7 117.7 15 1 116 25 11 103.4 54 13 70.4 | 10196N/A157117.739.7151116N/A2511103.426541370.433.1 | 10196N/A20157117.739.741.7151116N/A352511103.42620.8541370.433.128.4 | 10 1 96 N/A 20 N/A 15 7 117.7 39.7 41.7 23.6 15 1 116 N/A 35 N/A 25 11 103.4 26 20.8 10.7 54 13 70.4 33.1 28.4 26.1 | 10 1 96 N/A 20 N/A 0 15 7 117.7 39.7 41.7 23.6 38 15 1 116 N/A 35 N/A 27 25 11 103.4 26 20.8 10.7 19.6 54 13 70.4 33.1 28.4 26.1 11.6 | 10 1 96 N/A 20 N/A 0 15 7 117.7 39.7 41.7 23.6 38 23.5 15 1 116 N/A 35 N/A 27 N/A 25 11 103.4 26 20.8 10.7 19.6 10.6 54 13 70.4 33.1 28.4 26.1 11.6 12.2 | 10 1 96 N/A 20 N/A 0 0 15 7 117.7 39.7 41.7 23.6 38 23.5 8.9 15 1 116 N/A 35 N/A 27 N/A 18 25 11 103.4 26 20.8 10.7 19.6 10.6 26.5 54 13 70.4 33.1 28.4 26.1 11.6 12.2 13.6 <td>10 1 96 N/A 20 N/A 0 0 15 7 117.7 39.7 41.7 23.6 38 23.5 8.9 8.8 15 1 116 N/A 35 N/A 27 N/A 18 N/A 25 11 103.4 26 20.8 10.7 19.6 10.6 26.5 20.1 54 13 70.4 33.1 28.4 26.1 11.6 12.2 13.6 11.2</td> | 10 1 96 N/A 20 N/A 0 0 15 7 117.7 39.7 41.7 23.6 38 23.5 8.9 8.8 15 1 116 N/A 35 N/A 27 N/A 18 N/A 25 11 103.4 26 20.8 10.7 19.6 10.6 26.5 20.1 54 13 70.4 33.1 28.4 26.1 11.6 12.2 13.6 11.2 |

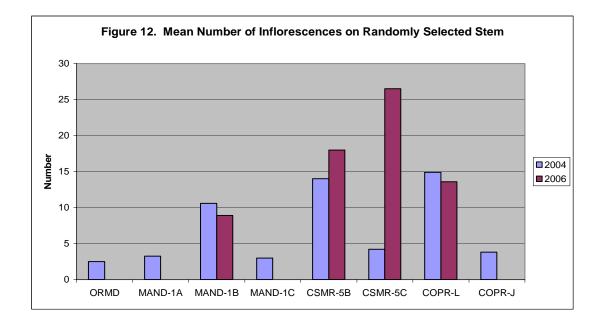
 TOTAL:
 127
 34

 * Plant 46-16- not examined, presumed dead in 2004, detected in spring 2006, with 2005 pods on 2005 stems

** No data collected





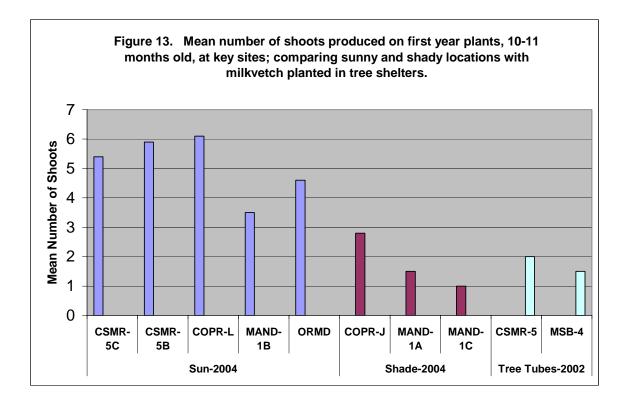


The 2004 outplants generally achieved their greatest size and vigor in their second summer, 2005 (**Figure 10 and Table 14**). This is consistent with the observations on the original outplants (**Figure 5 for 2003**). Milkvetch in their second year at more favorable growing sites exhibit the most growth, biomass and flowering in their second season, with gradual declines in subsequent years as the plants age. MAND-1B was an exception- this group in 2006, their third summer, was slightly taller, and produced 3-4 times the number of flowering shoots as they did in their second season.

In 2006, their third summer, the COPR-Lagoon plants exhibited more decline than the CMSR-5C group. The eleven remaining Lagoon plants were shorter, produced fewer flowering shoots, and around ½ as many flowers per randomly sampled stem, compared with the 13 remaining CSMR-5C plants. The one surviving individual in the CSMR-5B plot was large and vigorous in 2006 and is reshooting in 2007. Eight plants are reshooting at CSMR 5C as of May 2007.

We compared the mean number of shoots produced on first year 2004 plants growing in sunny and shady locations with the number of shoots produced in 2002 on individuals planted in tree shelters (**Figure 13**). The 2002 data is for reproductive individuals only as reported in Soza, et al. 2003. The 2004 outplants growing in sunny locations produced more shoots than the 2004 group planted in shady locations. Plants confined within tree shelters produced only 1-2 flowering shoots while plants in sunny locations produced means

ranging from 3.5 to 6. While the tree shelters reduced risk of loss from small mammals like rabbits, the tubes confined milkvetch and sunlight was reduced on the lower stems and plant bases. Sunlight appears necessary to stimulate more shoot production and flowering in first year plants. Plants at CSMR-5C in 2004 were shorter (mean height of 42 cm) than plants in the 2002 CSMR-5 reproductive group (mean height of 61.7 cm in flowering plants). But CSMR-5C produced twice as many flowering shoots and more vegetative shoots than the 2002 plants confined to tree tubes.



WILD SITE IN 2006

We monitored the population of VMMV at the wild site on August 24, 2006 and report these data in **Table 15**. Sixty reproductive adults were observed, and these were all located in the Colony B area (northern group). Five non-flowering juveniles, probably recruited in 2005, were observed in Colony A (southern group). The remaining non-flowering juveniles were located in Colony B. The Colony A area has generally declined over the last few years and it is less vegetated, overall, than the nearby Colony B location. Colony B appears to have contracted in areal extent, and milkvetch are growing in clumps and very crowded in some areas. Additional 2006 recruits have appeared to the north and northwest of the Colony B area- shrub cover is gradually increasing in the northwest area, which was almost barren in 1997 when milkvetch were first discovered. This location corresponds with the general direction of sheet flow and heavy rainfall in 2005 was observed sheeting in this general direction (northwesterly) which may have dispersed some milkvetch seed in that direction. Also, a gradual increase in shrub cover in this area is likely helping protect the milkvetch from some dry winds and may increase localized fog drip.

Adult VMMV at the wild site in 2006 were similar in mean height to the MSB-4 outplants in their second summer in 2003 (90.6 cm +/- 33.4 vs. 99.3 +/- 33.4) but the wild site produced around half as many shoots, flowering shoots and inflorescences per random branch compared with MSB-4 (**Table 9**). Despite their similar height, overall vigor and flowering was much reduced at the wild site compared to the introduced populations.

Comparison with measurements in Wilken and Wardlaw (2001) for summer of 2000 shows an even more striking difference in growth and flowering at the wild site compared with some of the more favorable introduction sites. Mean height of the longest shoot on reproductive plants at the wild site in 2000 was 46.2 cm (N= 23). Mean number of inflorescences per shoot (all shoots were counted) was 3.2. It therefore appears that when we compare the wild site in 2006 with similar data from summer of 2000, milkvetch were half as tall and produced ¼ the number of flowers in 2000. Rainfall in both 2006 and 2000 was near normal (**Figure 1**). But rainfall in the year prior was very different. Only 9.1 inches fell in 98/99, while 29 inches fell in 04/05. We hypothesize that there is a prior year, carry-over effect, and that high rainfall in the year prior produced better growth in the subsequent year at the wild site than when the prior year was very dry. This effect could arise from simply more favorable soil moisture conditions, better plant vigor going into the 2006 growing season, or a combination of both.

| WILD SITE | N | PERCENT FLOWERING | MEAN HEIGHT (CM) | STD. DEV. | MEAN # SHOOTS/ PLANT | STD. DEV. | MEAN # FLOWERING SHOOTS/ PLANT | STD. DEV. | MEAN # INFL PER RANDOM STEM | STD. DEV. |
|---|----|----------------------|------------------------|--------------|----------------------------|--------------|--------------------------------------|--------------|-----------------------------------|--------------|
| Colony "A" Adults | 0 | | | | | | | | | |
| Colony "A" juveniles | 5 | 0 | 5.6 | 3.2 | 2.6 | 1.1 | N/A | | | |
| Colony "B" Adults | 52 | 88% | 90.6 | 33.4 | 6.2 | 4.8 | 5.4 | 4.5 | 11.3 | 13.5 |
| Colony "B" subset larger than 60 cm | 40 | 98% | 105.3 | 22.9 | 6.92 | 5.1 | 6.4 | 4.7 | 13.1 | 14.8 |
| Colony "B" subset smaller than 60 cm | 12 | 58% | 37.5 | 4.9 | 2.8 | 2.4 | 1.9 | 1.2 | 5 | 2.5 |
| Colony "B" 2005 | | | | | | | | | | |
| Juveniles | 6 | 0 | 29.7 | 8 | 1.3 | 0.5 | N/A | | | |
| Colony "B" 2006 Juveniles | 37 | 0 | 3.2 | 2 | 1.2 | 0.5 | N/A | | | |

NATURAL RECRUITMENT

Recruitment of new milkvetch from seed produced by the introduced plants has been documented at most of the introduction sites at one time or another across the study period. Milkvetch seedlings have distinctive club-shaped cotyledons and are relatively easy to recognize (**Photo 32**). Seedlings generally appear following episodes of heavy rainfall. Seedlings are sometimes seen as late as June, suggesting germination can occur weeks after cessation of rainfall. Seedlings emerging early in the season often do not persist for very long and are vulnerable to complete removal by herbivores such as rabbits. Seedlings appearing after March are more likely to persist, possibly because other vegetation is more available to sustain herbivores. As the associated vegetation develops in springtime, it can also provide some visual cover, and young milkvetch growing in amongst other plants are sometimes left alone by herbivores (**Photo 33**). When associated vegetation becomes dense and lush, young milkvetch often decline as competition worsens and light becomes limiting (**Photo 34**).



Photo 32. Young juvenile milkvetch in twiggy litter. Club-shaped cotyledons are still present on most of these individuals. Note heavy condensation on leaflets from late spring fog. Wet conditions stimulated germination of numerous coyote brush seedlings also visible in this photo.



Photo 33. Milkvetch and arroyo willow juveniles are protected amongst stems emerging from below ground rhizomes of Mexican or Baltic rush. Juveniles may be less visible to herbivores like rabbits when they are in and amongst other vegetation.



Photo 34. This juvenile at MSB-3 is being overtopped by goldenrod and western ragweed. Low light conditions and competition is limiting growth and recruits in this area exhibited low vigor, did not produce multiple shoots, had symptoms of mites and eventually failed to establish.

Tables 16, 17, 18, and 19 show the survivorship of each annual cohort of seedlings and juveniles. Figure 14 shows the number of naturally recruited juveniles documented during each annual August monitoring session at all sites. Generally, very few naturally recruited seedlings/juveniles survived into the August monitoring period during the first two years following outplanting. A notable exception was the 2004 cohort produced at MSB-4 where 62 juveniles survived and matured in the plot. CSMR-5 in 2003 and 2004 produced the fewest seedlings with the lowest survival rate. In very wet 2005, seedlings were observed at all the study plots where seed had been previously produced in the plot, including drier sites with low adult vigor such as MAND-2 and MAND-3. Despite considerable seed production in 2004, not many seedlings were observed at CSMR-5 and COPR-Lagoon and few juveniles persisted there in 2005 despite the more favorable moisture conditions. Abundant growth of associated vegetation engulfed many seedlings/juveniles and probably contributed to their failure to establish (Photo 30). Poor juvenile survivorship at the CSMR-5 and COPR-Lagoon abruptly changed in 2006, when both sites produced hundreds of juveniles that matured and about 1/3 flowered and produced seed (Table 15) (Photos 35 and 36).

| 2003 cohorts | | | | | | 1 |
|--------------|---------|--------|--------|--------|--------|---------------------|
| SITE | Feb-03* | May-03 | Aug-03 | Apr-04 | Aug-04 | Comments |
| MSB-3 | 12 | 85 | 8(1) | 3 | 1 | drowned winter 2005 |
| MSB-4 | 32 | 92 | 29 | | 0 | |
| MSB-5 | 2 | 44 | 9 (1) | | 5(1) | drowned winter 2005 |
| CSMR-5 | 0 | 20 | 13 | | 0 | |
| | | | | | | |

Table 16. Naturally Recruited Cohorts- Survivorship and flowering of 2003, 2004 cohorts. (#) = number flowering.

* Reported in Soza, et al. 2003

2004 cohorts

| 2004 Cononta | | | | | | |
|--------------|--------|--------|------------------|--------|--------|-----------------------|
| SITE | Mar-04 | Jun-04 | Aug-04 | Aug-05 | Aug-06 | Comments |
| | | | | | | |
| MCD 2 | 1 | | $\mathcal{O}(4)$ | 0 | 0 | drown ad in winter OF |
| MSB-3 | 1 | | 3(1) | 0 | 0 | drowned in winter 05 |
| MSB-4 | 100's | 314 | 62(8) | 0 | 0 | drowned in winter 05 |
| MSB-5 | <10 | 0 | 0 | 0 | 0 | |
| CSMR-5 | 0 | | 2 | 1(1) | 1(1) | reshooting May 2007 |
| | | | | | | |

| SITE | Nov-04 | Dec-04 | Jan-05 | Feb-05 | Mar-05 | Jun-05 | Aug-05 | Oct-05 | Aug-06 | Comments |
|-------------|--------|--------|--------|--------|--------|--------|----------|----------|---------|-----------------|
| MSB-3 | 100's | 0 | 100's | 0 | 100's | 414 | 416(16+) | 126+(36) | 115(78) | |
| MSB-4 | 0 | 0 | 100's | 0 | 100's | 104+ | 33(10) | 31(12) | 31(29) | |
| MSB-5 | 100's | 0 | 100's | 0 | 100's | 759 | 435(6) | 289+(11) | 39(14) | |
| CSMR-5 | | | 49 | | 3 | 3 | 8 | | 2 | |
| MAND-1 | | | | | | 430 | 68(1) | | 24(3) | |
| MAND-1B | | | | | | | 5 | | 2 | |
| MAND-2 | | | | | | 10 | 1 | | 0 | plot desiccated |
| MAND-3 | | | | | | 3 | 0 | | 0 | plot desiccated |
| COPR Lagoon | | | 13 | | | 1 | 2 | | 0 | |
| COPR Pond | | | | | | | 43 | 23+(3) | 1 | flooded in 06 |

 Table 17. Naturally Recruited Cohorts- Survivorship and flowering in 2005 cohorts. (#)= number flowering.

(1) = number in cohort which flowered in their first and/or second year

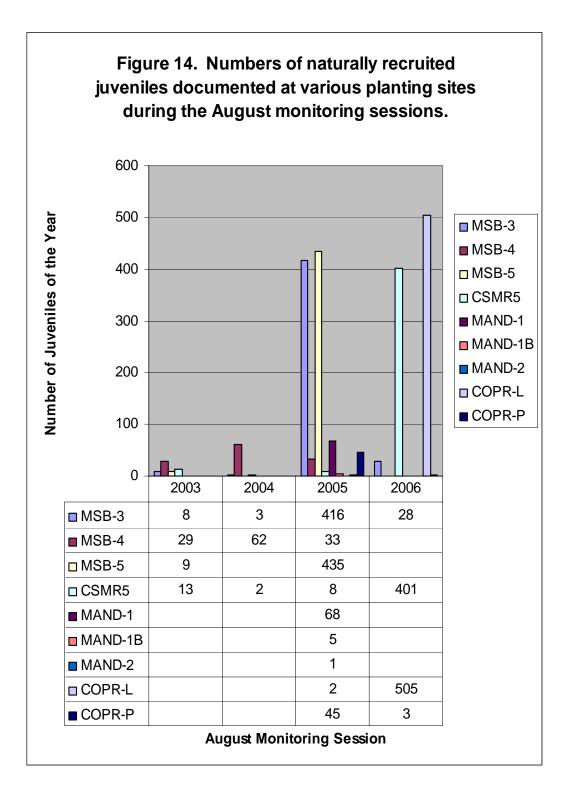


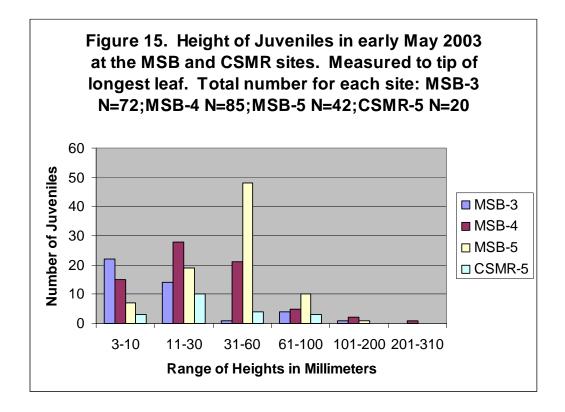


Photo 35. COPR-Lagoon in May 2007. A dense patch of naturally recruited milkvetch (recruited in 2006), contains hundreds of individuals. This patch corresponds to the area in red on **Photo 18**. The rest of the area is covered with dense western ragweed, annual grasses and white sweet clover. Vegetation gaps are largely gone. *Leymus triticoides* has begun to establish in the plot and is also expanding.



Photo 36. Masses of naturally recruited juveniles at CSMR-5C in mid-June, 2006 (center) growing amongst established 2004 supplemental plants (starting to flower and with previous years' annual growth stems still attached). Surrounding bright green vegetation is heliotrope. Absence of juveniles immediately adjacent to established plants may have occurred due to removal of germinating seedlings by an atypical bloom of isopods. Sow and pill bugs were extremely numerous in and adjacent to the root crowns of older milkvetch in 2006.

<u>2003 Observations</u>: Seedling/juvenile milkvetch were first observed in February 2003 at the MSB-3-5 plots (Soza, et al. 2003). By early May, CSMR-5 also had produced seedlings. We examined this cohort of juveniles in detail the first week of May. These juveniles were distributed in small patches and clumps, typically close to the parent plants. We measured the height of juveniles in millimeters, measured to the longest leaf and report these data in **Figure 15**. Height of juveniles ranged from very small, more recently germinated individuals with one or more true leaves (3-10 millimeters) to the tallest individual at MSB-4 which was 310 mm (31 cm;12 inches). The majority of juveniles were located in either microtopographic depressions (38%) or flat areas (34%), and few juveniles were observed on the top of small mounds (6%).



By August 2003, many of the juveniles observed in May were no longer present, and were probably removed by herbivores. At MSB-4, 29 juveniles were still present during the August monitoring session. By early September, these juveniles abruptly exhibited moisture stress, became desiccated and died.

<u>2004 Observations</u>: In spring of 2004, the MSB-4 site produced several hundred seedlings/juveniles while MSB-3, 5 and CSMR-5 had very few (**Table 17**). By June, these juveniles appeared larger and more vigorous than those observed in 2003. We haphazardly marked and numbered 27 milkvetch distributed throughout the plot and followed their growth and survival. Eleven of the 27 plants were already producing multiple stems by June 10, which seemed to be a sign of good vigor and maturation. By early August, only 3 out of the 27 had died. Eight of the 27 were documented flowering (33%). All eight were multistemmed plants greater than 44 cm in size. An additional 38 plants that had been marked with pinflags were still doing well in the plot, 4 flagged plants were missing and presumably herbivorized, and 18 were desiccated. This entire group of plants continued to persist through the fall of 2004 and the majority was starting to reshoot when the entire plot was inundated in early January 2005 and they drowned.

The difference in survival, growth and vigor between the 2003 and 2004 group at MSB-4 is striking, but difficult to explain. We assumed that we would get better juvenile survival in a wetter year such as 2003, which was about four inches above normal rainfall, but this was not the case. In contrast, rainfall in 2004 was about four inches below normal, yet many of the naturally recruited milkvetch at MSB-4 thrived. In addition to differences in total annual rainfall, spring 2003 experienced late rains, and two inches of rain fell in May (VCWPD, 2007). In contrast, no rain fell in April or May 2004. It is possible that competition with associated vegetation benefiting from the late spring rains may have reduced juvenile milkvetch survival.

It should be noted that the MSB-4 plot experienced severe herbivory in late-winter, early spring 2004, and most adult milkvetch in the plot were being herbivorized by rabbits and meadow voles. In contrast, herbivory on the juveniles of the year during April and May was low, suggesting that there is some difference in palatability between juveniles and adults.

<u>2005 Observations</u>: Very wet conditions occurred in water year 2004/2005 and carpets of seedlings were observed on several occasions at the MSB plots, starting in early November following the mid-October rains. These early-germinating cohorts were entirely removed within about two weeks of observing them, presumably by herbivores. All three MSB plots were inundated by McGrath Lake for about ten days on at least two occasions, about one month apart. Following the final dry down of McGrath Lake in early March, the MSB- 3-5 area was again carpeted by a new cohort of seedlings.

By summer of 2005, there were hundreds of juveniles at the MSB sites scattered throughout the original planting locations, new juveniles had emerged on flooded ground beyond the original planting sites at MSB-3 and 4, and juveniles were common underneath dense adjacent vegetation patches at MSB-3. Juveniles in shaded locations such as in the back of the MSB-3 plot (**Photo 37**) and MSB-5 plots persisted throughout the summer but did not put on appreciable growth – they exhibited low vigor, weak stems and may have been affected by mites (**Photo 34**).

Juveniles were common amongst the heavy milkvetch litter in the original MSB-3 planting location, often tucked away amongst the twigs and stems. In early summer, it appeared the heavy litter was providing some protection from herbivores, but eventually, most of these juveniles either disappeared or were engulfed by the mass of vegetation which

grew in the very moist original plot area. At the MSB-4 site, seedlings/juveniles that found themselves in the original planting area were engulfed in new growth of rhizomatous perennials such as salt grass, juncus and carex and quickly disappeared (**Photo 6**). Snail control in the MSB-4 area was nearly impossible due to moist conditions and dense herbaceous cover, and only the juveniles in the exposed, drier southern edge beyond the original planting plot were able to establish (see **Photo 25**).



Photo 37. Abundant milkvetch juveniles amongst competitors in March 2005 on previously inundated ground in the central MSB-3 plot. Following dry down, numerous species including arroyo willow, coyote brush, mugwort, and primrose also produced abundant recruits. The red runners are from an adjacent yerba mansa. None of these milkvetch juveniles survived the overgrowth of vegetation which occurred in this area over the subsequent growing season.

The MSB-5 plot is confined by McGrath Lake to the west (about 2-3 meters away), and surrounded by dense vegetated areas on all sides. Following flooding, we did not observe natural recruitment in these adjacent areas, and new seedlings were confined to the small amount of remaining open ground on the edge of the original planting plot (**Photo 15**).

We marked thirty juveniles of the year in early summer 2005 at each of the MSB sites, scattered throughout the area, and monitored them in early August. We assigned a unique identifier to the new areas at MSB-3 and 4 and these are labeled "E" referring to them

being a new extension of the original plots. Fifty eight percent of the 17 marked plants located in full sun in the MSB-3 E, north of the original planting plot, were in flower in early August. Thirty three percent of the 18 plants in the MSB-5 plot were flowering, and 38% of the MSB-4 E plants, located in the new area south of the original planting plot, also flowered. By October, a few more plants matured and were able to flower and produce some modest amounts of seed.

In 2005, seedlings/juveniles were also observed for the first time at the Mandalay plots. Juveniles were observed at all the Mandalay plots where seed had been produced, including the low vigor MAND-2 and MAND-3 locations. The only juveniles to persist were in MAND-1 and MAND-1B. Once the COPR-Pond site dried down, 45 juveniles were also observed in August. These juveniles were located in four small groups close to where the previous adults had produced seed in 2003 and 2004 (**Photo 19**). None of these juveniles were flowering on August 10, but by October 21, three plants were flowering and fruiting, ranging in height from 66-84 cm and had produced multiple flowering branches. These did not persist because the COPR-Pond overtopped its banks and the adjacent dune hollow supporting milkvetch was under water again for a period of time in winter 2006.

Both the CSMR-5 and COPR-Lagoon sites produced few juveniles in 2005 despite the wetter growing conditions (**Figure 14** and **Table 17**). Many of the seedlings and juveniles observed in the vicinity of the CSMR-5 plots were unable to compete with the growth of neighboring herbaceous vegetation (**Photo 30**). All eight of the CSMR-5 juveniles monitored in August were under 10 cm in size and were apparently unable to reach the size and vigor exhibited by the plants at the MSB and COPR-Pond sites.

<u>2006 Observations</u>: Rainfall levels in 2006 were close to normal in the general region (**Figure 1**). Relatively few juveniles were observed at the majority of the planting sites in 2006 (**Table 19**). At MSB-3, most of the juveniles produced were adjacent to the 2005 recruits in the north plot extension, and they became overtopped by vigorous growth of the previous year's cohort. Largely confined beneath the shade of the adult plants, they did not flower and most of them were left in small hardware cloth cages so that they would not be removed by herbivores. No seedlings were seen at the MSB-4E and 5 locations. Three additional juveniles were observed in summer at the COPR-Pond area in the vicinity of the previous 2003 planting area where seed had been produced.

| | 2006 | 2006 | 2006 | 2006 | Estimated % |
|---------------|---------------|-----------|---------|-------------|-------------|
| SITE | Non-flowering | Flowering | Counted | Estimated * | Flowering |
| | | | | | |
| MSB-3E | 26 | 1 | | | |
| MSB-3 | 1 | | | | |
| MSB-4E | 0 | | | | |
| CSMR 5 | 30 | | | | |
| CSMR 5B | 25 | | | | |
| COPR-Pond | 3 | | | | |
| | | | | | |
| COPR-Lagoon * | | | 146 | 200 +/- | 33% |
| CSMR 5C * | | | 236 | 269 +/- | 33% |

Table 19. Status and flowering in naturally recruited cohorts- 2006. (#) = number flowering.

2006 cohort

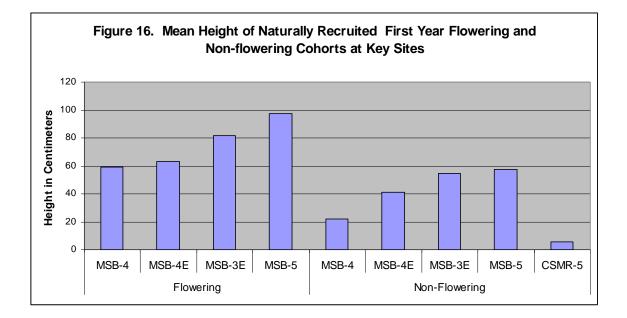
* Unable to count individuals due to large numbers of natural recruits very close together.

A dramatic increase in juvenile recruitment and survival occurred at the CSMR-5C and COPR Lagoon sites in 2006 (**Table19**). This was the first time that hundreds of seedlings persisted and developed into larger plants at these locations. At CSMR, most of these juveniles were growing in dense patches in the vicinity of the central area of the CSMR-5C plot and likely were from seed produced by the supplemental plants, rather than the original founders (**Photo 36**). The bulk of these juveniles were located in dense patches and quickly it became impossible to separate individual plants and accurately count them. We estimated about 1/3 of these plants matured and were flowering in August and September. While herbivores had removed some seedlings in certain areas, they also apparently left some of the denser patches alone. It again appeared that small mammal herbivores were not particularly interested in eating juvenile plants.

The COPR Lagoon also had hundreds of naturally recruited juveniles surviving into the summer months. However, they were distributed in a much more localized area than the CSMR site. The COPR Lagoon site has very little open ground left, and all the associated herbaceous vegetation has become much denser, limiting locations where seedlings can emerge in relatively open growing conditions (Photo 35).

The large pulse of recruitment at CSMR and COPR Lagoon in 2006 is interesting and again, difficult to explain. Both the CSMR-5 and COPR-Lagoon sites support a perched water table that is more distant from the surface elevation than the MSB sites (Figure 1). Observations to date suggest that sites with a water table closer to the surface, such as the MSB sites, exhibit better seedling survival than locations distant from the water table. The higher clay content at the CSMR sites and compacted texture in summer when surface soils are dry appears to slow juvenile development, but this was suddenly not a problem in 2006. One possible factor which may account for the difference in growth and survival seen at these sites in 2006, is the fact that the previous year was very wet. This may have resulted in a very well charged perched aguifer that may have provided hydrologic benefit that carried over into the next growing season. At the COPR Lagoon, for example, Parker was unable to hit saturation in fall 2004 and indicated the water table must have been more than 150 cm deep (Parker, 2006). In November 2006 we found a perched water table at two nearby sample locations at 84 cm and 123 cm below ground surface (Figure 2). This limited information suggests more moisture was likely available below ground in 2006 which may have improved survivorship and growth in this cohort.

<u>Comparison of First Year Natural Recruits</u>: **Figure 16** compares the mean height of the longest stem on flowering and non-flowering first year juveniles at selected locations. Data used for this figure compare the MSB-4 2004 group of 27 marked plants, and the 2005 marked cohorts of around thirty plants at the MSB-3E, MSB-4E and MSB-5 and all the surviving juveniles at the CSMR-5 location. All locations were relatively open and sunny, except the MSB-5 location which was becoming increasingly overtopped by tall willows. Overall, the flowering cohorts exhibited a mean height of 60 cm or greater while the non-flowering cohorts were shorter than 60 cm at all sites. The tallest flowering plants were located in MSB-5, and we believe these plants were taller, in part, because under lower light conditions, longer but weaker stems are produced as the plants are "leggier" as they struggle to reach more sunlight.



Seed Dormancy Observations

Over the course of observing the wild North Shore milkvetch population, some seedlings generally emerge every year. Wilken and Wardlaw (2001) note VMMV is capable of annual recruitment from a seed bank. Mature legume seeds generally require a treatment to break the hard seed coat and stimulate germination, and both seed coat nicking and boiling water have been used to stimulate germination of VMMV (Wilken and Wardlaw, 2001). They therefore speculate that under natural conditions, the amount of mechanical abrasion equivalent to that produced by standard scarification techniques seems unlikely to take place in the few months between maturity (late summer) and the onset of winter rains (fall and early winter). Weathering and the action of soil micro-organisms contribute to breaking dormancy. Consequently, Wilken and Wardlaw (2001) conclude it is likely not all seeds produced during any year may germinate in the following year.

Observations of seedlings emerging in the MSB plots in February 2003, a few months after seed matured for the first time at the introduced site, suggests that at least some portion of the previous year's seed production can germinate within a few months of hardening. It is possible that mechanical abrasion from human activity visiting and walking in the milkvetch area could have stimulated some seeds to germinate. However, seedlings in May 2003 were often observed close to and underneath parent plants, and sometimes within a few centimeters of the plant bases where trampling is unlikely to have occurred.

Smith and Sandoval (2001) examined seed dormancy in VMMV under controlled conditions at the COPR greenhouse. They investigated seed dormancy in two morphologically different types of fresh seed collected from plants at the COPR nursery. Dr. Cris Sandoval had previously observed two types of seeds- one type are small with a green seed coat and the others are larger, flattened and dark (**Photo 38**). They note that fewer dark seeds were produced but they did not document the proportion of dark to green seed.

RSABG had previously demonstrated reasonably good levels of germination by nicking each seed coat with a straight edge razor (66% germination from wild collected seed) (Soza et al. 2003). Smith and Sandoval (2001) were interested in whether dormancy could be broken using a simpler method like immersion in cold or hot water; and whether green and "black" seeds had different germination rates and dormancy mechanisms. They found that the black seeds started germinating within one week of planting without a presoak or hot water treatment. Within ten days, over 30% had sprouted and after 40 days, germination leveled off around 55%. In contrast, less than 20% of the green seeds not subjected to a cold presoak or hot water treatment had germinated seventy days later. When black or green seeds were subjected to the cold water presoak treatment, the black seeds again germinated rapidly (over 80% within 10 days). Around 80% of the green seeds eventually germinated, but it took about seven weeks to achieve the same germination rate as the black seeds. The hot water soak treatment stimulated the highest rates of germination- above 90% in the green seeds and leveling off around 75% for the black seeds. Smith and Sandoval (2001) conclude that the black seeds are better adapted for first year sprouting, and that the green seeds were viable but had more protracted dormancy. They hypothesize that under non-greenhouse conditions, the green seeds likely have a longer dormancy.



Photo 38. Milkvetch seed magnified 30 times, viewed through digital camera and eyepiece of compound microscope. Seeds are approximately two millimeters in length. One seed (center) exhibits an exit hole made by a seed weevil or predatory wasp. Four seeds in this view exhibit the blacker rough seed coat (abbreviated:BT). Fibers on the outer coat make these seeds appear whiter under artificial light. Note that the two uppermost blacker looking seeds are laterally compressed and less rounded. Blackened coats appear rougher, often have light colored fibers of some type on the seed coat, and exhibit more cracks and weak points. The flattened, blacker seed just above the seed with the weevil hole has a cracked seed coat along the bottom right edge.

In this study, we also observed some darker seeds in material collected from the introduction sites in 2003 and 2004. While the vast majority of mature milkvetch seeds are plump, and a smooth, dull brownish green color, we occasionally saw seeds we describe as slightly larger, with a blackened, rough looking seed coat and the seeds are less plump and slightly flattened/laterally compressed (**Photo 38**). We report the percentage of black seed (BT) from samples taken in 2003 and 2004 for selected introduction sites and the wild site (**Table 11a, 11b**). Black, rough-looking seed (BT) were observed at all collection sites in both years. Percent BT ranged from 2.4 to 13.8 percent of the seed sampled in 2003, and 6.7-12.5 percent of the seed observed in 2004.

Smith and Sandoval (2001) comment that the lower percentage of sprouting in green seeds under controlled conditions (under 20%) indicates that immersion may be necessary to break dormancy in first year seed under natural conditions. Our observations of very large amounts of seed germinating following episodes of heavy rain and flooding at the MSB sites would be consistent with their observations. We assume that the bulk of seed germinating in the plots in 2005 was produced in 2003, as far less fruit was produced in 2004 due to herbivory.

At least five waves of mass germination were observed at the MSB sites starting in November following almost 4 inches of rain in October 2004. If all the seeds possessed the same dormancy mechanism, they would all have germinated at the same time, unless they were still retained in pods or held above ground on annual stems. The batch of stubborn, later germinating seeds at the MSB sites, emerging after final dry down in early March 2005, saved the populations there from extirpation.

Large amounts of milkvetch can also germinate without immersion or extremely wet conditions. In 2006 at the CSMR-5C and COPR Lagoon sites (**Table 1**), hundreds of seedlings appeared and matured in areas not subjected to flooding or obvious extended periods of immersion. Rainfall in 2006 most closely approximated normal (**Figure 1**).

Seed Dispersal Observations

This study provided an opportunity to examine natural dispersal of milkvetch seed from introduced populations. We know that all observed seedlings came from seed produced by planted milkvetch and therefore did not arise from a pre-existing seed bank in those areas. In early May, 2003, we examined seedlings and juveniles at CSMR 5 and the three MSB plots and measured the distance from each juvenile to the base of the closest planted milkvetch in centimeters (**Table 4**).

Seedlings were generally distributed in patches and clumps, usually in close proximity to parent plants. We often observed that seedlings were most common beneath the flowering branch where seeds and or pods were concentrated and had fallen there due to gravity.

| Site | Ν | Mean Distance | Maximum Distance |
|--------|----|---------------|------------------|
| MSB-3 | 85 | 15.21 | 74 |
| MSB-4 | 92 | 11.85 | 38 |
| MSB-5 | 44 | 44.7 | 106 |
| CSMR-5 | 20 | 23.7 | 45 |

Table 20. Distance of 2003 juveniles from closest parent in centimeters.

Surface sheet flow may also disperse seeds beyond parent plants. Heavy rainfall in 2005 resulted in surface sheet flow at the wild site that apparently carried some milkvetch seed 6-8 meters beyond the area where seedlings are normally seen and generally in a northwest trending direction (Larry Lodwick, pers. comm.).

Milkvetch seed often remains in the pods for a considerable period of time, and some infructescences with pods still containing seed are commonly retained on the previous year's growth stems well into the next growing season and even through fall of the next year. The species *pycnostachyus* is described as having pods that are long persistent on the receptacle with very tardy dehiscence (Barnaby, 1964). It is common to observe intact pods/infructescences on the ground still retaining the seed. Pods retained on stems eventually start to break down if they don't fall, and the thin valve tissue tends to break down first while the thicker tissue associated with the ventral and dorsal suture remains intact. The top of the pod, oriented skyward, decays sooner than the bottom- one can some times observe seed visible through the breaking down valve walls with the relatively intact dorsal side of the pod appearing like a boat containing the seeds. Fewer pods are retained on old stems in winters that experience heavy rains and high winds, presumably because the stems get blown and pods/infructescences break off.

The tendency for seed to be retained in pods for a prolonged period suggests that the pod itself may serve as a dispersal unit. The small, lightweight pods are only slightly inflated.

When working in a milkvetch plot, it is common for dry, mature pods to catch on clothing and hair. Milkvetch pods are beaked at the apex by an incurved, persistent style and pods in our variety, *lanosissimus*, are usually thinly strigulose. Sometimes, dried calyx material and even dried floral tubes are retained on pods. These features combine to make mature dry milkvetch pods stick and snag on hair and clothing. It is therefore likely that milkvetch pods can adhere to mammal fur, providing an opportunity for pods with seed to be carried further away from parent plants. While we predict that seed could be dispersed in this manner, we have not observed milkvetch distant from the planting area that might have arisen from seed carried out of the plot by humans or small mammals.

Inundation and flooding which occurred at certain planting locations in 2005 provided an opportunity to observe flood-mediated milkvetch seed dispersal. At the MSB-3 and 4 sites, seedlings emerged along the edge of the flood line that extended several meters beyond the original planting sites out into more open ground on drier low dune areas (**Photo 25**). The edge of the flooding at MSB-3, for instance, carried detritus and light litter floated out to the margin during inundation and accumulated there (**Photo 23**). Numerous milkvetch emerged in this area following dry down, corresponding with the edge of inundation and litter accumulation (**Photo 24**). We hypothesize that milkvetch pods still retaining seed floated out of the ground litter. The slight inflation of the pods (reported by Barnaby (1964) as "a little inflated but hardly bladdery") may help pods float. The furthest distance milkvetch juveniles were observed following inundation at MSB-3 was 14 meters (46 feet).

At the COPR-Pond in August 2006, we discovered two etiolated non-flowering milkvetch emerging from robust dense skeletons of drying *Melilotus alba* mixed with dense coyote brush. We believe these two plants emerged from seed/pods that floated out following inundation in 2005. They were found west and about 22 meters (75 feet) from the original Pond planting site where seed was previously produced in 2003 and 2004. These plants were not seen during the subsequent visits.

In November 2006, Sheri Mayta with CDFG discovered a robust mature milkvetch in full flower and fruit, about 30.5 meters (100 ft) southwest of the original 2003 Pond planting site. It was growing in a low depression closer to the back side of the foredunes. The plant had been overlooked, probably because we simply had never wandered over in that particular direction, and based upon the plant's size and vigor (twelve large flowering stems) it had likely been growing there since the dry down following the 2005 inundation at the Pond. This location also corresponded to the outer edge of the 2005 inundation, but we

cannot rule out that seed was dispersed to these locations by some other means.

The ability of milkvetch seed to disperse to new locations facilitated by inundation during flooding events may represent an important clue to the habitat requirements of VMMV. Successful recruitment of juveniles along the exposed margins of inundation areas suggests adaptation to living adjacent to isolated palustrine and/or lacustrine wetlands. The recovery of inundated milkvetch populations at MSB 3 and 4 and spatial shift in location, for instance, occurred primarily because the very wet winter stimulated considerable seed germination and plants which emerged on the outer edges of inundation found favorable growing conditions combined with low levels of competition from other plants on the adjoining sparsely vegetated dunes. In contrast, seedlings that emerged in the original planting locations largely did not persist because of excessive growth of associated wetland plants, primary herbaceous rhizomatous perennials, stimulated by high rainfall (**Photos 6** and **37**). The subsequent gradual dry down that occurred as inundation subsided may also have contributed to survival of the new recruits- growth rates appeared to keep pace with the gradual dry down, allowing many of these juveniles to establish.

When COPR-Pond and McGrath Lake overtopped their banks, water levels gradually expanded beyond the main lake and pond basins- the water was not moving rapidly or flowing at high velocity like it would if it was associated with a riverine system. The combination of flooding with low water velocity may result in milkvetch remaining in the general area where previous plants were successful, but facilitates dispersal to localized areas nearby where vegetation gaps or low cover of herbaceous perennials occur. The potential for long term dormancy of some portion of the milkvetch seedbank also suggests that seed produced by milkvetch on the outer fringes of previously inundated areas could persist until the next inundation and associated very wet conditions that allow seeds to germinate and mature into juvenile and adult plants.

Another aspect of potential milkvetch seed dispersal involves retention of pods on the previous year's growth stems. We observed that when plots at MSB 3, 4 and 5 were inundated in 2005, the previous year's growth stems, still supporting some pods with seed, were standing above the water (the water was about 30 cm (1.5 ft) deep across the original planting sites) (**Photo 23**). We speculate that this may be an adaptation which might serve, in part, to anchor and retain a portion of the milkvetch seedbank within the vicinity of the parent plant. It may reduce loss of seed during flooding event and may help ensure that some portion of the seed produced remains in the general area where plants were previously

successful.

VMMV therefore appears to be capable of hedging their bet- producing seed that is retained within pods which facilitate dispersal during inundation and flooding to new growing sites while keeping the aerial seedbank portion anchored within a previously successful growing area.

We hypothesize that the aerial stems from the previous year's plants would probably not remain above the flood level in riverine systems subject to moving water, higher velocities and likely carrying woody debris and litter. The stems would snap and break or topple under those conditions. None of the planting sites to date occur adjacent to stream or The 1967 historic collection of a single plant across from the entrance to river systems. McGrath State Beach was on the extreme outer fringes of the floodway associated with the Santa Clara River and suggests VMMV also potentially occurred on the edges and fringes of riverine floodways. These fringe areas may represent temporary locations where milkvetch seed is deposited and able to grow for a period of time following flood events at locations with more open ground, possibly lower plant cover, and/or areas where fine sediments have been deposited. As these fringe areas dry down, milkvetch seedlings could potentially keep pace with the dry down and mature to older age classes and even potentially produce some viable seed in their first growing season. In low rainfall years, these same sites may be too arid or too distant from a perched water table for juveniles to successfully recruit, and during dry time periods, milkvetch may "blink out" and reside only as dormant seed bank.

VIII. MILKVETCH HEALTH AND DISEASES

Over the course of the study period, we have observed several different abnormal growth, stress and disease indicators and we describe these in more detail below. We do not know the definitive cause of most of these anomalies, and therefore, they are described below based upon the observed symptoms.

<u>Sudden Onset Decline in Full Flower on Whole Milkvetch Patch</u>: We have observed established plants in good vigor and in full bloom and fruit development, abruptly become stressed, older leaves drop, flowers and fruits stop maturing, and often, few seeds are produced (**Photo 26**). Eventually, the younger leaves and growth tips can also be affected.



Photo 26. Sudden loss of soil moisture or water table at COPR-Pond, August 2004. Mature plants were in full flower. Old leaves are drooping and graying and have died. Only the youngest leaves and tips are still green. Meanwhile, the flowers all turned black and died, and the pods blackened as live tissue abruptly died. Few mature seeds were produced.

When this occurs across the entire patch, it is generally an indicator of water stress. This phenomenon occurs commonly at the wild site and COPR-Pond. Moisture is limited at the wild site, and in drier years and later in the season, all the available soil moisture perched above the oil layer can be eliminated- growth, flowering and fruiting are subsequently arrested. This problem contributes to low fruit production at the wild site in drier years, and in most years, inflorescences produced late in the season do not form fruit while flowers produced earlier in the season, do. Plants do not necessarily die if this occurs in late summer and fall. Milkvetch appear to store sufficient reserves to be able to recover from late season drought when moist conditions resume.



Photo 31. Sudden decline of mature individual in full flower, CSMR-5, 2003. Declining plant has turned grey from tissue death. Adjacent plants on left unaffected. Plant in back on far right, also affected.

Sudden Onset Decline in Full Flower on Single Individuals: We have observed large, robust plants in full bloom abruptly lose vigor, the older leaves die and fall, and sometimes, even the younger leaves and green growth tips decline (**Photo 31**). This has occurred at several sites, in different years, and usually affects single individuals or sometimes, several individuals near one another. The symptoms usually come on rapidly. Flowering usually ceases and pods stop maturing when this symptom arises. Plants usually die and do not reshoot after this occurs, but not always. These symptoms were discussed with Cheryl Bloomcrist from the California Department of Food and Agriculture, Plant Health and Pest Prevention Services (pers. comm October 17, 2006). She indicated the symptom was likely some pathogen affecting the fine roots, possibly some type of root rot. A definitive determination would require excavating the root system and obtaining living samples of healthy and unhealthy fine root tissue for examination at the state lab. A problem in the root crown could also be a possible cause.

<u>Miniaturization</u>: We have observed odd growth in certain individual plants that we describe as "miniaturization". This has occurred primarily at the CMSR-5 planting areas. New shoots

seem shorter and narrower than normal shoots and the leaflets are smaller, narrower, with more acute tips (**Photo 29**). This condition continues as the plant matures (**Photo 28**). Plants have sometimes died the year following the onset of this condition, and sometimes are in good condition the next year. We believe this condition may be the result of air pockets in and around the root crown or upper portion of the plants root system. Air pockets are observed at CSMR associated with deep cracks in the heavy clay soil in summer, and gopher and mole activity was often present around the base of affected plants.



Photo 28. Miniaturization on right, normal growth on left. CMSR-5, 2003.



Photo 29. Miniaturization on reshooting CSMR-5 plant, 2004.

<u>Spots and Weak Chlorophyll</u>: Juveniles and adults growing in low light conditions sometimes exhibit low levels of chlorophyll (look less green) and small spots on the leaflets. This symptom was originally attributed to cucumber mosaic virus (Dr. Dieter Wilken, pers. comm.). Fresh samples sent to the state pathology lab indicated plants had likely had mites, but they were no longer present. Cheryl Bloomcrist (pers. comm. 2006) indicated that cucumber mosaic virus is an agricultural pest and unlikely to affect wild plants.

<u>Tissue Senescence and Blackening</u>: When VMMV were first discovered in August 1997 at the wild site in Oxnard, the plants were exhibiting low vigor and leaves were starting to decline. Leaflets had turned from sage green to a straw color (**Photo 27**) and then greyblack fungus fibers could be seen with magnification on the tissues' surface. There were also localized aphid infestations at the site in 1997, and where aphid honeydew forms on plants, sooty mold can grow which appears as a black irregular coating on the affected tissue. A number of subsequent reports describe milkvetch as being adversely affected by "sooty mold". We now understand that the mold which forms on honeydew secretions is termed "sooty mold" and that other molds grow on declining, dieing tissue are called "grey molds" (possibly some type of *Botrytis* or *Cladosporium*, Cheryl Bloomcrist, pers. comm.



Photo 27. Moisture stress showing stages of color loss as vigor declines- from pale sage green to washed out beige, then grey/black with dead, mold-affected tissue (MSB-2).

We rarely see aphid problems on introduced milkvetch and therefore, sooty mold is rarely seen. Aphids were a problem on plants installed in tree shelters (Soza et al. 2003). Tissue senescence and blackening, perhaps more appropriately described as "greying" occurs commonly and is the result of tissue death, but not the cause (**Photo 27**). When flowers abruptly die from lack of water, for instance, the entire inflorescence can also turn entirely black (**Photo 26**).

IX. HERBIVORY

Herbivores have substantial effects on vegetation within habitats where milkvetch were introduced. Non-native snails, small mammals including rabbits, isopods, caterpillars, seed beetles and others, feed on milkvetch and other co-occurring vegetation. Small mammal herbivores like rabbits thin and remove vegetation which directly benefits juvenile

2006).

and adult milkvetch, by creating more open, less competitive growing conditions. Without herbivores, milkvetch would likely be unable to compete, especially at more mesic introduction areas with high vegetative cover and little bare ground. At certain locations and at certain times of year, however, direct herbivory on milkvetch removes seedlings and juveniles and can be severe enough to prevent new growth and lead to failure in adult plants. Herbivory management is therefore a critical component of efforts to establish additional milkvetch populations.

In the following section, we describe various types of herbivores and their effect on milkvetch growth and survival. We also describe the management approaches we have used to deal with herbivory problems. Animal species identifications are tentative and based upon field guides, local lists and personal observations unless otherwise indicated. We also describe other direct and indirect effects animals have on milkvetch.

GASTROPOD HERBIVORY

<u>Snails</u>

Non-native terrestrial snails were identified at the wild site as a cause of herbivory problems in milkvetch shortly after the plants were rediscovered. Impact Sciences, (1998) noted there was no evidence of herbivory on milkvetch leaves in August 1997 although "garden snails" were observed on plants. They found a high number of garden snails in the spring of 1998 feeding on milkvetch. A snail specimen was identified by staff at the USFWS as the milk snail, *Otala lactea* (USFWS, 2000). We typically encounter snails that look like milk snails at most introduction sites. The common brown garden snail, *Cantareus aspersus* (previously *Helix aspersa*), is less frequently encountered.

Terrestrial snails consume tender plant tissue including developing new shoots, leaflets, buds, inflorescences and developing pods and ovules. They can also chew into thick stems and cause stems to collapse or break off at the point of injury. It is likely that snails consume seedlings, although we have not directly observed this. Snail "shine" (dried mucus), is a tell tale sign that herbivory symptoms are likely caused by non-native snails.

Left unchecked, snail herbivory can potentially strip milkvetch of leaves, damage new growth and compromise plant vigor, especially if severe herbivory occurs in spring and early summer. Fall herbivory may be less of a problem to milkvetch survival, as established plants seem to be better able to recover from fall herbivory. However, fall herbivory can be serious when developing fruits are removed, thereby reducing seed production.

Terrestrial non-native snails favor moist, protected habitat conditions. Areas of dense herbaceous vegetation and mats of non-native iceplant near wetlands harbor large populations of snails. Seasonally, there is little snail herbivory during the cool winter months, but they seem to start feeding in February and can become more active on warm winter days. During hot dry weather or in dry years, snails move around less on the ground and stay on vegetation. Sometimes they seal themselves inside with a mucus plug. Snail activity is most noticeable in wetter years, and snail populations appear lower during years of lower annual rainfall. Snail herbivory is most problematic at the MSB and CSMR introduction sites, while snail herbivory has been minimal at the Mandalay and COPR-Lagoon and COPR-Pond sites.

<u>Snail Management:</u> Snail management has focused on reducing the suitability of milkvetch areas to harbor snails, periodic use of *Deadline* snail bait, and hand removal, particularly during the dry season and in years of low rainfall. Iceplant has been removed from areas adjacent to plots at the MSB sites. *Deadline* liquid bait is applied in small drops either near plants exhibiting symptoms of snail damage, or along the moister edge of plots where they abut denser herbaceous wetland vegetation or iceplant mats likely harboring snails. Bait is used as little as possible, and only applied when damage seems to be excessive and hand removal has not been effective.

Hand removal is effective at reducing the numbers of snails and essential in dry years or during dry months when snails seem to stop moving around and do not encounter the bait. To remove snails, we generally look at the plot for a few minutes and as one's eyes adjust, snails start to become noticeable. We find that on any given visit, snails tend to favor a particular zone within the milkvetch area. For instance, on one visit, we may tend to see snails near the tops of plants, hanging on the underneath side of a leaf. On another visit, we may tend to find snails in the middle of plants, on stems and on rebar (installed to support the tree shelters on the 2002 outplants). This tendency suggests the snails are thermoregulating in response to ambient conditions. When a lot of snail shine and tissue damage is concentrated in a particular location on a milkvetch- continued perseverance generally results in finding the snail. In very dry springs, snails tend to reside in the bases of reshooting milkvetch and this can damage new growth unless these snails are located and removed by hand. Areas that tend to harbor snails can include rebar used to mark plants, the plastic, collapsed flags on pin flags, hardware cloth cages and adjacent woody and herbaceous vegetation. Adjacent vegetation should be examined to locate nearby snails. Plants with large leaves at the ground surface also can support snails and should be examined when adjacent to milkvetch areas with snail problems. A single large wild radish (*Raphanus sativus*) that was over one meter tall was harboring hundreds of snails adjacent to CSMR- 5 when it was removed (see pink flowering individual in background in **Photo 16**). As long as non-native terrestrial snails persist at more mesic planting locations, periodic snail management will likely be needed.

<u>Slugs</u>

Slugs are far less frequently encountered than terrestrial snails. They differ from snails in that they spend more time underground or at ground level feeding on roots, buried seeds and seedlings and are therefore less likely to be seen. We have occasionally observed a small, 2-3 cm long dark slug lacking patterns removing cotyledons from seedlings, and assume they contribute to the loss of seedlings germinating in cool, moist winter conditions. These slugs were occasionally seen under cardboard and newspapers placed adjacent to milkvetch at CSMR-5 in 2006, when we experienced severe herbivory on new growth shoots from large numbers of sow and pill bugs. We also very rarely found a larger slug, tentatively identified as the gray garden slug (*Agriolimax reticulatum*) (Hogue, 1993). Captured slugs were removed from the site. Snail baiting should reduce slug numbers in localized treatment areas.

SMALL MAMMAL HERBIVORY

Small mammalian herbivores including gophers, rabbits and meadow voles are common at milkvetch planting sites and play a role in removing seedlings and herbivorizing established plants. However, we have never directly observed small mammals grazing on milkvetch and therefore, the observations in this next section are speculative and based upon professional judgment to some degree. Further direct observation would require trapping, track plates, movement-triggered photography and other intensive study to confirm which exact species is causing a specific herbivory effect. We describe various potential herbivores and their effects separately below, but it should be noted that some herbivory symptoms, especially failed, cropped new annual growth shoots, may be the result of combined effects from different herbivores, all taking advantage of milkvetch at a vulnerable stage.

Gophers

Gophers were identified early on as a problem at the CSMR plantings sites. They are likely Botta pocket gopher (*Thomomys bottae*) (ESA, 2003; Laudenslayer, et al. 1991). Failure of CSMR-1 was attributed to gophers eating a 2 inch diameter hole in the tree shelters and consuming the above ground milkvetch plants (Soza, et al., 2003). The CSMR sites exhibited more gopher activity than the MSB planting sites, and therefore, most of the milkvetch at the original 2002 CSMR plots were planted in below-ground chicken wire cages.

Failure of CSMR-1 early on may have been exacerbated by the planting site location, as it abutted a raised access roadway, which was a few feet higher than the adjacent habitat, providing additional drier ground in which to overwinter and burrow. Further out into the salt marsh, the chicken wire cages rusted away from the corrosive salts and gophers were active in the rye grass meadows just above the mud/salt flats in summer.

Because gophers were common at the CSMR planting sites, battery-operated gopher thumping devices were also used in 2002. They appear to be ineffective- an active device was found in CSMR-5 engulfed in fresh turbated soil, suggesting the vibrations did not deter gopher activity.

Another planting site that experienced excessive herbivory by gophers was South Ormond Beach. Gopher activity was not observed in springtime when the site was initially planted, but later in the summer, gophers removed many of the plants. This planting area was also adjacent to a small hill of higher ground- the gophers may have been residing in this adjoining area and expanded into the planting area when soils became drier in early summer.

Gophers are generally absent from the wild site, presumably because below-ground oil contamination limits their ability to survive at this location. Gopher activity seems more common in areas supporting herbaceous vegetation including annual grasses. The CSMR sites experienced more gopher herbivory than the McGrath sites, and CSMR supports more extensive areas dominated by annual grasses such as *Lolium* and *Polypogon*. Gophers are least common at locations with a perched water table near the surface. We assume that

gophers do not survive well at locations where tunnels are prone to flooding. There are also annual variations in gopher activity tied to cycles of lower precipitation. We note, for instance, that the baseline July 2002 photograph for MSB-3 documented in Soza et al. 2003, showed extensive turbation on the north side of the planting plot, on the drier ground. Since that time, we have observed very little gopher activity at this location, and the site overall appears to have an expanded wetland transitional area compared to 2002 when the plants were originally installed.

Because loss of milkvetch to gophers was identified as a problem in 2002, we selected supplemental planting areas that exhibited low levels or no gopher activity. Milkvetch should generally never be installed at locations that appear moderately to heavily invaded by gophers. To date, we have had minimal loss of milkvetch attributed to gopher activity. Low levels of gopher activity appear to be a good "indicator" of potential suitability for milkvetch. The only location where we have planted milkvetch into below-ground cages was the supplemental plants installed at CSMR-5B and 5C. Since installation, gopher activity overall seems to have lessened in this general area as well, and natural recruits not protected by cages were doing well in 2006.

Rabbits

Herbivory on milkvetch attributed to rabbits has been one of the most difficult challenges facing this project. Rabbits play a major role in removing palatable plant species and plant parts. On the one hand, consumption of herbaceous biomass within milkvetch planting areas by rabbits allows milkvetch to persist in an otherwise highly competitive environment. However, when rabbits or other small herbivores remove seedlings and damage established plants, the potential exists for the entire milkvetch population to be eliminated. Since small mammal herbivores are native to these sites, their removal or control is not an option.

Black-tailed jackrabbit, cottontail and brush rabbits all occur at MSB (ESA, 2003). Black tailed jackrabbits (*Lepus californicus*) are rarely seen and have only been directly observed at Mandalay State Beach, about 0.75 miles south of the MSB planting sites. Their pellets (scat) are substantially larger than cottontail or brush rabbit pellets- this large scat is also rarely seen. The local coastal area around MSB likely supports a remnant population that may occasionally receive new individuals from the nearby Santa Clara River corridor. It is unlikely blacktailed jackrabbits exist in densities sufficient to impact milkvetch survival. Desert cottontail rabbits (*Sylvilagus audubonii*) are abundant and commonly observed in and near milkvetch planting areas at all locations and brush rabbits (*S. bachmani*) have also been observed at CSMR-5.

<u>Herbivory Symptoms Attributed to Rabbits:</u> We have never directly observed a milkvetch eaten by a rabbit. However, signs of rabbit are abundant within planting areas, including footprints, pellets, nesting spots, paths and dust baths. Rabbit paths can generally be seen in sandier locations leading from areas of dense brush cover out to more open ground extending 2-4 meters from the edge of dense protective cover. Rabbit paths created by consuming herbaceous vegetation can also be seen, and these "cleared paths" widen over the summer and fall months as rabbits continue to harvest vegetation along the cleared route. Rabbit pellets can represent a significant component of ground litter at certain sites.

Herbivory on milkvetch attributed to rabbits includes removal of carpets of seedlings in winter and early spring, cropping of new growth shoots attempting to emerge from the woody bases in late winter, and the consumption of above-ground growth stems on mature plants. The rapid disappearance of cohorts of seedlings has been generally attributed to rabbits, and to a lesser degree, slugs and snails.

Severe cropping of new growth shoots is the most troubling result of intense herbivory because it can result in death of that individual if herbivory does not die down on its own. Severely herbivorized milkvetch root crowns will continue to attempt to produce shoots until, presumably, their energy reserves are used up. Severe chronic herbivory results in the root crown producing cropped shoots over most of its surface area, and sometimes appearing like moss on the ground surface (**Photo 12**). In 2004, severe herbivory occurred at MSB-3 and MSB-4, while very low levels of herbivory had occurred the year before. The only plants which survived in MSB-3 were several that were caged and a group of individuals on the north edge of the plot exposed to open low dunes. Plants caged to exclude herbivores as late as mid-May have recovered from severe cropping and produced subsequent growth and flowering.

Rabbits commonly harvest lengths of milkvetch stem, remove the leaves and leafy tips and consume the stem. This symptom was first clearly understood upon examination of planting plots in early winter. We noted that stems of western ragweed reshooting from below-ground rhizomes in winter were disappearing as the ground became covered in discarded leaves and leafy tops (**Photo 10**). Since observing this, I directly observed rabbits harvesting black sage and manzanita in this way at the Burton Mesa Ecological Reserve in Santa Barbara County. In 2006, we noticed that coyote brush and mule fat shoots at the wild site had been cropped around the bases with rejected leaves lying about on the ground and the removed stems largely absent.

<u>Rabbit Herbivory Effects on Vegetation at Planting Sites:</u> Rabbits herbivorize a variety of plants associated with milkvetch planting sites. As a result, there are localized areas of cleared ground with sparse cover of herbaceous plants that commonly abut areas of dense shrub cover, such as stands of coyote brush, willow, dune lupine, spiny rush, and poison oak. We describe these localized areas of open ground as "severe herbivory zones". Rabbits are known to be reluctant to go out into areas that are wide open and distant from hiding cover due to their vulnerability to being spotted by predators. Therefore, they tend to intensively herbivorize localized areas adjacent to dense protective cover where they can quickly escape if predators appear. Coyotes have been seen emerging from the dense arroyo willow dune hollow at MSB near the planting sites on several occasions.

When hardware cloth cages are placed at milkvetch planting sites, one can start to appreciate the affect of herbivory on surrounding vegetation (**Photo 30**). Herbivory cages and exclosures quickly become engulfed in vegetation, especially in wetter years, while areas outside the cages are cleared and open. Herbivory cages are useful in allowing observation of which species are most favored and removed by rabbits or other herbivores that do not enter the cage.

The original 2002 microsites at CSMR and MSB were selected by RSABG for plant installation in 2002. It is now apparent that certain locations with open ground and less competing vegetation were, in fact, locations of severe herbivory adjacent to dense shrub cover. MSB 3, 4, and 5, in particular, had sparse to low cover apparently maintained by herbivores, and, as a consequence, milkvetch installation at these locations placed them under the direct management of herbivores like rabbits. The initial RSABG plantings utilized tree shelters that generally prevented direct herbivory until they were removed in November of 2002.

Most of the 2004 supplemental plantings sites where milkvetch were installed also turned out to be areas subject to severe herbivory by rabbits and possibly others. Since there had been only low levels of rabbit herbivory on milkvetch installations in 2003 and little rabbit herbivory at the wild site, we did not anticipate the degree to which milkvetch would be herbivorized. We also did not use tree shelters for a variety of reasons, but this decision left new installations vulnerable to herbivory shortly after installation.

<u>Vegetative Structure Favoring Rabbit Herbivory:</u> Over the course of this study it has become apparent that the architecture, or vertical and horizontal structure of vegetation in the introduction areas, influences where small mammal herbivory is most severe. The architecture of the milkvetch stand itself can also influences herbivory levels.

We observed that stands of woody vegetation like coyote brush, or taller dense vegetation like a mugwort or goldenrod patch, harbor resident rabbit populations who then venture out of the stand into the surrounding area and graze. Where a dense stand of taller vegetation occurs on only one "side" of a milkvetch planting area and the remaining area is open and comprised of low growing herbaceous species, there tends to be less rabbit herbivory on seedling, juvenile and adult milkvetch. Where shrubs and even mature milkvetch form screens or otherwise block views, rabbit herbivory is generally more severe.

For example, we lost most of the plants installed in CSMR 5B in 2004 to herbivory while most of the plants in 5C were ungrazed. CSMR-5B had been previously weeded, and was largely surrounded by tall goldenrod and coyote brush. CSMR-5C abutted dense coyote brush but opened out onto low growing vegetated areas in the salt marsh and herbivory on the initial installation there was low.

Where milkvetch occur in a patch and are successful at maturing there, milkvetch plants themselves create a visual screen and become areas where rabbits reside and feel safe, particularly later in the growing season (**Photo 16**). Standing litter from previous years' annual growth stems lay about and milkvetch growth becomes dense and blocks views into dense patches. It is common to see rabbit dens beneath dense milkvetch patches. One can look beneath a patch and see where rabbits have removed most competing palatable associates and have consumed low-growing milkvetch stems. Herbivory worsened in 2006 along one edge of MSB-3E where plants grew together and eventually screened off this area from view to the open dunes and, presumably, predators like coyotes and fox.

We therefore recommend avoiding planting milkvetch in "patches" that may result in a dense, close together stand architecture. Planting in narrow staggered bands or linear arrays may be better. Once natural recruitment occurs, however, issues of spacing become

moot, as plants come up where conditions are suitable and survive where herbivores leave them alone. Thinning of taller shrubs and removal of dead biomass can open up sites that have become visually screened, and calm herbivory to some degree.

<u>Seasonal Variations in Rabbit Herbivory and Milkvetch Response:</u> Late fall/early winter represents the onset of difficult conditions for herbivores, since some of their most favored plant species are now dormant and lack fresh growth. In early winter, herbivory on emerging seedlings appears intense. For example, carpets of seedlings which germinated in early rains in water year 04/05 at the McGrath sites lasted only about two weeks

By January of most years, some rainfall has occurred and new growth of favored browse plants like western ragweed begins. However, their favored parts, the stems, are scarce and require more time to develop. Milkvetch adults generally tend to reshoot starting in early January. If, by the end of January, new growth shoots are not seen, this is generally an indication that herbivory is removing new shoots, or that the plant is no longer alive. We note that in 2007, the McGrath plants were reshooting well, until herbivory suddenly started in mid-April.

Sometimes winter-spring herbivory on new annual growth shoots subsides on its own without caging. Established milkvetch seem resilient to some degree and plants caged in late April or otherwise experiencing a reduction in herbivory develop reasonably good subsequent growth and flowering compared with un-herbivorized individuals. We hypothesize that the woody base of milkvetch may serve as a repository for stored energy reserves which help the plant persist in areas subject to periodic herbivory.

As winter progresses into springtime, additional waves of germination usually follow episodes of heavier rainfall. Survival and persistence of seedlings seems to improve as spring conditions appear, suggesting that herbivores shift to more robust vegetation. This provides the window of opportunity for seedlings to develop into juveniles that may persist into the summer months. Our observations over the last few years suggest that there is likely strong selective herbivore pressure on early germinating milkvetch.

Once milkvetch seedlings start to produce true leaves, direct herbivory seems to slow down or stop. Since rabbits prefer milkvetch stems, rather than leaves, there is a tendency for young compact juveniles with woolly leaflets to be left alone in spring and early summer (**Photo 10**). Where juveniles are establishing in sunny open ground, they tend to remain

compact for a period of time and produce multiple stems, but with little elongation. The tendency for maturing juveniles of the year to be less herbivorized than established adults provides an opportunity for some milkvetch to mature at more favorable growing sites and produce seed in their first year.

Adult milkvetch seem to tolerate moderate levels of summer herbivory where they are otherwise in good vigor. While some stems are harvested, others are left alone. Where milkvetch occur in a dense patch, less accessible areas of the patch are less herbivorized than exposed edges adjacent to areas of herbivore activity.

Rabbits and other potential herbivores can continue to graze milkvetch into the fall months. Fall herbivory remains a concern because of removal and loss of stems containing pods and seeds. We have noticed that plants of the year severely herbivorized in fall can reappear the next spring in very healthy condition- suggesting sufficient energy has been stored over the growing season to withstand late season herbivory.

When established milkvetch experience severe herbivory of annual growth shoots which subsequently subsides in late spring, successful reshooting and flowering does occur. Since herbivory forces the milkvetch root crown to continue to push up shoots, once herbivory stops, previously grazed plants tend to exhibit more shoots than ungrazed plants. For instance, data from the MSB sites in 2004 indicated that milkvetch in the herbivorized plots (MSB-3 and 4) produced about twice as many shoots as did MSB-5 (**Table 10**) but fewer of them flowered.

Rabbit Herbivory at the Wild Site: Since its discovery in August of 1997, milkvetch at the wild site have exhibited very low levels of herbivory by rabbits and/or other small mammal herbivores. The only year that small mammal herbivory caused substantial damage to milkvetch was in 1999, which was a relatively dry rainfall year following the extremely wet 97/98 El Nino. Our assumption was that rabbit numbers were higher than normal due to the wet conditions of the previous year and the subsequent very dry winter produced low levels of palatable vegetation. Hungry rabbits therefore appeared to consume milkvetch. Some cages were installed by the wildlife agencies in 1999 at the site, but we were subsequently not allowed back onto the property for several critical months, during which time snails and rabbits severely damaged the population. The lowest number of known adult reproductive milkvetch plants at the wild site occurred that year, consisting of 12 individuals (CDFG files).

Since 1999, direct herbivory by rabbits on milkvetch at the wild site has been low and not a serious problem. Wilken and Wardlaw (2000) asserted that during 2000, they did not observe any evidence of rabbits (fecal pellets, tracks) within the fence-enclosed site. We consistently see evidence of rabbits within the fenced enclosure. We have observed dens, pellets and dust baths in and around the milkvetch areas. We have seen low levels of rabbit herbivory on mature milkvetch in localized areas. We have also observed evidence of herbivory on coyote brush, mulefat, everlasting and rabbit's foot grass. The chain link fence exclosure (a rent-a-fence) can be breached by animals that can scoot under it at certain locations.

The chain link fence was replaced in 2006 and a 1.66 acre area containing the wild population is now inside a six foot high chain link permanent fence, buried three feet deep to prevent burrowing and entry during the North Shore bioremediation period. Silt fencing is also attached to the bottom of the fence to further prevent small animals from entering the area. This silt fence will be removed when construction is completed. It will be important to watch carefully how the wild site behaves with this new fencing installed which may alter herbivore behavior and restrict predators from entering the area.

Meadow Voles

Meadow voles were identified as a potential herbivore on milkvetch at MSB 4 in 2004, when severe herbivory removed annual growth shoots attempting to emerge from the root crown. The MSB-4 planting area has far more herbaceous vegetative cover than does MSB 3 and 5. Rushes, sedges, salt grass and annual grasses are common here but are scarce at the other MSB sites. Consequently, we observed "runways" (cleared paths) about 2 inches wide in the herbaceous vegetation, and they frequently lead to the bases of severely herbivorized milkvetch.

Herbivore activity attributed to meadow voles also occurred at COPR-Lagoon site in 2005. Narrow runways were visible in patches of salt grass and annual rabbits foot grass areas in the south end of the plot, and a completely cropped "lawn" effect arose beneath a patch of milkvetch that had been directly seeded into a small area in 2003. No successful reshooting occurred at this location in 2005.

The California meadow mouse (*Microtus californicus*), a type of meadow vole, is reported to occur at MSB (ESA, 2003) and likely occurs at other sites as well. Nine other

species of mice are reported at MSB. The only mouse ever directly observed was a dead *Peromyscus* lying near MSB 3 in 2004.

When the runways visible in MSB-4 in spring of 2004 were raked up and disrupted, and several cages installed around the most severely damaged individuals, herbivory on the bases declined to tolerable levels and most plants recovered. We assume meadow voles can readily climb up the hardware cloth cages but may be reluctant to do so if they feel exposed to predators.

Ground Squirrels

Ground squirrels we tentatively identify as the California ground squirrel (*Spermophilus beecheyi*) are abundant at the MSB site and their dens are frequently seen associated with extensive mats of iceplant on the open low dunes. Pieces of iceplant and rejected pieces of iceplant "figs" or fruit, are common laying about outside the entrances to ground squirrel dens.

We have never observed a ground squirrel herbivorizing milkvetch, and therefore, we do not know whether they contribute to the herbivory symptoms we see. However, something is able to remove the tops of mature milkvetch that are inside hardware cloth cages that are usually at least 24 inches tall. The plants are topped at about the same level as the cages, leaving the lower portion of the stems alone. It seems likely that something is able to climb the hardware cloth cages and chew off stems, and it seems unlikely that rabbits or meadow voles would be able to do this. We considered that this symptom may be caused by deer. It seems possible that mule deer may occur now and then at the MSB area, but we have never seen one or any tracks or scat. Ground squirrels seem like the most likely explanation. This type of herbivory generally does not cause the complete loss of established plants, and is typically localized.

Milkvetch Palatability and Selenium

Given that rabbits and ground squirrels occur at the wild site but have not damaged milkvetch in recent years, we wonder why there is such a difference between herbivory levels at this location compared with other planting areas like McGrath State Beach. Our current theory is that there is something reducing the palatability of milkvetch at the wild site, and potentially, certain other coastal planting locations. It is possible that the presence of oil waste sludge in soil below the milkvetch plants results the uptake of substances that reduce its palatability.

Some species in the genus *Astragalus* are known to be poisonous to ruminants. Locoweed poisoning has been attributed to three general toxins- 1) selenium, which is absorbed from the soil; 2) nitrogen-containing sugar compounds called nitroglycosides such as miserotoxin; and 3) swainsonine, an alkaloid that can lead to the classic symptoms of locoism in ruminants (Armstrong, 1998). Locoweed indicators of selenium-rich soils are sometimes referred to as poison vetches and may contain selenium levels of several hundred to 10,000 ppm (Cheeke and Shull, 1985 *in* Armstrong, 1998).

University of California, Santa Barbara graduate student Sophie Parker postulated that Ventura marsh milkvetch may be selectively uptaking selenium. Selenium is a trace element found in coal, crude-oil, oil shale, and coal conversion materials such as liquefaction oils and synthetic gasses, and the waste products of such materials (Lemly, 1997 *in* Parker, 2006). In addition, selenium may be found in naturally high concentrations in soils that are derived from Cretaceous shale parent materials (Terry and Zayed, 1998 *in* Parker, 2006). Parker hypothesized that high levels of selenium could poison competing plants and their pollinators, giving VMMV a competitive advantage in areas where selenium concentrations are high, and if VMMV bioaccumulates selenium, VMMV may grow distasteful, deter herbivores and gain a competitive advantage over neighboring plants.

Parker found VMMV to be a moderate bioaccumulator of selenium (SE), based upon samples collected from milkvetch and soil at the Coal Oil Point Reserve nursery site (Parker, 2006). Non-accumulators such as grasses typically have less than 25 milligrams of SE per kilogram of dry weight, while VMMV had well over 100 mg SE in its stems and leaves. Parker found no soluble selenium in surface soils beneath VMMV, but did find soluble selenium in surface soils beneath VMMV, but did find soluble selenium in surface soil beneath nearby dune lupines, suggesting milkvetch were taking up the soluble, ionic form of SE. Litter beneath VMMV contained higher concentrations of SE, and Parker suggests that recycling of SE, coupled with increases in uptake of new SE as the root systems of individuals grow and have access to greater volumes of soil, could result in VMMV accumulating high concentrations of selenium in their tissues over a period of several years.

Sites with lower levels of rabbit herbivory include the COPR-Pond and Mandalay State Beach. Herbivory at COPR-Lagoon and CSMR-5 also has not been as severe

compared to the McGrath Lake sites. An active oil seep is present in the immediate area where milkvetch were planted at COPR-Pond and the underlying geology is Monterey Shale, from which petroleum can be extracted (University of California, 2004). There may therefore be more selenium in the soil in this general area. The Mandalay planting locations exhibit high numbers of rabbits, as the ground is littered with pellets and the general planting areas appear severely herbivorized, but milkvetch are frequently left alone here as well. Mandalay is across the street and down slope from the North Shore oil contamination, and it is possible that some of the subsurface contaminants from the oil waste sludge may have migrated offsite or moved through the perched aquifer. The worst herbivory has occurred at the MSB sites since 2004- perhaps the perched water table and lake itself dilute soluble selenium that might otherwise be present in the soils there.

Given the challenges in establishing new populations of milkvetch, we wonder how the plants survived at natural sites throughout their historic range. Herbivores like rabbits are ubiquitous to coastal habitats and therefore, finding areas without them seems unlikely. How, then, did milkvetch survive at these locations? Review of historic collections suggests that many of these coastal sites are locations where oil wells and oil geology underlies the area. Areas like Ballona wetlands, Bolsa Chica, and Ventura, have active oil fields in localized areas. Active wells also occur adjacent to Mandalay State Beach and McGrath State Beach – oil wells can be seen within view of McGrath Lake on private inholdings just west of the lake itself.

In reviewing causes of mortality in recently introduced milkvetch, we noticed that new nursery-raised milkvetch plants were sometimes herbivorized shortly after installation. At those same sites, the plants that remained have persisted with relatively little herbivory. For example, at MAND-1B, we lost seven plants in the first months following installation, but since that time the remaining seven plants have survived, unherbivorized, for three years. We hypothesize that nursery-raised and watered plants may be more palatable than plants that emerge on their own at these coastal sites.

Further study is warranted to determine if selenium levels are higher in milkvetch at the wild site and locations with low herbivory compared to sites with higher herbivory levels like McGrath State Beach. It is possible that historic VMMV habitat sites provided both suitable growing conditions and contained higher amounts of selenium allowing milkvetch to persist within an environment strongly influenced by herbivores.

Small Mammal Herbivory- Management Strategies

A variety of methods have been used to reduce small mammal herbivory on milkvetch, with mixed results.

<u>Chemical Herbivory Deterrents</u>: We used several herbivory deterrents in spring of 2004 at MSB- 3 and 4. Products used included *Liquidfence*, which contains putrified eggs and *Shakeaway* (fox and bobcat urine granules). We also prepared a hot pepper spray ourselves, made from three chopped up yellow onions, five diced jalapenos and several teaspoons of cayenne pepper- this was cooked with water for a couple of hours and filtered through a fine screen, producing about one gallon of liquid. This was applied to plants with a hand-held pump sprayer. We applied these substances sequentially over several weeks to both plots in the general area where milkvetch were located. Over the next few weeks, herbivory started to calm down in MSB 4 while it did not calm down in MSB-3. One reason that the liquid herbivory deterrents may have been less effective at the MSB-3 site was the general lack of herbaceous vegetation in the area to apply the spray to. Once most of the milkvetch new growth has been hammered, there was little biomass to receive smell or tastebased sprays. At this point, we are not convinced these products are particularly effective.

Individual Caging of Milkvetch: Placement of hardware cloth or chicken wire cages around individual milkvetch can slow herbivory and sometimes causes it to stop, but not always. Twenty-four or 30 inch tall hardware cloth with holes ½ inch or less was typically used. Cages were placed at ground level and anchored with stakes or wire. We recommend using the ¼ inch hardware cloth in order to reduce the chances that reptiles may get stuck in holes in the wire. Chicken wire is particularly dangerous for lizards and snakes and should be avoided. We had two western fence lizards stuck in ½ inch hardware cloth. We successfully released one by carefully cutting it out of the wire, only to have it turn and get stuck again moments later (we then freed it again). Cages and enclosures made of wire need to be carefully installed where the wire meets in order avoid creating where reptiles can lodge.

Caging of individual plants is time consuming and particularly difficult when plants are from prior years and have a lot of annual stems which often still support milkvetch pods containing viable seed. Because milkvetch can produce stout stems which spread laterally and robust plants are sometimes almost two meters across, large amounts of wire can be required and there may not be sufficient space to install it where plants are naturally recruited or close together.

We have occasionally used shorter pieces of left over hardware cloth to make short cages placed around vulnerable juveniles. On these shorter cages, we usually place a piece of hardware cloth on the top for additional protection. Typically, we don't use tops on the cages because it reduces light and milkvetch growth on maturing plants in summer usually extends far beyond the height of the cage.

<u>Large Exclosures</u>: We have sometimes placed hardware cloth or chicken wire around large patches of milkvetch to reduce herbivory. As more extensive pieces of ground are placed behind wire, problems occur when the topography is uneven and it can be difficult to ensure that the exclosure is flush with the ground. We have tried to minimize sinking wire below ground because it causes soil disturbance.

Larger exclosures can be installed more quickly than individual cages, but appear to be easier to breach. In other words, herbivores seem to find their way into the exclosures, anyway. None-the-less, enclosures seem effective at reducing some direct herbivory or at least keeping it at tolerable levels. Again, hardware cloth is preferable to chicken wire.

<u>Overgrowth of Vegetation in Cages and Exclosures:</u> Where cages and exclosures prevent herbivory on milkvetch, they also reduce or prevent herbivory on associated vegetation. This can quickly become a serious problem for milkvetch if they become engulfed in competing vegetation. Attempts to prevent loss of juveniles and adults by caging have led to death from excessive competition as the area inside the cage becomes overgrown (**Photo 30**). This is particularly a problem in more mesic years when growth can be difficult to manage. Cool shaded conditions inside cages can result in herbivory from snails and slugs. When individual cages are used, it can be difficult to reach the interior of the cage to remove competitors, snails and monitor plants.

To prevent overgrowth, we therefore recommend that competitors be removed when they occur inside an area that requires an individual cage. Competitors should be removed by the taproot when at a young stage. Plants like western ragweed shooting from belowground rhizomes will re-appear if pulled or broken off. Plants like annual grasses, which have fibrous roots, should generally not be pulled out as too much soil disturbance can occur unless they are very young. When larger exclosures are used, more associated vegetation can remain, as it is easier to get inside the exclosure if necessary to trim back excessive growth later on.

<u>Duration of Caging and Exclosures</u>: We generally start watching in late January- early February for problems with excessive herbivory on new growth shoots. We have avoided caging plants as much as possible. If it appears that severe herbivory is continuing, we generally install cages or exclosures by March and remove them once milkvetch have obtained enough robust growth to hopefully be resilient, typically in early to mid-June. Occasionally, a cage is left in place where the plants occur in locations that are particularly vulnerable to herbivory or where plant vigor is low. Generally, growth of associated vegetation and milkvetch by mid-summer is sufficient to sustain some level of small mammal herbivory and removal of cages and enclosures is desirable.

<u>Disruption of Vole Runways</u>: A steel rake was used to break up obvious runways in MSB-4 in 2004 when runways were very visible going from base to base of milkvetch. Combined with caging and herbivory deterrents, a reduction in herbivory occurred in the general area by late April. Thirteen of the thirty plants located in this plot recovered.

ARTHROPOD HERBIVORY

Moth and Butterfly Caterpillars

Adult butterflies are commonly seen around milkvetch patches in late spring through summer. The most commonly seen butterfly attracted to milkvetch patches is the Common Hairstreak (*Strymon melinus pudica* per Mattoni, 1990). This butterfly is seen at most sites and has been directly observed ovipositing amongst buds on young developing inflorescences. Despite seeing this butterfly often, mature caterpillars of Common Hairstreak are seen very rarely (**Photo 7a**). Common hairstreak caterpillars were numerous at the wild site in 1998 (CDFG files).

Marine blue butterflies (*Leptotes marina* per Hogue, 1993) have also been observed visiting milkvetch and ovipositing on developing inflorescences (**Photo 5b**). We have never seen a Marine blue caterpillar on milkvetch. We occasionally observe very small green caterpillars in developing inflorescences. They are likely consuming young tissue including buds and developing flowers- but this has generally gone un-noticed.

Numerous other caterpillars are seen in milkvetch plots in summer and fall. By far the

most numerous caterpillars are a grey woolly bear with shorter orange hairs (**Photo 7b**). This is probably a moth caterpillar and may be some type *Arachnis*. This caterpillar is most commonly seen at the CSMR site and sometimes is found on summer mustard nearby. Beetle larvae are also sometimes seen.

<u>Damage to Milkvetch:</u> We have directly observed caterpillars consuming developing ovules inside maturing pods which have not yet hardened. Caterpillars chew an irregular hole in the pod valves, typically about 2-3 mm in diameter, stick their head in and then consume the ovules inside. This occurs when the pod is still green and turgid, and when the ovules/seeds are swollen but not hardened off. Usually, all the ovules are consumed, but not always. The pod itself is usually left in place.

Photo 7a provides detail on localized herbivory by a Common Hairstreak caterpillar on fruits at CSMR-5 in October of 2006. One can see pods in all stages of development, and entry holes on certain pods. This caterpillar seems to have moved around quite a bit, skipping some pods and hitting others- it is not uncommon to see rows of pods damaged by caterpillars. No leaf damage is visible in the immediate area on this photo, and we have not observed much leaf damage from caterpillars on milkvetch.

Infructescences were examined in 2003 and 2004 to document damage to developing fruits with information summarized in **Tables 11a, 11b**. The proportion of pods exhibiting damage that was primarily caused by caterpillars ranged from 3 to 11 percent in 2003 at various sites, with the exception of much higher damage at Mand-1 with 38 percent. While around 90% of the pods at the MSB and CMSR original 2002 planting sites produced seed, only 57% of Mand-1 and 68% of the wild site did. Due to small mammal herbivory in 2004, fewer sites were sampled. The percent of damaged pods attributed primarily to caterpillars was between 9-12%.

Overall, the reduction in milkvetch seed caused by caterpillar herbivory on ovules is measurable and likely varies between years and sites. Some level of caterpillar use of milkvetch is a natural occurrence, and we have not removed or otherwise controlled their numbers in any active way. Should a substantial increase in damage occur, it is possible that a caterpillar-specific biological control agent could be applied to reduce their numbers with little potential effect on non-target insects. It would be important to ensure that no sensitive or rare butterflies or moths could be affected if such treatments are implemented.

Seed Beetles

Bruchid beetles (Family Bruchidae), a type of seed beetle, were observed emerging from seed collected from the wild site for conservation purposes in 1997 and 1998. They have been tentatively identified as *Macrohoptus* (Wilken and Wardlaw, 2001). A small wasp reported to parasitize developing bruchid beetle larvae was also occasionally observed emerging from collected seed. This wasp has been tentatively identified as *Bruchophagus mexicanus* (Michael Wall, RSABG, pers. comm.) Adult seed beetles are seen now and then wandering about on milkvetch during the flowering period at introduction sites.

Bruchid beetles are attracted to legumes (Borror and White, 1970). Adult bruchids are reported to actively search for oviposition sites during the early stages of flower and inflorescence development (Robbin Thorp, pers comm. in Wilken and Wardlaw, 2001). Another source regarding tropical legumes and bruchid beetles list a variety of adaptations suggesting ovipositing can occur on scattered seeds, mature seeds, and on embryonic immature seed (Barbour et al. 1987). I do not know to what extent these strategies might apply to our case. Regardless of when adult bruchids oviposit into ovules or seeds, the result is the developing larvae consume the contents of the seed at the host's expense. In fall, when seeds are hardened off and dry, adult beetles have been observed to emerge by cutting an exit hole in the seed wall. The seed wall appears to be about two layers thick- the exposed edges of these layers can be seen under 30 power magnification. Envelopes containing infested seeds often had the entire intact circular "escape hatch" lying in the bottom of the envelopes- presumably made by either the bruchid beetle or the wasp, escaping. The exit hole is less than one millimeter in size (Photo 38). Similar sized exit holes are seen now and then on mature dry milkvetch pods. These pods may still contain some viable seed, as it is common to find bruchid-damaged seed and undamaged seed in the same pod (Wilken and Wardlaw, 2001).

In fall of 1997, I noticed a seed in an envelope on my desk that had an exit hole but the bruchid beetle had not yet emerged from the hole. This escape hole was apparently still too small. Over a period of at least ten days, I observed the bruchid beetle working on enlarging the hole, leaving a scalloped edge from bite marks. There would be periods of activity and inactivity- I thought it had died at one point, only to see it reworking the hole several days later. Eventually it freed itself and wandered away.

In 2003 and 2004 we quantified the percentage of seed lost to bruchid beetle

(**Tables 2a, 2b**). From our samples, percent seed with exit holes in 2003 was very low, under 1%. In 2004, about 2% of the seed sampled from McGrath 4 and Mand-1 was damaged.

Wilken and Wardlaw (2001) reported high levels of bruchid beetle damage in seeds from the wild site in 2000- 50% of the pods and 30% of all seeds were classified as infested or damaged as a result of beetles. Data collected by Diane Steeck and I in 1997 found seed to be heavily infested at the wild site and seed production may have been reduced by about 44 percent (USFWS, 2001). This was reported as follows: "most fruits produced at least four seeds, but seed predation reduced the average number of undamaged seeds to only 1.8 per fruit". Wilken and Wardlaw (2001) reported the mean number of all seeds (full plus empty) at the wild site (Oxnard) in 2000 was 2.8. If 30% of the seeds were damaged by beetles as reported, then the mean number of sound seeds produced was around 2 per pod, which is similar to the results we found in 1997.

It is likely bruchid beetle infestation levels will vary by sites and years, tied to climate and other variables. To reduce infestation levels would likely require use of some type of insecticide- this could have undesirable collateral effects on non-target species and would likely not be a viable management tool at most sites. At this time, we don't propose bruchid beetle control.

Aphids, Argentine Ants and Milkvetch

Colonies of ants are common in the milkvetch habitat area at most sites. We have tentatively identified the ants of concern as the non-native Argentine ant (*Linepithema* (*Iridomyrmex*) *humile*) (Suarez and Case, 2002). This native to South America has been studied extensively due to the profound effect it can have on the native arthropod community in invaded habitats, which can then lead to broader negative ecosystem effects (Human and Gordon, 1997). The combination of disturbed ground and higher levels of soil moisture favor establishment of Argentine ant colonies (Suarez, et al. 1998). Since most of the milkvetch planting sites are adjacent to or near wetlands, are located in the coastal fog belt, and are near urbanized areas where Argentine ants are already entrenched, it appears the ecosystems where milkvetch has been planted are severely invaded by Argentine ants. We note that red harvester ant colonies (*Pogonomyrmex californicus*) a declining native ant species, are still present in the adjacent drier dunes at MSB and the wild site, indicating Argentine ants have not yet entirely displaced this species in drier soil locations near the

coast.

Argentine ants have a generalized diet that includes nectar, insects, seeds, carrion and honeydew secreted by Homopterans (Suarez, et al. 1998). Therefore, they can potentially affect milkvetch directly by consuming nectar intended for use by pollinators, and by tending aphids to obtain honeydew.

Aphids were noted on milkvetch at the wild site in 1997 (Impact Sciences, 1998). Aphids were reported as a problem in the summer of June and July 2002 on milkvetch planted in tree shelters (Soza et al. 2003). Black-looking sooty mold formed on the surface of milkvetch where aphid secretions were deposited. To reduce aphids and ants, RSABG applied diluted dish soap and JMS Stylet oil, released aphid predators like lady beetles and lacewings, and used bait to reduce Argentine ants (Soza et al. 2003).

In this study, we have experienced no noticeable aphid infestations requiring treatment. The only site which has exhibited a visible infestation with extensive sooty mold on leaves, flowers and maturing pods was at MSB-4, in the fall of 2003 and 2004. The aphid problem was restricted to only a few plants, and fruit appeared to be maturing despite the presence of aphids. Consequently, we made no effort to control the aphids.

The tree shelters used in 2002 on the original outplants were made of a fine plastic mesh with 1/8 inch openings, were 60 cm (24 in.) tall and 15 cm (6 in) wide, and were fastened to a rebar stake (Soza, et al. 2003). These narrow tubes created cooler moist conditions around the plant bases and milkvetch biomass which we hypothesize exacerbated aphid and Argentine ant problems. This was one of the reasons we chose not to use tree shelters in 2004 for the supplemental installations.

<u>Other Argentine Ant Effects on Milkvetch</u>: Despite not having an appreciable problem with aphids affecting milkvetch, Argentine ants are extremely common and are frequently found adjacent to the bases of established milkvetch. It seems likely that these colonies are harvesting nectar from milkvetch and individual ants are observed now and then wandering through inflorescences. Further study is warranted to determine the degree to which Argentine ants are contributing to reduced seed set and possibly lowering levels of crosspollination by interfering with pollinator behavior.

Another effect Argentine ants may have on milkvetch relates to the presence of

colonies around the plant bases and their potential to occupy weakened tissue in the root crown on older established plants. We have occasionally seen ants emerging from cracked areas and broken down tissue areas on the woody root crowns- this is most noticeable on planted individuals where the root crown was exposed, rather than the normal condition found on naturally-recruited individuals, where the root crown is not visible. There may be negative effects on health and vigor of older individuals related to damage to the root crown tissue by Argentine ant colonies

<u>Management Recommendations for Argentine Ant Control:</u> I investigated the feasibility of using baits for control of Argentine ant invasions in wildland preserves in 2001. Generally, baiting of wildland areas has been shown to be ineffective for a variety of reasons. Blachly and Forschler (1996) looked at the effectiveness of baiting for late season control of Argentine ants and found baiting to be reasonably effective over a short time frame around treated buildings in Georgia, but complete eradication did not occur. Krushelnycky and Reimer (1998a) examined the efficacy of applying Maxforce bait for control of Argentine ants in a national park in Hawaii. Ant numbers were substantially reduced following treatments, but eradication did not occur. Foraging ant numbers recovered modestly in large treated areas within a month and a half after treatment, indicating some nests had survived. These results suggest that complete control, even in a confined setting over the short term, is not practical.

Baiting programs may not be effective in an ecological setting because baiting only reduces the numbers of ants, rather than eliminating them (Krushelnycky and Reimer, 1998a; Blachly and Forschler, 1996). Blachly and Forschler (1996) cite several other studies which found a) chemical barriers were often ineffective because they kill or repel foragers while having little effect on the colonies' reproductive activity; and b) nest treatments can eliminate entire colonies but are impractical for dense or large area infestations. Dr. Deborah Gordon, Stanford University, indicated that baiting natural areas is not likely to be practical and factors which may be of concern in an ecological setting include effects on non-target arthropods (Dr. Gordon, pers. comm.). There is no bait specific to the Argentine ants- baits will attract and kill non-target organisms (Dr. John Klotz, University of California, Riverside, pers. comm).

This information suggests that baiting is not desirable and likely not effective at controlling Argentine ant invasion levels. Existing management options therefore appear limited. If an effective biological control specific to Argentine ants can be found, it would be

important to implement control at milkvetch sites.

ISOPOD HERBIVORY

Sow and pill bugs are common in milkvetch planting sites. Isopods eat both young and decaying plant material, particularly in winter and springtime or in damp areas. In early spring 2006, the CSMR-5, 5B and 5C planting areas experienced a dramatic increase in sow and pill bugs. These were tentatively identified as the common pill bug (*Armadillidium vulgare*) and dooryard sow bug (*Porcellio laevis*) (Hogue, 1993). Pill bugs are more rounded in appearance, darker slate colored, and can roll themselves into a ball, while sow bugs are slightly larger, flatter, lighter gray, and have two prominent tail-like appendages.

In March 2006, we observed that many of the remaining original 2002 milkvetch plants and 2004-supplementals at CSMR-5 were not exhibiting reshooting. New annual growth shoots were generally not visible or were uncommon. This was the first time we had experienced a plot-wide failure to reshoot at the CSMR sites. Examination of the root crowns showed they were teaming with sow and pill bugs. Shoots attempting to emerge on the root crown were severely cropped, appearing as very short stubby nubs with tips and leaves stripped off. The appearance of the cropping was basically similar to that attributed small mammal herbivory like rabbits or voles. But the presence of 100's of isopods suggested they were contributing to failure to reshoot.

The milkvetch root crowns on older plants can contain crevices, cracks and decaying tissue associated with previous year's growth (**Photo 3**). This condition creates moist, shady three dimensional areas at the soil surface where decaying or very young new plant growth is vulnerable to removal and damage by isopods. The CSMR site also has firm soil with more clay content- this creates occasional deep cracks which also had large numbers of isopods hiding in them. Some gopher tunneling locations and areas where moles had traversed the near-surface also exhibited cracks near milkvetch root crowns supporting large numbers of isopods. The milkvetch root crowns also had areas where soil clumps appeared, somewhat like worm castings, only in this case, these soil masses were impregnated with isopods.

<u>Management Response</u>: To reduce isopod levels, we first tried sprinkling diatomaceous earth powder around the milkvetch bases. Diatomaceous earth, when dry and powdery, gets on the exoskeletons of isopods and reportedly will reduce their numbers by causing irritation and also blocks respiratory function. We discovered that under moist coastal conditions, with

periodic rain and fog, the diatomaceous earth quickly became moist, then hardened and formed clumps. The isopods seemed unphased, and were living in and amongst clumps of hardened diatomaceous earth, suggesting it was ineffective.

We then resorted to simply placing pieces of cardboard or newspaper adjacent to the bases of milkvetch, usually just one by each plant, and generally, a piece about 12 inches square. After a few days, we came back and lifted the cardboard/newspapers. Hundreds of isopods were underneath- we scraped the isopods clinging to the cardboard/newspaper into a bucket. We then used a large spoon and scooped up isopods and the friable soil they were intermingled with. When the trap is lifted, they scurry quickly, and we observed them trying to escape back toward milkvetch bases and into adjacent cracks in the soil where they are difficult to remove, so one must move quickly to get as many as possible. The traps were put back on the same ground again and we revisited and removed isopods several times over the next few weeks. Isopod levels appeared to drop, but most importantly, new shoots soon began to emerge and the new annual growth recovered.

We found that the cardboard lasted longer and seemed to work better than the newspaper (brown paper bags also worked). It appeared the isopods preferred to actually feed on the cardboard, less so the newspaper. We occasionally saw an etiolated germinating milkvetch seedling under the traps. Where traps prevented successful germination, those germinating milkvetch were lost. For that reason, it is important that traps remain localized, stay on the same piece of ground, and be limited in size.

In summer, we noticed that there were commonly cleared zones around the established milkvetch lacking juveniles. We hypothesize that the outbreak of isopods may have also affected young seedlings, resulting in their removal adjacent to infested root crowns (see **Photo 36**).

<u>Other Organisms Found under Isopod Traps</u>: We observed a variety of other creatures under the traps at the CSMR sites. These were generally left alone. Jerusalem crickets (*Stenopelmatus* sp.) were common. We also saw millipedes, centipedes, small earth worms and occasional webless spiders. Colonies of ants tentatively identified as Argentine ants based upon their size, habit and lack of formic acid odor, were also common.

142

OTHER ANIMAL DIRECT AND INDIRECT EFFECTS ON MILKVETCH

<u>Moles</u>

Evidence of mole activity is common at certain sites, particularly the MSB, CSMR and COPR Lagoon sites. Broad-handed mole (*Scapanus latimanus*) has been identified at MSB and likely occurs at other sites (ESA, 2003). Moles are small mammals that feed primarily on soil invertebrates such as earthworms but they are reported to also eat roots. Food items are encountered as they burrow shallowly through moist surface soil, leaving characteristic ridges of loosened earth tracing their foraging paths.

Mole ridges/burrowing can be readily observed. Mole burrows are particularly noticeable at the CSMR site and have been observed going up to near the bases of planted individuals still in chicken wire or hardware cloth below-ground cages. We have observed air pockets by the milkvetch bases and in the surrounding hardened clay soils in summer at CSMR. We hypothesize that some abnormal growth and abrupt declines in vigor observed in summer on otherwise robust milkvetch may be caused by air pockets associated with mole and gopher burrows, exacerbated by periodic deep cracks in the summer-hardened clay soils. It is also possible that there are secondary effects caused by these air pockets, such as increased access by sow and pill bugs attracted to these protected, moist areas, and they may increase Argentine ant colonization as well. Since moles are native to the planting sites, their control is not an option.

<u>Skunks</u>

On several occasions, we observed dug up surface soil at milkvetch planting sites in winter months. This has been observed primarily at MSB-5 and at CORP-Lagoon. In both 2005 and 2006, the top several inches of soil across extensive areas of the milkvetch patch at CSMR-Lagoon were observed to be superficially "plowed up". Occasionally, divots were also seen. Plowing was generally limited to sparsely vegetated areas. Seedlings were present in the surrounding undisturbed ground, but missing from the plowed up soil. Additionally, no subsequent germination of new seedlings on the plowed ground was seen in the following weeks, although new seedlings were observed in adjacent areas. We hypothesize that a) any seedlings that had germinated or were starting to germinate in the loosened soil likely died from desiccation; and b) the loosened soil remained problematic, again perhaps because it dried out more rapidly than adjacent, more compact native ground.

At MSB-5, we observed juveniles of the year dying adjacent to more localized areas of plowed ground- their roots were likely damaged when nearby areas were disturbed.

Reserve Manager Dr. Cris Sandoval first observed these skunk foraging symptoms at the COPR Lagoon. It should be noted that we saw no evidence of skunk foraging at this site during the previous two years. It is possible that as native vegetation has re-taken this site since the removal of *Melaleuca* in 2002, more soil invertebrates are present here than in the first couple of years following removal, which may be attracting skunks. It is also possible that skunk populations have simply increased based upon natural fluctuations. Dr. Sandoval (pers comm.) indicated skunks caused high mortality at a nearby Snowy Plover colony in the dunes in 2006, which had not occurred in previous years.

Skunk damage is another problematic issue that lacks a viable management solution. Since skunks seem to prefer digging into more open ground, it may be possible to reduce digging and plowing by increasing woody litter on the ground (like old milkvetch stems). We have not tried this approach.

X. ASSOCIATED VEGETATION

The majority of experimental introduction sites with favorable milkvetch establishment and survival represent ecotonal areas and are generally transitional between wetlands (such as salt marsh, lagoon, lake, pond, dune swale) and uplands (low backdunes, foredunes, coastal prairie). Favorable planting sites typically support the phraeatophytic shrub coyote brush (*Baccharis pilularis*), either as scattered individuals, small groups, or adjacent larger patches. Mature arroyo willows (*Salix lasiolepis*) are often adjacent or nearby. **Tables 4** and **5** list herbaceous, shrub and tree species closely associated with milkvetch growing sites.

NATIVE AND NATURALIZED SPECIES

Western ragweed (*Ambrosia psilostachya*), a facultative-wetland rhizomatous perennial herb, is ubiquitous to virtually all the favorable introduction sites. Western ragweed appears less tolerant of saline conditions than some of the other rhizomatous associates and is absent from failed plots installed in areas of high salt marsh (CSMR-1, 3 and 4; COPR-Jaumea). Western ragweed was also absent from some of the driest sandy dune sites where milkvetch vigor was low (MSB-1, 2; MAND-3). Coyote brush is also ubiquitous to

most planting sites but was absent from the central dune hollow at COPR-Pond, potentially due to more frequent episodes of winter inundation. Locations supporting coyote brush and western ragweed but lacking the presence of facultative wetland or obligate wetland plants are likely too dry for successful milkvetch recruitment.

The distance to a perched water table appears to influence the composition of obligate and facultative wetland herbaceous and shrub associates (**Figure 2**). Introduction sites more distant from the perched water table such as the CSMR-5 and COPR-Lagoon support more cover of annual non-native grasses and have lower levels of rhizomatous perennial obligate and facultative wetland species compared with more mesic locations such as at McGrath Lake. At some drier sites, rhizomatous wetland plants are present, but in very low numbers and are intensively cropped by rabbits (e.g. MAND-1,1B).

Ventura marsh milkvetch grow best at locations where rhizomatous wetland perennials such as salt grass (Distichlis spicata), Mexican and Baltic rush (Juncus balticus and mexicanus often co-mingled), slender carex (Carex praegracilis), and horsetail (Equisetum laevigatum) occur sparsely and have not formed dense areas with continuous thatch. Favorable conditions typically occur on the drier outer margin of wetland/upland ecotones. Sparse cover is sometimes maintained by herbivores. Hydrologic fluctuations can also achieve this result. At COPR-Pond, loss of the local water table in dry years appears to reduce the ability of rhizomatous wetland plants to form dense patches and thatch. The dune hollow where milkvetch occur at COPR-Pond is subject to episodes of inundation and drought, which has combined to produce an herb-dominated "meadow" with an unusual mix of natives and non-natives (Photo 19; 20). Salt grass is present but generally does not form dense patches, while low growing cud weed (Gnaphalium luteo-album), rabbit foot grass (Polypogon monspeliensis) and western ragweed dominate the area but localized vegetation gaps occur where milkvetch come up from seed relatively easily and can keep pace with growth of the adjacent herbaceous vegetation (Photo 21). Periodic inundation appears to prevent the establishment of coyote brush, which is present only around the outer limits of inundation. Shrub cover is therefore more distant from the main COPR-Pond planting area, which could contribute to low levels of rabbit herbivory. Snail control has also never been needed at this location.

| | | | | | | | N | ILKVET | CH SITE | s | | | | |
|---|-------------------------|-----------------------|------|---------|------------|-------------|-------------|-----------|-----------|-----------|------------|------------|------------|------------|
| | Common Name | Wetland Indicator* | WILD | ORM | MAND- 1 | MAND- 1A | MAND- 1B | MSB- 3 | MSB- 4 | MSB- 5 | CSMR- 5 | COPR- L | COPR- J | COPR- P |
| | | | т | REE ANI | D SHRUB | ASSOCIA | TES | | | | | | | |
| | | | | | | | | | | | | | | |
| Artemisia californica | California sagebrush | | х | | | | | | | | | | | |
| Baccharis pilularis | coyote brush | phraeat. | х | х | Х | Х | Х | х | х | Х | Х | х | Х | х |
| Baccharis salicifolia | mulefat | FACW | х | | | | | | | | | | | х |
| Lotus scoparius | deerweed | | х | | | | | | | | | | | |
| Malosma laurina | laurel sumac | | х | | | | | | | | | | | |
| Myoporum laetum | myoporum | phraeat. | х | | | | | | | | | | | |
| Salix lasiolepis | arroyo willow | FACW | х | | х | Х | Х | х | х | х | | х | х | |
| Salix exigua | sandbar willow | OBL | | | | | | | | х | | | | |
| Solanum douglasii | Douglas's nightshade | FAC | | | | | | х | | х | | | | |
| Toxicodendron | - | | | | | | | | | | | | | |
| <i>diversilobum</i> * Source: Reed, 1988 | poison oak | phraeat. | Х | | | | | Х | | Х | | | | |

* Source: Reed, 1988
 **Bromus madritensis spp. rubens, B. hordeaceous, B. diandrus, Vulpia myuros, Lolium multiflorum, Schismus barbatus
 *** Source: USDA; Mason 1957

| | Common Name | | | | | | | Ν | /ILKVE | ICH SIT | ES | | | | |
|------------------------------|---------------------------|-----------------------|-----------------------|------|-----|------------|-------------|-------------|-----------|-----------|-----------|------------|------------|------------|-----------|
| | | Wetland Indicator* | Alkaline Sites *** | WILD | ORM | MAND- 1 | MAND- 1A | MAND- 1B | MSB- 3 | MSB- 4 | MSB- 5 | CSMR- 5 | COPR- L | COPR- J | COPF P |
| HERBACEOUS ASSOCIATES | ; | | | | | | | | | | | | | | |
| Rhizomatous Perennials: | | | | | | | | | | | | | | | |
| Ambrosia psilostachya | western ragweed | FAC | | | Х | х | х | | х | х | Х | х | х | | х |
| Anemopsis californica | yerba mansa | OBL | Х- | | | | | х | х | х | х | | | | |
| Artemisia douglasiana | mugwort | FACW | | | | | | | х | | | х | | | |
| Carex praegracilis | slender carex | FACW- | х | | | х | | х | | х | х | | | | Х |
| Distichlis spicata | salt grass | FACW | х | | Х | | | | | х | х | х | х | | Х |
| Equisetum laevigatum | horsetail | FACW | | | | х | | | | | х | | | | |
| Euthemia occidentalis | western goldenrod | OBL | | | | | х | | х | | | х | | | |
| Frankenia salina | alkali heath | FACW+ | х | | | | | | | х | | | х | х | Х |
| Heliotropium curassavicum | heliotrope | OBL | х | | | | | | | | | х | | | х |
| Jaumea carnosa | jaumea | OBL | х | | Х | | | | | | | | х | х | х |
| Juncus acutus ssp. Leopoldii | spiny rush | OBL | х | | Х | | | | | | х | | | | |
| Juncus mexicanus/balticus | Mexican or Baltic rush | FACW/OBL | х | | | Х | х | х | | х | х | | | | |
| Potentilla anserina | silverweed | OBL | х | | | | | | х | | Х | | | | |
| Perennial Subshrub | | | | | | | | | | | | | | | |
| Cressa truxillensis | alkali weed | FACW | х | | х | | | | | | | | | | |
| Biennial | | | | | | | | | | | | | | | |
| Gnaphalium ramosissimum | pink everlasting | | | х | | х | х | х | х | | х | | | | |
| Oenothera elata ssp. hookeri | Hooker's evening primrose | FACW | | | | х | | | х | х | Х | | | | |
| Annual | | | | | | | | | | | | | | | |
| Annual Grass Group** | | | | х | | | | | х | х | | х | х | | х |
| Conyza canadensis | horseweed | FAC | | х | | х | х | | х | | Х | х | х | | х |
| Cotula coronopifolia | brass buttons | FACW+ | X +/- | | Х | | | | | | | | | | |
| Gnaphalium luteo-album | weedy cudweed | FACW- | | | | | | | | | | | | | х |
| Parapholis incurva | sicklegrass | | х | | Х | | | | | | | | | | |
| Polypogon monspeliensis | rabbit foot grass | FACW+ | | х | | | | | | Х | Х | х | х | | х |
| Xanthium strumarium | cocklebur | FAC+ | | | | | | | | | | | | | х |

* Source: Reed, 1988
 **Bromus madritensis spp. rubens, B. hordeaceous, B. diandrus, Vulpia myuros, Lolium multiflorum, Schismus barbatus
 *** Source: USDA; Mason 1957

Locations which support dense patches of wetland obligates such as yerba mansa (*Anemopsis californica*) and silverweed (*Potentilla anserina*) are not favorable, primarily because these species tend to form continuous patches that exclude milkvetch and tend to harbor snails which cannot be effectively controlled. Milkvetch grow well at locations which produce late spring and summer eruptions of heliotrope (*Heliotropium curassavicum*) such as CSMR-5 (**Photo 36**). This plant provides excellent cover for juvenile milkvetch in summer, attracts pollinators, and yet does not appear to competitively exclude them.

Western goldenrod (*Euthemia occidentalis*) and mugwort (*Artemisia douglasiana*) are rhizomatous perennials which produce taller individual growth stems and, like western ragweed, they do not form continuous ground thatch. Milkvetch does not grow well where these plants have produced a dense patch, but seems to tolerate scattered individual stems. These plants are similar to western ragweed, in that in wet years, they produce more individual stems, much taller individual stems, and they produce more side branches, leading to closed conditions and little or no light penetrating the stand which is unfavorable for milkvetch. Mugwort only occurs as a single patch at MSB-3 and a single small patch at CSMR-5. We observed in 2005 that new mugwort juveniles produced from seed were common at MSB-3 and 4, but over time, they were removed by herbivores. This may explain why mugwort is not common at these sites. Western goldenrod has expanded into additional habitat as a result of removal of invasive competitors at CSMR-5 (**Photo 17**) and could potentially overtake the milkvetch area over time. This species co-occurs with the Northern brine milkvetch (*Astragalus pycnostachyus* var. *pycnostachyus*) at the Pescadero artificial dike occurrence in San Mateo County (Wilken and Wardlaw, 2001).

The biennial Hooker's evening primrose (*Oenothera elata* ssp. *hookeri*) has been somewhat problematic at the McGrath State Beach sites. It occurred here and there as occasional individuals in 2002 when the sites were originally planted. Wetter conditions appear to have resulted in a substantial expansion of this species, such that it is now forming an almost continuous band along the lake's wetland transition zone, corresponding closely with areas favored by naturally recruited milkvetch. New evening primrose plants recruit abundantly in wetter years and grow rapidly, forming a low-growing broad rosette that can exclude juvenile milkvetch and harbor snails and isopods. Occasionally they will bloom in the first year, but typically, they bolt in the second year and can be over 1.5 meters in height, substantially reducing open growing conditions as a result. Some local botanists feel this plant is very weedy and they are reluctant to introduce it in revegetation projects. We have

thinned this plant at MSB-3 when they become extremely numerous and large adjacent to milkvetch.

Both Hooker's evening primrose and California horseweed (*Conyza canadensis*) are species that are commonly seen in coastal areas growing along roadsides. Horseweed is common at most planting sites and is a problem for milkvetch primarily in wetter years when it produces very numerous, tall individuals with a lot of side branches. Roadsides are locations where there is additional moisture as a result of runoff from adjacent impervious surfaces and lower plant cover due to periodic roadside maintenance. Northern brine milkvetch occurs as roadside populations along Highway 1 in San Mateo County at several locations (Wilken and Wardlaw, 2001). Ventura marsh milkvetch may similarly be capable of occupying near-coast locations adjacent to roadsides, and introduction into this type of habitat may be worth further investigation.

The wild site at North Shore supports a different mix of associated vegetation compared with the introduction sites (**Tables 15** and **16**). The wild site is vegetated primarily by phraeatophytic or facultative wetland shrubs, entirely lacks rhizomatous perennials and has the lowest richness of herbaceous species of all the sites examined (**Photo 13a, b**). The absence of rhizomatous perennials may be due to the presence of oil sludge 10 or more inches below ground. The only native herbs that co-occur with milkvetch at the wild site are pink everlasting (*Gnaphalium ramosissimum*), and western tansy (*Descurainia pinnata* ssp. *glabra*). Pink everlasting, a biennial, has increased in the last couple of years potentially due to wetter conditions which provided recruitment from seed and sufficient numbers of plants such that rabbits did not remove all of them.

In 2004, Impact Sciences used a Trimble GPS unit and plotted all milkvetch growing in the wild site that fall, and mapped the perimeter of shrubs. They have not yet calculated total shrub cover from these data, but they provided me with a map. The Colony "B" area where the majority of milkvetch now grow, occupies a band about 9 meters wide and 24 meters long. Mule fat (*Baccharis salicifolia*) dominates and closely corresponds with the area where Colony "B" milkvetch occurs, while the adjacent slightly more upslope area has more coyote brush, and the area further down slope and extending out into the old oil basin also is dominated by coyote brush. Shrub cover appears to be around 50%, ignoring overlap, cover of milkvetch and cover of small new juvenile shrubs. About 80% of that cover is mulefat. Mulefat typically does not occur at the introduction sites.

NON-NATIVE WEEDS

Non-native weeds are present at all the introduction sites to varying degrees (**Table 6**). Chronic weediness is particularly problematic at the COPR-Lagoon and CSMR planting sites. Both locations are unfortunately near their respective natural reserve boundaries, and abut largely unmanaged offsite weed populations. Onsite weed management at both natural reserves is not adequately funded and staffed. At CSMR, the Southern Pacific Railroad right-of-way is severely invaded by castor bean (*Ricinis communis*) and poison hemlock (*Conium maculatum*)and the area adjacent to Santa Monica Creek is periodically disturbed by flood control activities and supports areas dominated by summer mustard (*Hirschfeldia incana*), poison hemlock and Italian thistle (*Carduus pycnocephalus*). At CSMR, poison hemlock tends to expand into the weeded area. Castor bean has been controlled every year since 2002 and continues to produce new plants from its long-lived seed bank.

Herbivory in localized areas limits the expression of weeds at the CSMR site to some degree (**Photo 16; 30**). Rabbits will consume poison hemlock, Italian thistle and mustard when in a young stage. This reduces the density of these weeds to some degree within localized severe herbivory zones. The COPR-Lagoon is adjacent to coastal prairie (these areas may have experienced past agricultural activity) and is invaded by Harding grass (*Phalaris aquatica*) and bristly ox-tongue (*Picris echiodes*). Summer mustard, Italian, and milk thistle (*Silybum marianum*), are also common. Sweet clover (*Melilotus alba*) is a common problem in wetter years at the MSB, COPR-Lagoon and wild site. In wet 2005, some sweet clover plants achieved the size of small trees.

Non-native iceplant (*Carpobrotus edulis* and possibly *C. chilensis*) is a problem primarily at the McGrath State Beach sites (**Photo 24**). Iceplant produces extensive mats that can competitively exclude milkvetch and harbor snails. Iceplant mats are present both on adjacent low dunes as well as scattered patches throughout the wetland lake margins. Iceplant in the wetland margins largely drowned during episodes of inundation that occurred in winter 2005 but unfortunately, there was no effort to prevent its recovery once the area dried down so it is re-growing. We have controlled iceplant by hand removal and limited glyphosate applications adjacent to MSB-3, 4 and 5. A small area of iceplant adjacent to

| | | | MILKVETCH SITES MAND- MAND- MAND- CSMR- CO | | | | | | | | | COPR- COPR- | | |
|--------------------------|---------------------|------|---|------------|-------------|-------------|-------|-------|-------|---|---|-------------|------------|--|
| LATIN NAME | COMMON NAME | WILD | ORM | MAND- 1 | MAND- 1A | MAND- 1B | MSB-3 | MSB-4 | MSB-5 | 5 | L | J | COPR- P | |
| | | | | | | | | | | | | | | |
| Carduus pycnocephalus | Italian Thistle | | | | | | | | | х | х | | | |
| Carpobrotus chilensis | Sea Fig | | | | | | х | х | х | | | | | |
| Carpobrotus edulis | lceplant | х | | | | | х | х | х | | | | | |
| Centaurea melitensis | Tococlote | х | | | | | | | | | | | | |
| Conium maculatum | Poison Hemlock | | | | | | | | | х | | | | |
| | | | | | | | | | | | | | | |
| Hirschfeldia incana | Summer Mustard | | | х | х | х | | | | х | х | | | |
| Melilotus alba | White Sweet Clover | х | | | | | Х | | | | х | | х | |
| Melilotus indica | Sourclover | | | | | | | х | | | х | | | |
| Phalaris aquatica | Harding Grass | | | | | | | | | | х | | | |
| Picris echioides | Bristly Ox Tongue | | | | | | | | | | х | | | |
| Rhaphanus sativus | Wild Radish | | | | | | | | | х | х | | | |
| Ricinis communis | Castor Bean | | | | | | | | | х | | | | |
| Rumex crispus | Curly dock | | | | | | | | | | | | х | |
| Silybum marianum | Milk Thistle | | | | | | | | | х | х | | | |
| Sonchus asper ssp. asper | Prickly Sow Thistle | | | | | | | | | х | х | | | |
| Sonchus oleraceus | Common Sow Thistle | | | | | | х | х | | х | х | | | |
| Vicia sp. | Vetch | | | | | | | | | х | | | | |

MSB-5 became dominated by yerba mansa and silverweed soon after the iceplant was sprayed with glyphosate. Complete removal of iceplant from the McGrath State Beach wetland margins and adjacent dunes is critical to the long term ability of milkvetch to establish and persist in the area and would benefit both the dune and wetland habitats there. All iceplant has recently been removed from the adjacent McGrath 28 acre private parcel which is currently being restored by the North Shore developers and they are obligated to maintain the 28 acre mitigation area free of iceplant for the next decade.

Several introduction sites were the locations of recent weed control and restoration activities. The Ventura County Parks Department, who manage the Mandalay State Beach area, undertook an extensive effort in 2002 and 2003 to control iceplant and myoporum at the park. Some localized areas that had been entirely iceplant dominated have been restored to native dune scrub, including an area about 50 feet south of the MAND-1, 1A and 1B planting sites. Currently there is no funding for follow-up treatments, and both species may gradually return unless maintenance can be addressed.

Several large *Melaleuca* trees were previously removed from the COPR-Lagoon in 2002, and some localized native revegetation occurred there (**Photo 18**). COPR-Pond supported a dense stand of pampas grass (*Cortaderia* sp.) that was also removed in 2002.

The wild site has chronic weed problems and supports entrenched populations of tocolote (*Centaurea melitensis*) and white sweet clover. Most of the iceplant has been removed over the last few years and will be entirely removed within the new 1.66 acre milkvetch exclosure area in the near future. The area between the previous small milkvetch exclosure (approximately 36 m X 60 m) and the new permanent exclosure is being revegetated with dune scrub species pursuant to approved plans for the North Shore project. The scattered large myoporum were removed in 2006. Control of tocolote and sweet clover has occurred sporadically but has made little dent in the populations. During one weeding episode in summer 2003, bumble bees were observed visiting the sweet clover, and as the patch was removed, they started to visit the nearby milkvetch. This suggests that these weeds can, on the one hand, attract pollinators to the area, but may excessively compete with milkvetch for pollinator service. The wild site would benefit from some type of restoration to create a more favorable herb layer, but we do not yet know what native herbs would grow there while tolerating the below ground oil and we cannot risk introducing a species there which could excessively compete with milkvetch.

GENERAL VEGETATION TRENDS ACROSS THE STUDY PERIOD

Overall growth of associated vegetation at introduction sites is influenced by broader rainfall trends across the study period. Review of rainfall patterns across the period indicate that milkvetch were introduced in relatively dry years (**Figure 1**).

Most of the planting sites have exhibited an overall increase in wetland margin vegetation since installation in April 2002. The arroyo willows and coyote brush are taller and wetland rhizomatous perennials have expanded and formed denser and larger patches. At MSB-5, the sand bar willow (*Salix exigua*) which makes new shoots from below ground rhizomes has expanded and has overtaken the original planting area, shading out milkvetch (**Photo 15**). A single branch from a large established arroyo willow on the banks of McGrath Lake also expanded and overtopped ½ of the original MSB-5 planting location within three years.

Extremely wet conditions in 2005 resulted in a substantial expansion of wetland vegetation, particularly at the McGrath Lake sites. Herbaceous vegetation has also closed in at the COPR-Lagoon, (**Photo 35**), which was very open and sparsely vegetated when we first installed plants there in January 2003 (**Photo 18**). It is likely that the *Melaleuca* trees had suppressed herbaceous vegetation either through shading or other factors and, once removed, herbaceous vegetation has more or less continuously expanded. The Mandalay sites, which exhibited extremely low cover of herbaceous plants in the first few years of installation now has considerably more cover of plants such as everlasting, heliotrope, yerba mansa and western goldenrod. From observing these areas, we have seen that wetter-thannormal conditions provide a window of recruitment in part because the herbivores now have so much more vegetation upon which to graze, and species they might otherwise entirely remove from the area have a window of opportunity to establish.

At the McGrath sites, the original planting plots were almost unrecognizable in summer of 2005 (**Photo 6**). Following inundation, many of the adult coyote brush died. Seedlings of coyote brush and arroyo willow appeared across most of the flooded ground and many of them continue to grow. Assuming these individuals persist, much of the herb-dominated wetland margin areas at MSB 3 and 4 may change and become dominated by arroyo willow and coyote brush. If overtopped by willow, it is unlikely milkvetch will persist in those areas. Very dry conditions are likely for the summer of 2007, and so we will see if some loss of these newly recruited shrubs and willows occurs.

Seasonal Variation in Vegetative Cover: Cover of herbaceous vegetation varies seasonally across the growing season. Milkvetch therefore encounter different degrees of vegetative cover in associated vegetation across a single growing season. Efforts to readily characterize cover in milkvetch plots are stymied by a) seasonal variation from winter through spring and summer; b) the transitional, ecotonal nature of the planting area; and c) removal of vegetation through weeding or herbivory. These ecotonal areas are difficult to define as a discrete stand of vegetation which is the common means through which an area is described and its vegetative cover estimated.

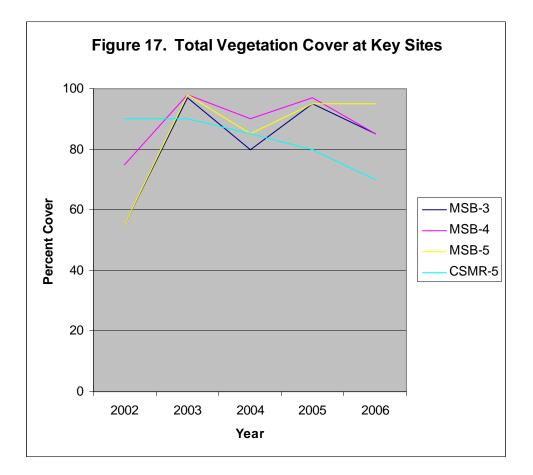
Adult milkvetch in winter exhibit low vegetative cover since plants have died back, but by summer, mature plants in full development can cover substantial areas where milkvetch are close together. Individual milkvetch can reach up to two meters in width when moisture is not limiting. Milkvetch seedlings and juveniles emerge in open growing conditions near parent plants in winter only to later be overtaken by growth in the adults. When growing close together, milkvetch itself creates shrub cover that can result in herbivores like rabbits and meadow voles living underneath the patch and this can exacerbate removal of seedlings and juveniles.

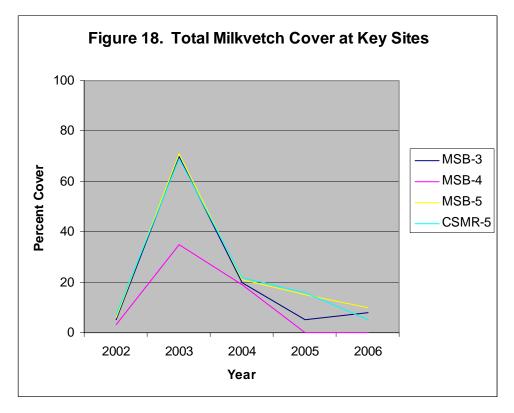
Cover of western ragweed, the most common herbaceous associate at milkvetch planting sites, varies from winter through summer. Western goldenrod and mugwort exhibit a similar pattern. Established stems drop their leaves and are dormant in late fall to early winter. Milkvetch seedlings can emerge and milkvetch can reshoot and find more open growing conditions and light gaps at this time. New annual shoots are produced from the below ground rhizomes, and these generally start to appear by early January. These annual shoots grow gradually taller and leafier and become more numerous. At the same time, western ragweed, in particular, is a favored browse of cottontail rabbits and frequently herbivorized, especially in winter (Photo 10). Eventually, more shoots are produced to replace those cropped by rabbits in winter. If moisture is limiting, such as locations like MAND-1, very little western ragweed survives due to removal by herbivores and it persists as widely spaced, short individual shoots. In very wet years, mugwort, ragweed and goldenrod achieve considerably more overall growth and can overtop and competitively exclude adult and juvenile milkvetch. Milkvetch juveniles are often able to keep pace with the growth of sparse to moderately dense western ragweed, but do not compete well when western ragweed is particularly dense and forms continuous cover.

Cover in milkvetch planting sites generally increases across the spring and summer months unless herbivory levels are high or concentrated in localized areas. At the same time, planting sites which require considerable weeding, such as the CSMR sites, exhibit less cover because we removed the most aggressive invasives. Cover in the planting areas is strongly influenced by herbivores, especially rabbits, and localized areas of severe herbivory can experience removal of almost all herbaceous vegetation produced there over the course of the growing season.

General Trends in Milkvetch and Vegetative Cover at Key Sites: Figure 17 shows the estimated total vegetative cover at key sites across the study period. The MSB sites exhibited similar general trends across the period. Vegetative cover is highest in wetter years and drops in drier years. These cover values do not depict the shift in species composition that typically occurs between wetter and drier years. High cover at the MSB-5 plot in 2006 occurred because the planting site has been largely overtaken by arroyo and sand bar willow, which overtop the plot. The CSMR-5 location exhibited a slightly different overall trend. This occurred primarily because CSMR-5 had supported continuous cover of annual grasses in 2002 when the original outplants were installed, but since that time, grass cover is generally sparse and patchy. This shift appears to be the result of modified architecture in the CSMR-5 area relative to herbivory. The July 2002 plot photograph shows that CSMR-5 was open to the salt marsh and the herb layer was dominated by dense cover of Lolium multiflorum (70% cover, Soza et al. 2003). By July 2003, tall vegetation blocked views into the plot from the salt marsh and milkvetch grew in size, creating the equivalent of a visually screened brush patch within which rabbits intensively herbivorized the herb layer and removed most grasses (Photo 16).

Figure 18 shows the estimated total cover of milkvetch at key sites across the study period. The general trend shows that milkvetch cover peaks in the second year after planting and declines in subsequent years. MSB-4 exhibited the lowest overall milkvetch cover in its second year primarily because milkvetch were more widely spaced when planted at this location. MSB-4 exhibited no milkvetch cover after 2005 because milkvetch no longer occupied the original planting area. Few milkvetch remain at the MSB-3 original plot and the majority of plants in 2006 were in the north extension area, which we now define as MSB-3E (**Photo 24**). At CSMR-5, most of the adults had declined by 2006, and most of the new recruits were located in the adjacent CSMR-5C location, while the original plot supported scattered juveniles and three milkvetch recruited in previous years.





JUVENILE ESTABLISHMENT

Milkvetch seedlings come up in a wide range of plot conditions, but their ability to grow and establish can be impaired by excessive competition from neighboring plants. We estimate that optimal vegetative cover for establishment and maturation of juveniles is generally around 50% or less. Intervening vegetation gaps where sunlight can reach the ground surface are important and allow juveniles to develop multiple stems early in their growth period and likely enhances development of a vigorous below-ground root system (**Photo 10**). Juveniles grow best at locations where they can keep pace with the height of adjacent associated herbaceous vegetation and are not overtopped. At COPR-Pond, during years of higher rainfall, naturally recruited milkvetch have been able to keep pace with the height of adjacent herbaceous vegetation and have established in areas with 90-95% cover (**Photo 35**). Under low light conditions, milkvetch juveniles are leggy, induced by efforts to reach more light and they put on little growth (**Photo 34**). In this situation, they are vulnerable to mites, further reducing vigor and often they experience more nibbling (herbivorized leaflets and cotyledons).

Milkvetch seedlings can emerge on open sparsely vegetated ground with little or no litter. They can also emerge in locations with twiggy litter (**Photo 32**) and have sometimes been seen in iceplant litter several centimeters thick. Herbivores like rabbits will sometimes avoid removing milkvetch seedlings/juveniles where they are closely associated with other vegetation and are protected to some degree (**Photo 33**).

ASSOCIATED VEGETATION AT POORLY PERFORMING DRY SITES

Table 7 lists the most abundant plant species associated with poorly performing, failed planting sites that appeared too dry. Both MSB-1 and MSB-2 were located in low dunes east of McGrath Lake and had sandy soils and low levels of litter. No obligate, facultative, or phraeatophytic plant species occurred in these planting sites. MSB-2 was very flat and exhibited high cover of annual grasses. MSB-1 was located on a seaward rise in a low dune, and supported more typical dune scrub vegetation. At both locations, planted milkvetch established during the 2002 period with supplemental irrigation. Both sites started reshooting in December, 2003. It seems that early reshooting is observed at locations where plants are more stressed. Milkvetch put on new growth which abruptly declined in early summer of 2003 when moisture apparently became too limiting. The combination of very sandy dune soils, exposure to drying winds, reduced fog drip, and greater distance to the perched water table (which we could not reach to sample) are the factors we believe

contributed to failure of these plots.

MAND-2 and MAND-3 also performed poorly, although milkvetch exhibited better overall vigor here compared to MSB-1 and 2. Plants were installed in January, 2003. MAND-2 was a shallow dune swale with a slight topographic depression that supported a mix of wetland and upland dune species. MAND-3 was adjacent to established phraeatophytes like coyote brush, spiny rush and poison oak, but otherwise was flat and supported high cover of summer mustard. Plants here were largely unherbivorized, no snail control was needed, and they exhibited some modest flowering and fruiting in 2003 and 2004. MAND-2 supported much more wetland vegetation in 2005 when wetter conditions ensued, and milkvetch juveniles were observed at both sites in early summer 2005, but by the end of the season, all the adults and juveniles died.

The perched water table was slightly nearer the surface at MAND-2 and 3 compared with MAND-1, yet plant vigor, persistence, recruitment and survival has been much better at the MAND-1 sites. It is therefore likely that factors such as lack of protective shrub cover, reduced fog drip, and exposure to drying winds may have played a role in poor performance at MAND 2 and 3. The large dense band of established arroyo willow immediately north of the MAND 1, 1A and 1B locations with more favorable milkvetch growth would block direct exposure to dry Santa Ana winds in summer and fall and evapotranspiration likely raises humidity in the immediate area (**Photo 8**).

| | | | low d | lunes | dune swales | | |
|--|-------------------------|-----------------------|-------|--------|-------------|-----------|--|
| LATIN NAME | COMMON NAME | Wetland Indicator* | MSB-1 | MSB-2 | MAND- 2 | MAND 3 | |
| Shrubs-Subshrubs | | | | | | | |
| | | | | | | | |
| | hard have seen | | | | | | |
| Ambrosia chamissonis | beach bur-sage | | х | Х | | | |
| Baccharis pilularis | coyote brush | phraeatophyte | | | х | Х | |
| Croton californica | croton | | | Х | | | |
| Ericameria ericoides | mock heather | | х | | | | |
| Eriogonum parvifolium | seacliff buckwheat | | | | х | | |
| Heterotheca sessiliflora ssp. sessiliflora | hairy golden aster | | | Х | | | |
| Juncus acutus ssp. Leopoldii | spiny rush | obligate | | | | Х | |
| Lotus scoparius | deerweed | | | | Х | | |
| Lupinus chamissonis | dune bush lupine | | Х | | Х | | |
| Opuntia littoralis | prickly pear cactus | | | | | Х | |
| Toxicodendron diversilobum | poison oak | phraeatophyte | | | | Х | |
| Herbs and Grasses | | | | | | | |
| | | | | | | | |
| Ambrosia psilostachya | western ragweed | | | | | Т | |
| Bromus hordeaceous | soft chess | | | Х | | | |
| Bromus madritensis ssp.rubens | red brome | | Х | Х | Х | | |
| Cakile maritima | sea rocket | | | Х | | | |
| Camissonia micrantha | suncup beach evening | | Х | х | | Х | |
| Camissonia cheiranthifolia | primrose | | | Х | | | |
| Carpobrotus edulis | iceplant | | х | Х | Х | | |
| Conyza canadensis | horseweed | | | | | Х | |
| Cryptantha sp. | cryptantha | | Х | Х | | | |
| Cirsium occidentale | cobweb thistle | | | | | Х | |
| Descurainea pinnata | tansy mustard | | | | Х | Х | |
| Erodium cicutarium | filaree | | х | | | | |
| Heliotropium curassavicum | heliotrope | obligate | | | Т | | |
| Herniaria hirsuta | herniaria | | х | х | | Х | |
| Hirschfeldia incana | summer mustard | | | | х | х | |
| Juncus mexicanus | Mexican rush | facw | | | х | | |
| Lastarriaea coriacea | lastarriaea | | | х | | | |
| Lotus purshianus | Spanish clover | | | | х | | |
| Malva cf. parviflora | cheeseweed | | | х | | | |
| Melilotus indica | sourclover | | х | x | | | |
| Phacelia distans | wild heliotrope | | x | X | | | |
| Phacelia ramosissima | rambling phacelia | | ~ | ~ | х | | |
| Schismus barbatus | schismus | | Y | v | ~ | х | |
| Schismus barbalus Vulpia myuros | slender fescue | | Х | X X | | ^ | |
| T = trace, very low levels | | I | | ^ | | | |

XI. RECOMMENDATIONS FOR FUTURE MODIFICATIONS

Suggested Revisions to Methods and Protocols:

A number of modifications should be considered to improve the effectiveness of experimental planting trials.

<u>Seed germination</u>: Winter/early spring outplanting of nursery-raised plants is desirable and corresponds with more favorable moisture conditions and natural rainfall. In both the 2002 and 2004 projects, we anticipated that plants would be ready for delivery and outplanting in January, but milkvetch root systems were still fragile. Plants raised from seed germinated in August-September did not produce sufficient growth to be ready for delivery and outplanting in January. To address this problem, milkvetch seed for outplanting efforts should be germinated earlier, around the beginning of June. Milkvetch would therefore be about seven to eight months old if outplanted in late January, early February.

<u>Outplanting dates</u>: Outplanting should occur in late January to early February and should not occur until rainfall is sufficient to produce new growth of associated vegetation needed to sustain herbivores.

<u>Direct Seeding Trials</u>: Direct seeding could be considered for initial testing of outplanting sites, and we still have some bulked seed of unknown maternal line that could be used for this purpose. Another seed bulking effort could also be undertaken to produce additional seed of known maternal line for recovery purposes. Timing of direct seeding trials is likely important. Direct seeding in late January of 2003, following relatively good rainfall, was successful in certain locations at the COPR. We recommend seeding after sufficient rainfall has occurred to stimulate new growth of associated vegetation needed to sustain herbivores. Early winter direct seeding may lead to losses of seedlings from herbivores.

<u>Herbivory Exclosures</u>: Herbivory exclosures could be used for initial outplanting of containerraised plants, but a different design is needed which does not reduce sunlight and confine milkvetch growth. Research to determine if the palatability of nursery-raised milkvetch can be reduced should be explored, as this could provide a simpler management tool since caging is extremely time consuming and increases management effort and costs.

Planting Configuration: Stand architecture is critical. Based upon observations to date, we

recommend avoiding planting milkvetch close together or in a patch. Instead, plant in narrow bands, linear arrays or scattered individuals. Arrays can be staggered to occupy different micro-sites if conditions warrant and replicate installations should be used.

<u>Ongoing Management is Critical</u>: Provisions for ongoing management of introduced populations must be addressed. Until such a time as coastal sites are free of non-native weeds and introduced snails, for example, some level of active management will likely be needed.

Site Selection Criteria:

Wilken and Wardlaw (2001) evaluated a series of potential VMMV introduction sites utilizing five criteria they felt were important to the survival of milkvetch and we summarize these criteria below:

- Dominant vegetation composed of a single canopy of native shrubs forming 50-75% cover;
- Absence of competitive native plants and annual exotic plants;
- A fresh or brackish water table judged to be in close proximity and near the surface;
- Relatively compact, stable sandy substrates with overall physical and chemical properties consistent with the Oxnard (wild) site;
- A buffer or border of natural habitat that might support medium or large sized bees.

Based upon our recent efforts to establish plants at new growing sites, the criteria above should be further refined. These criteria were developed prior to observations of severe milkvetch herbivory, and therefore, additional criteria must be developed to ensure that stand architecture and herbivore activity are considered during the site selection process. Locations with a single canopy of native shrubs but lacking native competitors and exotic annuals rarely exist in the coastal sites we have examined. Where this condition does exist, low herb cover has been maintained by herbivores. The high end of shrub cover also likely would result in higher levels of herbivory due to visual screening provided by high shrub cover, and unless milkvetch palatability is low at the site or can be reduced, such sites are not likely to be successful without continued intensive management.

Utilizing the observations obtained through this study, we offer the following additions and modifications to the site selection criteria:

- 1. Planting locations are near the coast with a maritime influence, mild temperatures, higher humidity and periodic fog.
- 2. The planting area is located in a transitional zone between wetlands and drier habitats with lower herbaceous cover and generally on flat to gently sloping ground.
- 3. A perched fresh or brackish water table is present and preferably between 50-120 cm from the surface topography.

Extreme fluctuations and potential for seasonal loss of the water table would be a concern.

- 4. Bare ground or bare ground with light to moderate litter should range from 5-25% for optimal seedling/juvenile recruitment. This may be maintained by herbivores.
- Gopher turbation is absent or occurs at very low levels.
 Keep in mind that gopher activity may increase in drier years or during the dry season.
- Planting sites adjacent to taller vegetation (over 0.5 meters) should be open to low growing vegetation stands, preferably on three sides, to reduce herbivore activity on milkvetch.
- 7. Locations distant from shrub cover or dense patches of taller herbaceous vegetation (over 0.5 meters tall) may experience reduced rabbit herbivory.
- 8. Such locations may be more exposed to drying Santa Ana winds and potentially less condensation from fog.
- 9. Shrub cover ranging from 20-40 percent may be optimal.

The presence of shrubs may not be entirely mandatory provided sites are not too exposed to dry winds or open sandy soils. The dominant shrub will likely be coyote brush, but other phraeatophytic shrubs may also occur, such as poison oak. Shrubs should occur as either single individuals or small groupings. Plots placed adjacent to dense shrub cover will likely experience herbivory. This will be a problem if milkvetch palatability is not reduced by possible trace elements in the soil such as selenium. Milkvetch should not be planted within a dense shrub stand.

- 10. Rhizomatous perennial facultative and obligate wetland thatch-producing plants (e.g. salt grass, juncus, carex, horsetail) are present, but exhibit either low overall cover or localized patchy cover.
- 11. Western ragweed is present and if so, areas supporting facultative wetland or obligate wetland herbs should be nearby as they indicate more favorable moisture conditions.
- 12. Some non-alkaline wetland indicator species should be present in the area, and areas dominated by halophytes and salt marsh indicators should be avoided.
- 13. If the planting location is vulnerable to occasional flooding, milkvetch should be able to adjust their location following flooding episodes through re-distribution of the seed bank and should not be topographically confined.
- 14. Soils contain some clay and silt particles, plus organic matter which improves water holding ability compared with coarse sands.
- 15. Salinity should be lower than 10 mmhos/cm and hypersaline conditions should not occur or remain within a mild range.

Based upon these criteria, the COPR-Pond site would be considered risky due to its tendency for wide fluctuations in the local water table. Ironically, these fluctuations have created a situation in which the dune hollow there supports optimal cover of annual and perennial herbaceous vegetation with some bare ground. Shrub cover is distant due to episodes of inundation- this seems to contribute to low levels of rabbit herbivory at this location. Therefore, COPR-Pond exhibits an unusual mix of conditions which we believe may still be favorable for milkvetch, at least in certain years. Plants continue to come up on their own from seed produced in 2003 and 2004, and occasionally, juveniles have been able to mature and flower in their first year (2005; 2006). If we can successfully build up the seed bank there, the COPR-Pond may remain a viable introduction site.

These criteria would have eliminated the MSB-1, MSB-2 and MAND-3 failed planting locations, which were located in dunes and were too removed from wetland margins or dune swales where facultative and obligate wetland herbs grow. MAND-3 lacked shrub cover on the northeast side and therefore was exposed to Santa Ana winds during dry hot periods. Because they were topographically higher in elevation than other nearby sites, the perched water table was beyond the optimal range at MAND-3 (we could not measure it at the MSB

sites).

MAND-1 and nearby sites are also marginal for milkvetch recruitment, probably due to the distance to the water table. That distance appears to have been moderated by the presence of an established band of arroyo willow in the dune swale there, which blocks Santa Ana winds, increases local fog drip and likely transpires more moisture. Milkvetch vigor and persistence was much better at the MAND-1 plots compared to MAND-2 and 3.

The salinity criteria would have eliminated the CSMR-1, 2, 3 and 4 planting sites. Dominance of pickleweed (*Salicornia* sp.) was an indicator of high salinity at CSMR-3. Dominance of rye grass (90%) and lack of bare ground (100% vegetation cover) at CSMR-4 also indicated unsuitable conditions, even if salinity had been within tolerable levels. It is doubtful milkvetch juveniles could have emerged within the rye grass meadow lacking vegetation gaps.

The salinity criteria may have eliminated both the South Ormond (salt marsh portion) and CORP-Jaumea planting sites. We did not measure salinity at these locations, but we assume, based upon the presence of halophytes and alkaline indicators, salinity at these locations probably exceeds tolerance by the milkvetch. High continuous cover of *Jaumea*, an alkaline indicator, and absence of fresh water indicators, suggested these areas were unsuitable, especially at South Ormond. It should be noted that we tried planting in these *Jaumea*-dominated areas, in part, because this species co-occurred with northern brine milkvetch at locations in Drakes Estero, Marin County. The habitat there is generally described as *Distichlis spicata-Frankenia salina-Jaumea carnosa* Association (Keeler-Wolf, 1999). At Drakes Estero, northern brine milkvetch (*Astragalus pycnostachyus* var. *pycnostachyus*) grow on the spine of narrow spits which jut out into the estero at various locations and lie between tidal and bay mud flats and pickleweed salt marsh. These areas do not exhibit continuous cover as our planting areas did, and localized lower salinity is reported to occur due to freshwater input or leaching (Keeler-Wolf, 1999).

MSB-5 would have likely met all the Wilken and Wardlaw (2001) site selection criteria, yet the site has ultimately proven to be a poor location for milkvetch for several reasons. It exhibited the recommended shrub cover and lack of exotics and competitors in the immediate area. Milkvetch were installed within/adjacent to a stand of sand bar willow, albeit there were fewer individual stems and it was more open at the time of introduction. We did not anticipate that sand bar willow would expand and become as competitive as it did over the next few years following milkvetch installation. Also, following episodes of flooding in

2005, most of the surrounding area where milkvetch seed may have been carried by expanding floodwaters was densely vegetated with primarily wetland plants or iceplant mats. The site therefore lacked a transitional area of drier and/or more sparsely vegetated habitats adjacent to the planting area. In contrast, MSB-3 and 4 were adjacent to drier sparsely vegetated areas, the seedbank was not confined by topography, and milkvetch emerged and established in adjacent new areas following flooding.

Other Potential Outplanting Situations:

We should continue to remain open to trying different types of planting situations in coastal locations for Ventura marsh milkvetch. The criteria suggested above should not be viewed as limiting or otherwise restricting efforts to experiment with other possible milkvetch introduction sites.

The wild site is an anomaly when compared to nearby, less-modified natural habitat areas where milkvetch has been introduced to date. A combination of unusual conditions occurs at the highly-modified wild site. The below-ground oil serves to create a perched water table by preventing infiltration of rainwater. The site can therefore support facultative wetland plants, but oil wastes seem to have resulted in no below-ground gopher activity and no rhizomatous wetland perennial competitors. The oil may release substances that reduce milkvetch palatability to herbivores. Shrubs tend to dominate the site, protecting it from dry winds and increasing fog drip, but do not reach their typical size due to oil contamination and therefore, shrub stands still retain areas with more open growing conditions-shrub skeletons and vegetation gaps are common. Seedlings are able to come up in most years at the wild site unless rainfall is very low. This set of conditions has proven difficult to duplicate in more natural coastal environments.

Historic collection sites, habitats and co-occurring species were largely not described on most herbarium labels and confusing labels and changes in place names serve to confound efforts to pin down geographic locations and potential habitats where VMMV once occurred. *Marsh* (Ballona Marshes, Chandler-1902); *cienega, Los Angeles County* (Spanish for marsh and sometimes applied to springs, Grant-1904); *meadow near seashore* (possibly Santa Monica, Parish 1882); La Bolsa (type locality, Parish 1882); *roadside along Harbor Blvd...near the mouth of the Santa Clara River* (Chase, 1967)- are examples of the limited habitat information documented from historic collections. Additional information and opinions about these and other historic collection sites can be reviewed in Jensen (2007), Wilken and Wardlaw (2001), Barnaby (1964); Critchfield, (1978); and USFWS (2001). In reviewing CDFG files while preparing this report, I discovered some interesting information overlooked in all published accounts I have seen regarding historic collection sites. My files contained a copy of E.O. Essig's August 1911 collection label from Ventura, California. This specimen and label are housed at the University of California, Jepson Herbarium (Accession # 74601). The label indicates the collection site was at an elevation of 200 feet, the plants were 1-2 feet tall, and the words "Rare plants" are typed on the bottom of the label. It was also given the common name of "locoweed, salt weed". Information accompanying this label indicates Essig was the State Horticultural Commissioner in Ventura County from 1910-1911.

An elevation near Ventura of 200 feet suggests the possibility that VMMV were not necessarily growing on the immediate coast. They could have been growing on eroded bluffs, benches, in barrancas, in nearby hills around the Ventura River, or down on the alluvial plain in east Ventura, but distant from the Santa Clara River. A plant height of 1-2 feet (30-61 cm) suggests plants were short and may not have been growing in a wet area, or moisture was otherwise limiting. "Rare plants" is revealing- either Essig had never seen these plants before, or few plants were growing in the location where he collected them - three herbarium sheets worth of milkvetch were reportedly collected. Further investigation into historic conditions in the Ventura area at the time of this collection may reveal other clues to where VMMV may have once occurred.

I have not had an opportunity to examine historic herbarium collections of VMMV, but the overall plant height reported by Barnaby (1964) ranged from 40-90 cm which is often shorter than the height of mature plants at our introduction sites. It would be worthwhile to examine the collections to determine if entire stems were collected and what the stem height and internode lengths were at different collection sites. Shorter overall stem lengths may indicate the collection site was not in a wet area, or plants were growing on more compacted soils or in years of drought.

Many of the historic VMMV collection sites were associated with coastal wetlands, but they may also represent sites where oil geology and oil wells occur. The distribution of VMMV may have been tied to locations which provided both the habitat and growing conditions necessary for milkvetch survival, and soil with higher levels of selenium leading to reduced herbivory.

The vicinity of Ventura and local hills support oil geology, active wells, and

asphaltum/oil springs. Combinations of surface water seepage and oil occur on Highway 150 between Ojai and Santa Paula, and west Sulphur Mountain. There may be other coastal locations where these features combine and could possibly support milkvetch. A map available for online viewing shows numerous "oil springs" in the San Buenaventura (Ventura) area, all north of the Santa Clara River, in 1866 (David Rumsey Collection, 2005). This same map shows numerous asphaltum wells in the vicinity of Ballona near Santa Monica, an historic collection site for VMMV.

Coastal upland terraces adjacent to the immediate coast may be potentially suitable habitat for experimental planting trials. In San Mateo County, the northern brine milkvetch occurs along the edges of Highway 1 near Pomponio State Beach (Wilken and Wardlaw, 2001), along the edges of a parking lot in localized patches of open ground and along the edges of a foot path bisecting dense coastal scrub habitats at Pescadero State Beach (Mary Meyer, pers. obs.). The trail bed has some friable soil, but beneath it is firm and compacted with more fine particles. Mowing for trail maintenance may reduce cover benefiting northern brine milkvetch at the Pescadero Dike location (provided the plants themselves are not damaged, which has reportedly occurred in the past). Dr. Dieter Wilken provided me with photographs of several of these locations taken in fall, 2006, and most show northern brine milkvetch occupying low competition sites where they are not submerged in continuous vegetative cover and are often located where disturbance has created open ground and sparse shrub and herb cover. Several of the San Mateo County occurrences are not affiliated with wetlands per se, and plants are shorter and less robust than northern brine milkvetch I have seen at Drakes Estero in Marin County, rooted in wetland habitats.

Coastal terraces adjacent to ocean cliffs in the vicinity of Carpinteria, Goleta, and Gaviota may support similar conditions to those seen in San Mateo County and should be further evaluated for possible milkvetch planting trials. Recent restoration at the Carpinteria Bluffs has replaced the coyote brush stands which previously supported considerable annual grass and ruderal weed cover with a mix of coastal shrubs species and the area now looks much more like the type of diverse coastal scrub habitat seen in San Mateo County. It will be interesting to see if the restored coastal scrub habitat results in a stable, self perpetuating community over time. Introduction of VMMV along the trail edge there would be interesting to try, and disturbance associated with trail users may be tolerated by VMMV to some degree.

XII. CONCLUSION

This report documents our initial efforts to establish test plantings of Ventura marsh milkvetch at coastal sites in Ventura and Santa Barbara County. We have been able to expand our understanding of potentially suitable habitats for this enigmatic plant by observing the response of VMMV to various planting sites, habitat conditions, weather fluctuations and interactions with native and non-native plants and herbivores. Observations of natural recruitment from seed produced by founder plants have also provided important insights into the unique ecological requirements of VMMV.

Habitats examined to date include palustrine/lacustrine wetland margins within coastal dune systems and upland/wetland transitional areas adjacent to active salt marshes and coastal lagoons. Most sites support perched water tables which vary from relatively stable to highly fluctuating. Over the study period, outplanting sites have been subjected to extreme rainfall patterns, including very wet and very dry periods. The density and biomass of competing vegetation fluctuates dramatically in response to wet and dry periods, influencing survivorship of seedlings, juveniles and adult plants.

Native herbivores such as rabbits play a powerful role by removing certain competitors entirely, and thinning others, leading to the creation of vegetation gaps which milkvetch can sometimes exploit. Severe herbivory by native herbivores on reshooting adults and established plants during the growing season has occurred at certain sites but is uncommon at others, suggesting milkvetch palatability varies between sites due to unknown factors. Non-native snails have invaded some locations and can also damage seedling, juvenile and adult plants, their flowers and developing fruits. Therefore, monitoring and active management will continue to be necessary to sustain introductions at most locations. Further investigation into factors influencing milkvetch palatability across different study sites is also warranted. Selenium concentrations may vary between sites, potentially influencing palatability and therefore, follow-up to Parker's research (Parker, 2006) should be conducted.

The drier outer margins of the wetland transition zone adjacent to the east side of McGrath Lake represent probably the best location to date for milkvetch as expressed by the size and vigor of planted individuals, successful recruitment of new plants from seed, evidence of recovery following floods and natural dispersal to nearby, previously unoccupied areas. However, this site has consistently experienced the most severe herbivory over the study period, requiring temporary caging and considerable hands-on-management.

Juvenile, nursery-raised milkvetch used in this study can establish and persist for a period of time at most introduced locations, including locations which may be too dry for successful seedling establishment. But seedlings require more favorable moisture regimes to establish and grow sufficiently to reach moist soil above perched aquifers during the dry summer months. As with many plant species, seedling survival and recruitment to older age classes is a limiting life history phase and is critical to establishment of self-perpetuating populations. The successful production of new annual growth shoots on adult plants is also an extremely vulnerable stage.

The life history strategy of VMMV differs from that of most other vegetation at these introduction sites. Rhizomatous perennials are able to respond to episodes of drought by reducing the density and height of stems and through spatial contraction. When more favorable moisture regimes return, below ground rhizomes can expand in response. As a short-lived perennial, VMMV cannot rapidly respond to spatial shifts in habitat conditions. Its ability to produce an abundant seed crop, variations in seed dormancy, and ability for at least some juveniles to become reproductive in their first year at more favorable introduction sites, has allowed VMMV populations to recover from flooding from the seed bank, and shift spatially to more suitable neighboring areas in response to a changing environment. The retention of seeds in pods and infructescences on the previous year's growth stems also contributes to the adaptability in these dynamic environments, especially adjacent to depressional wetlands prone to periodic flooding.

Milkvetch dispersal to new sites has been observed at locations where localized flooding occurs, but at locations like CSMR and COPR-Lagoon, milkvetch have yet to be observed beyond the general introduction area. At both sites, low competition growing sites are scarce and active management to suppress existing weedy competitors has been necessary.

From observing these outplanting sites over a period of time, it becomes very apparent that these are highly variable environments and growing conditions are neither stable nor predictable. Assuming that our test outplantings sites reflect conditions at historic locations, VMMV could be viewed as an example of a plant that exists at locations which are only suitable for temporary periods of time. VMMV likely existed as a metapopulation within the coastal systems where it historically occurred, and colonization and extinction events were probably common. Certain locations may have functioned as core population sites while at other sites, populations are more transitory. By producing an abundant seed crop, including some seeds with more prolonged dormancy, VMMV may re-appear at locations

when favorable growing conditions return. The concept that VMMV populations should exhibit a stable population trend and maintain predictable above-ground populations year after year at the same exact location is likely biologically and ecologically flawed. To further recovery, efforts should focus on establishing multiple VMMV colonies within a given coastal system that will likely fluctuate spatially and temporally. Further work to increase the number of occupied microsites, enlarge overall population size and build seed bank reserves, will be necessary at most locations to improve the potential for long term viability at introduction sites.

ACKNOWLEDGEMENTS

I would like to recognize the contributions of two scientific aides who assisted in this project. Anne Macleod worked on the project from 2003-2005 and Sheri Mayta has been assisting since 2006. Both have contributed tremendously to the success of this project. I would also like to thank Dr. Dieter Wilken, Dr. Cristina Sandoval, and Michael Wall, all of whom have provided advice and support throughout this endeavor. I would also like to specifically thank Trish Munro and the volunteers she has periodically helped organize to assist in planting and weeding efforts over the last several years.

XII. LITERATURE CITED

- Armstrong, W.P. 1998. <u>WaynesWord: http://waynesword.palomar.edu/ww0703.htm</u>. Locoweeds, Wild and Crazy Plants. Vol. 7 (3) Fall 1998.
- Barbour, M.G., J.H. Burk, W.D. Pitts. 1987. Terrestrial plant ecology. CA: Benjamin Cummings Publishing Company. 634 pp.
- Barnaby, R. 1964. Atlas of North American *Astragalus*. Memoirs, New York Botanical Garden 13:1-1188.
- Blachly, J.S. and B.T. Forschler. 1996. Suppression of late-season Argentine ant (Hymenoptera:Formicidae) field populations using a perimeter treatment with containerized baits. Journal of Economic Entomology 89(6):1,497-1,500.
- Bloomcrist, Cheryl. 2006. California Department of Food and Agriculture, Plant Health and Pest Prevention Services. Personal communication with Mary Meyer, California Department of Fish and Game, October 17, 2006.
- California Regional Water Quality Control Board (CRWQCB), 2000. Waste Discharge Requirements for North Shore at Mandalay Bay, Order No. 00-180. Los Angeles Region.
- Cole, F. R., A C. Medeiros, L. L. Loops, and W. W. Zuehlke. 1992. Effects of the Argentine ant on arthropod fauna of Hawaiian high-elevation shrubland. Ecology 73(4): 1313-1322.
- Critchfield, W.B. 1978. Rare Plant Status Report- *Astragalus pycnostachyus* Gray var. *lanosissimus* (Rydb.) Munz and McBurney. California Native Plant Society. Unpublished report. 3 pp.
- Environmental Science Associates (ESA), 2003. McGrath State Beach Natural Resources Management Plan. Unpublished report submitted to the California Department of Parks and Recreation. Channel Coast District. April 2003.
- Ferren, W. R., Jr., H. Page and P. Saley. No date. Management Plan for the Carpinteria Salt Marsh Reserve, A Southern California Estuary. Prepared for the University of California Natural Reserve System.

- Gordon, Dr. Deborah 2000. Department of Biological Sciences, Stanford University. Personal communication with Mary Meyer, California Department of Fish and Game. June 6, 2000.
- Hickman, J. C. 1993. The Jepson manual: higher plants of California. University of California Press. 1400 pp.
- Human, K.G. and D.M. Gordon. 1996. Exploitation and interference competition between the invasive Argentine ant, *Linepithema humile*, and native ant species. Oecologia 105:405-412.
- Ikeda, D. and M. Meyer. 2000. Report to the Fish and Game Commission on the status of the Ventura marsh milkvetch (*Astragalus pycnostachyus* var. *lanosissimus*). Status Report 2000-1, Sacramento, California. 50 pp.
- Impact Sciences. 1997. Summary of Ventura Marsh milk-vetch research at the North Shore site, Oxnard California. Unpublished report. 7 pp.

______. 1998. Ventura Marsh milk-vetch Mitigation and Preservation Plan (June 12, 1998). Unpublished report, 38 pp.

______. 2004. Wetland Creation/Dune Enhancement Plan for the McGrath Parcel, Ventura County, California. Unpublished report prepared for the County of Ventura. December 3, 2004. 61+pp.

- ______. 2005. North Shore Resource Protection Area/Milk-Vetch Preservation Plan. North Shore Project Site, Oxnard California. Unpublished report prepared for the City of Oxnard. June 17, 2005. 83+pp.
- Integrated Water Resources, Inc (IWR). 2004. Final Draft Hydrology Report, McGrath Parcel, Oxnard, California. Unpublished report contained in Impact Sciences, 2004. 10+ pp.
- Jensen, Nicholas J. 2007. The Habitat of Astragalus pycnostachyus var. lanosissimus (Ventura marsh milk-vetch) and an Assessment of Potential Future Planting Sites.
 Unpublished report submitted to David Magney, Channel Islands Chapter, California Native Plant Society. April 26, 2007. 95 pp.

- Jones and Stokes, 1994. Final Draft South Ormond Beach Wetland Restoration and Management Plan. Unpublished report prepared for the City of Oxnard, Community Development Department. November 1994.
- Keeler-Wolf, T. 1999. Summary of habitat requirements for the two populations of *Astragalus pycnostachyus* var. *pycnostachyus* observed in Drakes Estero, Marin County, on July 30, 1999. Unpublished memorandum, CDFG files.
- Klotz, Dr. John. 2000. Department of Entomology. University of California, Riverside. Personal Communication with Mary Meyer, Department of Fish and Game. June 19, 2000.
- Krushelnycky, P D. and N. J. Reimer. 1998a. Efficacy of Maxforce bait for control of the Argentine ant (Hymentoptera:Formicideae) in Haleakala National Park, Maui, Hawaii. Environmental Entomology 27(6) 1473-1481.
- Laudenslayer, William Jr., William Grenfell, Jr., and David Zeiner. 1991. A Check-list of the Amphibians, Reptiles, Birds, and Mammals of California. California Fish and Game 77(3): 109-141.
- Matonni, Rudi. 1990. Butterflies of Greater Los Angeles. Illustrated Guide. The Center for the Conservation of Biodiversity/Lepidoptera Research Foundation, Inc., Beverly Hills, CA.
- Munz, P.A. 1974. A Flora of Southern California. University of California Press. Berkeley, CA.1086 pp.
- Parker, Sophie. 2006. Assessment of Soil Properties and Environmental Conditions Related to the Successful Propagation of *Astragalus pycnostachyus* var. *lanosissimus*.
 Unpublished report. 12 pp.
- Rumsey, David, 2007. Website: <u>www.DavidRumsey.com</u>. Historic map collection. Image # 1070020, U.S. General Land Office. California, 1866.

Santa Barbara County Flood Control District (SBCFCD), 2007. website: www.countyofsb.org/pwd/water/hydro.htm.

- Soza, Valerie, Michael Wall and Dylan Hannon. 2003. Experimental Introduction of the Ventura Marsh Milkvetch (*Astragalus pycnostachyus* var. *lanosissimus*) at Carpinteria Salt Marsh Reserve and McGrath State Beach. Unpublished report submitted to the California Department of Fish and Game, South Coast Region. March 13, 2003. 30+pp.
- Suarez, A.V., D.T. Bolger, and T.J. Case. 1998. Effects of fragmentation and invasion on native ant communities in coastal southern California. Ecology 79(6):2,041- 2,056.
- United States Fish and Wildlife Service ((USFWS), 2001. Endangered and Threatened Wildlife and Plants; Final Rule for Endangered Status for *Astragalus pycnostachyus* var. *lanosissimus*(Ventura marsh milk-vetch). Federal Register vol. 66, No. 98:27901-27908.
- University of California, Santa Barbara. 2004. Coal Oil Point Reserve Management Plan. June 22, 2004. 169 pp.
- Ventura County Watershed Protection District, 2006. Website, <u>www.vcwatershed.org/hydro/data/216B.htm</u>. Visited November 24, 2006.
- Ventura County Watershed Protection District, 2007. Website, <u>www.vcwatershed.org/fws/specialmedia.htm-</u> Historic Driest and Wettest Years for Ventura # 66. Visited May 2 2007.
- Wilken, Dieter and Tricia Wardlaw, 2001. Ecological and Life History Characteristics of the Ventura Marsh Milkvetch (*Astragalus pycnostachyus* var. *lanosissimus*) and Their Implications for Recovery. Unpublished report submitted to the California Department of Fish and Game, South Coast Region. March 1, 2001. 55 pp.
- WRCC website, <u>http://www.wrcc.dri.edu/</u>. Oxnard, CA (046569), Period of Record Monthly Climate Summary, Period of Record: 07/1/1948 to 12/31/2003; visited March 5, 2004 (www.wrcc.dri.edu/cgi-bin/cliMONtpre.pl?caoxn

Appendix A: Maternal Lines Producing Seed at the Outplanting sites during at least one year. 2002 introductions reflect lines which produced seed in 2003.

| | 2002 Original | Outplants pro | oducing seed in | <u>n 2003</u> . | |
|------------------|---------------|---------------|-----------------|-----------------|--------|
| MATERNAL LINES # | MSB-3 | MSB-4 | MSB-5 | CSMR-2 | CSMR-5 |
| 2 | | | | | |
| 6 | Х | Х | Х | | х |
| 19 | Х | Х | Х | | Х |
| 20 | | | | | Х |
| 21 | Х | Х | Х | | Х |
| 37 | Х | Х | Х | | Х |
| 43 | Х | Х | Х | | Х |
| 46 | Х | Х | Х | | Х |
| 52 | Х | Х | Х | | Х |
| 54 | | Х | | | |
| 61 | | Х | | | |
| 75 | Х | Х | Х | Х | Х |
| 80 | Х | Х | Х | | Х |
| 85 | х | Х | Х | | х |
| 96 | Х | | | | |
| 106 | | | | | Х |
| 107 | Х | Х | Х | | Х |
| 114 | Х | Х | Х | Х | Х |
| Total: | 13 | 14 | 12 | 2 | 1 |

| | 2003 Outplants- Maternal Lines Log | | | | | | | | | | | |
|-----------------|------------------------------------|--------|--------|--|--|--|--|--|--|--|--|--|
| MATERNAL LINE # | MAND-1 | MAND-2 | MAND-3 | | | | | | | | | |
| 6 | Х | | Х | | | | | | | | | |
| 19 | Х | Х | | | | | | | | | | |
| 21 | Х | Х | Х | | | | | | | | | |
| 37 | Х | Х | | | | | | | | | | |
| 43 | Х | | | | | | | | | | | |
| 46 | Х | | Х | | | | | | | | | |
| 52 | Х | | | | | | | | | | | |
| 75 | Х | | | | | | | | | | | |
| 85 | Х | | | | | | | | | | | |
| 107 | Х | | | | | | | | | | | |
| 114 | Х | | | | | | | | | | | |
| Total: | 11 | 3 | 3 | | | | | | | | | |

| | | 2004 Supp | lemental Ou | utplants- Mat | ternal Lines I | Log 2004 | | | |
|-----------------|--------|-----------|-------------|---------------|----------------|----------|-------|--------|-------|
| | | MAND- | MAND- | MAND- | | CARP- | CARP- | | COPR- |
| Maternal Line # | ORMOND | 1A | 1B | 1C | JAUMEA | 5B | 5C | LAGOON | Р |
| 6 | х | | | | | х | | х | |
| 19 | | | | | | х | х | х | |
| 21 | | х | | х | | | | х | |
| 37 | | х | х | | х | | | х | |
| 43 | | | х | | х | | х | х | |
| 46 | | | | | х | Х | Х | х | х |
| 52 | | | | | | Х | Х | | |
| 65 | | х | | | х | | Х | х | |
| 75 | х | | | | | | | х | |
| 77 | | х | х | | | Х | Х | х | |
| 79 | | | | | | | х | х | |
| 80 | | | х | | | | | х | |
| 85 | | | х | | х | | х | | |
| 106 | | | | | | | Х | х | |
| 107 | | | | | | | Х | х | |
| 114 | | | | | | | Х | х | |
| Feral | | | | | | | | | Х |
| unknown | | | | | | | | | Х |
| Total: | 2 | 4 | 5 | 1 | 5 | 5 | 11 | 14 | 1+ |