

DEPARTMENT OF FISH AND GAME

1416 NINTH STREET

P.O. BOX 944209

SACRAMENTO, CALIFORNIA 95814-2090

(916) 324-8348



August 2, 1990

Mr. Richard Spjut
World Botanical Associates
PO Box 2829
Laurel, MD 20708-0829

Dear Mr. Spjut:

Enclosed is the dried Astragalus agnicidus material that my assistant, Ms. Laurie Wickenheiser, discussed with you recently. As she indicated, we are interested in having a preliminary screening analysis conducted on this material in order to determine if this endangered species exhibits any potentially valuable biological activity. We understand that the first test will be a very preliminary assessment and that if potential value is detected, a more thorough chemical analysis will be needed. We also understand that an additional supply of plant material would be needed to conduct these more thorough test. We are willing to assist in making this material available if it is needed in the future.

We are interested in having A. agnicidus tested for several reasons. The fact that so many of the pharmaceuticals in use today were derived from plants, yet only a small fraction of all plant species have ever been tested for their potential benefits, makes us realize how little is known about our flora. As with many of the members of the genus Astragalus, this species is reported to have toxic properties (the specific epithet agnicidus is derived from the Latin for lamb killer), suggesting that it might contain compounds that could be diluted or modified into medicinally useful forms.

Another reason for our interest is that A. agnicidus is an extremely rare and endangered species about which very little is known. Until recently A. agnicidus was thought to be extinct as it had not been reported since the 1950s. Its rediscovery in 1987 was exciting and highly publicized. (See the enclosed articles.) This species is still extremely rare; its world wide distribution is limited to one population in California. Because it is vulnerable to chance events that could eliminate the sole population, A. agnicidus is officially listed as endangered under the California Endangered Species Act. The screening tests will certainly contribute to the scientific knowledge about this sensitive plant. In addition, the owners of the ranch where A. agnicidus grows have been extremely cooperative and are particularly interested in the potential medicinal values of the plant. Positive results also have the potential to benefit conservation efforts on a broader scale by demonstrating the need to protect natural diversity.

Mr. Richard Spjut

-2-

August 2, 1990

The A. agnicidus material that is enclosed was grown in a greenhouse at Humboldt State University (HSU), in Arcata, California, as part of a study examining the light requirements of this species. Unfortunately this study did not yield 100 grams of material to send for this preliminary analysis, but we hope that the enclosed is enough to get started. Since Ms. Wickenheiser spoke with you, we have found out that there is some additional material being used for another study that may be available to send. However, the individual who is working with it is out of town for a few weeks; we will contact you again as soon as we have more details on how much may be available and when the material will be harvested and dried. We thought it best to send you the material we have now and let you judge whether it is better to proceed with the testing with this sample or wait to combine it with any additional material that we may be able to send you. Please let us know what you think is most appropriate.

The seeds from which the enclosed material was grown were collected from the native population (Humboldt County, California) on August 6, 1989. They were sown in the HSU greenhouse in March 1990; the resulting plants were harvested in late May/early June 1990 and oven dried. This material includes shoots and roots, which we have kept separate in case this is useful for the analysis. We have also kept the samples separate by the light regime under which they were grown (no shade, medium shade, or maximum shade) in the event that this is useful. The plants were collected before they flowered, so the samples labeled "shoots" contain only stem and leaves.

Thank you very much for your assistance in having this endangered plant species screened. We look forward to learning about the results of the preliminary tests. Please feel free to contact me at the number listed above, or call Ms. Laurie Wickenheiser at (916) 327-0715 if you have any questions or need additional information.

Sincerely,



Ken Berg, Coordinator
Endangered Plant Program
Natural Heritage Division

Enclosures

cc: Ms. Laurie Wickenheiser
Endangered Plant Program

Ms. Lynn Lozier
The Nature Conservancy

Ms. Andrea Pickart
The Nature Conservancy

August 10, 1990

Dr. Ken Berg, Regional Coordinator
Endangered Plant Program, Natural Heritage Division
Department of Fish & Game
1416 Ninth Street
P.O. Box 944209
Sacramento, California 95814-2090

Dear Dr. Berg:

This is to acknowledge receipt of the following plant material of *Astragalus agnicidus* in six small paper bags as follows:

A5-R roots (Group A) No shade. 25 g.
B12-R roots (Group B) Medium shade. 10 g
C16-R roots (Group C) Maximum shade, 80-100% cover. 1.0-2.0 g.
A6-S shoots (Group A) No shade. 8 g.
B13-S shoots (Group B) 30-60% cover. 5 g.
C13-S shoots (Group C) Maximum shade. 1.0-2.0 g.

Due to the paucity of material, these samples will be combined into a single sample. The material has been forwarded to NCI for anti-AIDS screening, and possibly antitumor screening against as many as 60 cell lines, depending on the amount of extract obtained from the sample. I expect to have the AIDS screening data in one-two months, and the antitumor screening data within four months.

Active agents in plants are often present in minute quantities. If activity is discovered in your sample, an initial re-collection of 5 kg would be required. The fact that a sample exhibits biological activity in one or bioassays may have little significance unless the activity can be related to biologically active compounds. Because many of the NCI screens are relatively new, there is little information on the kinds of compounds that show activity. In the past, certain extraction procedures and bioassays have been sensitive to ubiquitous compounds considered to have little therapeutic potential. Isolation and identification of active compounds can require 20 kg or more; the fractionation process involves many steps that has to be guided by bioassay results, which plant material is consumed in the process.

Sincerely yours,

Richard Spjut, Director, Plant Explorer & Consultant

FAX/Phone/Recorder 301-498-5067

October 18, 1991

Dr. Ken Berg, Regional Coordinator
Endangered Plant Program, Natural Heritage Division
Department of Fish & Game
1416 Ninth Street
P.O. Box 944209
Sacramento, California 95814-2090

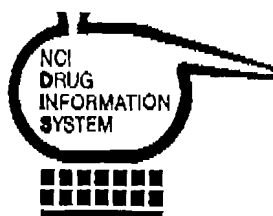
Dear Dr. Berg:

I am forwarding a copy of the test results for your samples of *Astragalus agnicidus*. The NCI decided to combine your sample parts into one sample, 52 g. This material was extracted and tested against HIV. The top graph indicates toxicity, and the lower graph shows response to HIV. No activity is evident, but toxicity is indicated at the higher doses.

Apparently no further tests were conducted due to the paucity of material. I hope these results will be of some use to you.

Sincerely yours,

Richard Spjut, Director, Plant Explorer & Consultant



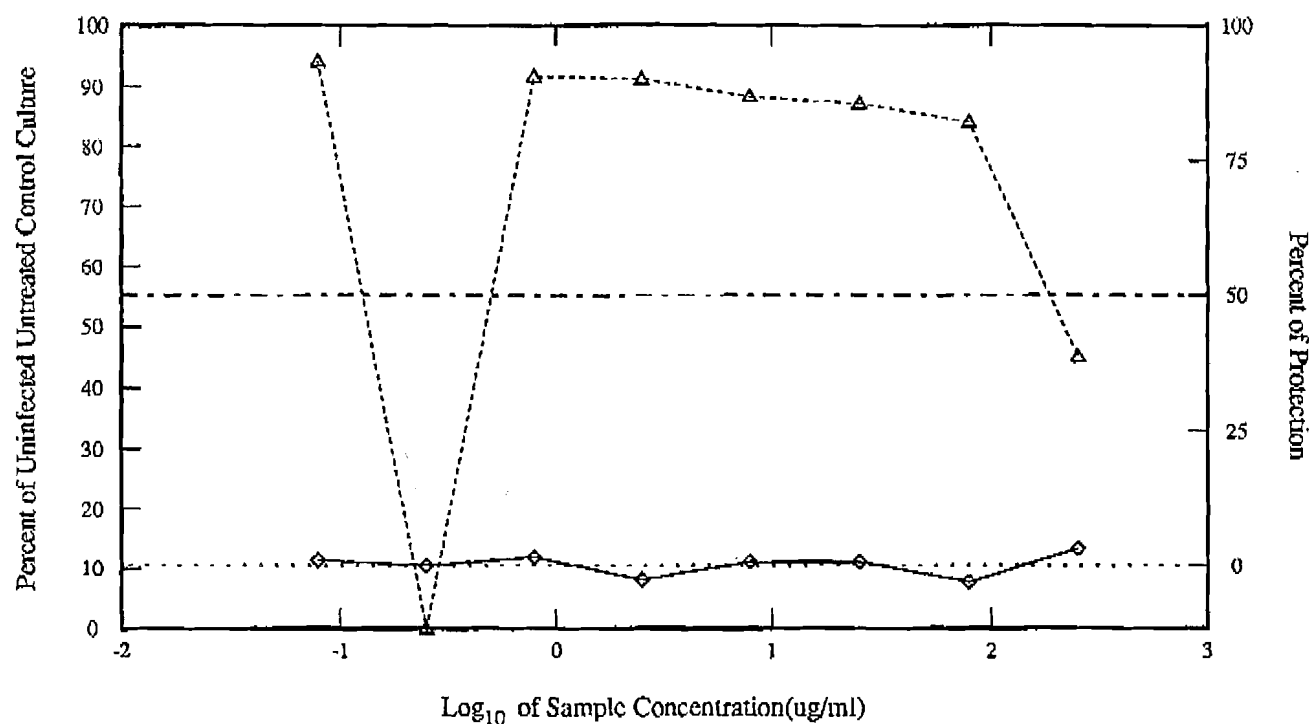
National Cancer Institute

Developmental Therapeutics Program

In-Vitro Testing Results

NSC: N 20845/ 1	Plate: 7397	Lab: 90	Assay:
Test Date: January 17, 1991	SSPL:	COMI:	
Report Date: October 11, 1991	Cell Line: CEM-1W	Solvent: DMSO < .25%	

IN VITRO ANTI-HIV DRUG SCREENING RESULTS



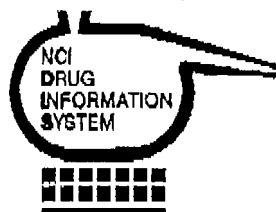
0%, 50% Reference Lines

Viral Cytopathic Effect

Infected Treated Culture

Uninfected Treated Culture

SUMMARY		DOSE	INFECTED RESPONSE	UNINFECTED RESPONSE
Index	Concentration	(ug/ml)	Percent of Control	Percent of Control
IC50 (ug/ml)	2.20×10^{-2}	7.90×10^{-2}	11.40	94.14
EC50 (ug/ml)		2.50×10^{-1}	10.46	0.00
TI50 (IC/EC)		7.90×10^{-1}	11.88	91.61
Conclusion		2.50×10^0	8.09	91.30
		7.90×10^0	11.09	88.33
		$2.50 \times 10^{+1}$	11.06	87.19
		$7.90 \times 10^{+1}$	7.68	84.09
		$2.50 \times 10^{+2}$	13.36	45.11



National Cancer Institute

Developmental Therapeutics Program

In-Vitro Testing Results

NSC: N 20845/ 1

Plate: 7417

Lab: 90

Assay:

Test Date: January 17, 1991

SSPL:

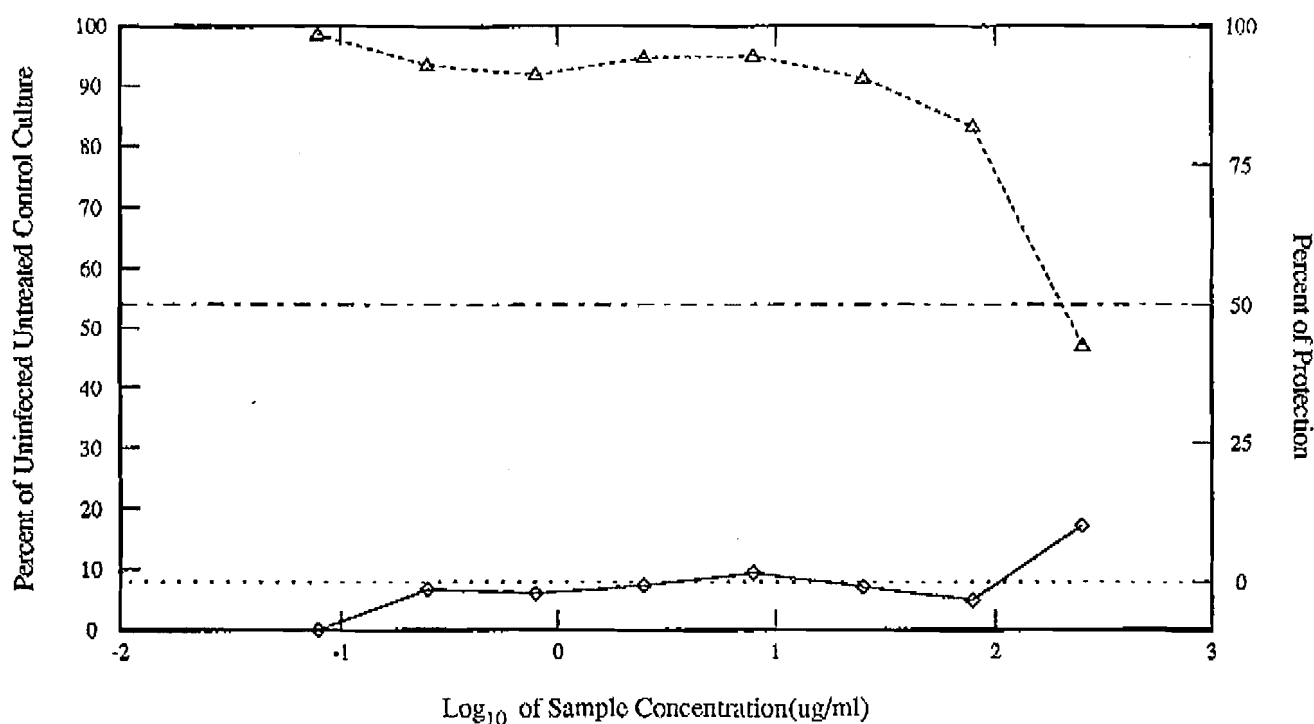
COMI:

Report Date: October 11, 1991

Cell Line: CEM-IW

Solvent: DMSO < .25%

IN VITRO ANTI-HIV DRUG SCREENING RESULTS



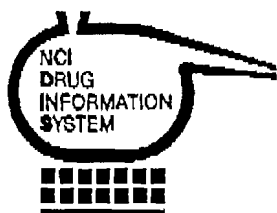
0%, 50% Reference Lines

Viral Cytopathic Effect

Infected Treated Culture

Uninfected Treated Culture

SUMMARY		DOSE	INFECTED RESPONSE	UNINFECTED RESPONSE
Index	Concentration	(ug/ml)	Percent of Control	Percent of Control
IC50 (ug/ml)	2.30×10^{-2}	7.90×10^{-2}	0.00	98.60
EC50 (ug/ml)		2.50×10^{-1}	6.62	93.44
TI50 (IC/EC)		7.90×10^{-1}	5.94	91.89
Conclusion		2.50×10^0	7.27	94.62
		7.90×10^0	9.29	94.98
		$2.50 \times 10^{+1}$	7.03	91.30
		$7.90 \times 10^{+1}$	4.84	83.11
		$2.50 \times 10^{+2}$	17.13	47.07



National Cancer Institute Developmental Therapeutics Program In-Vitro Testing Results

NSC: N 20846/ 1

Plate: 7396

Lab: 90

Assay:

Test Date: January 17, 1991

SSPL:

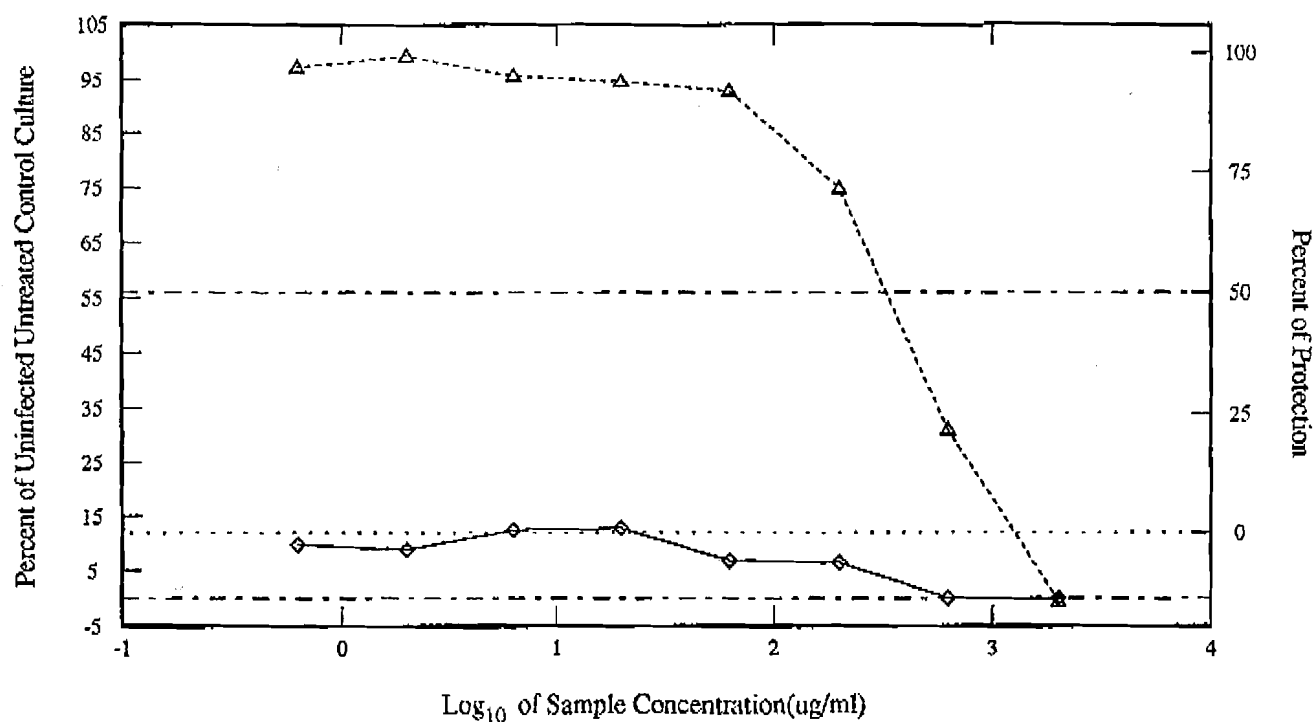
COMI:

Report Date: October 11, 1991

Cell Line: CEM-IW

Solvent: Saline < .9% (MEDIA)

IN VITRO ANTI-HIV DRUG SCREENING RESULTS



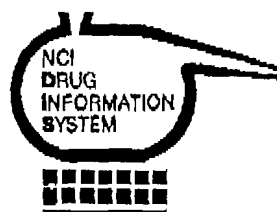
0%, 50% Reference Lines

Viral Cytopathic Effect

Infected Treated Culture

Uninfected Treated Culture

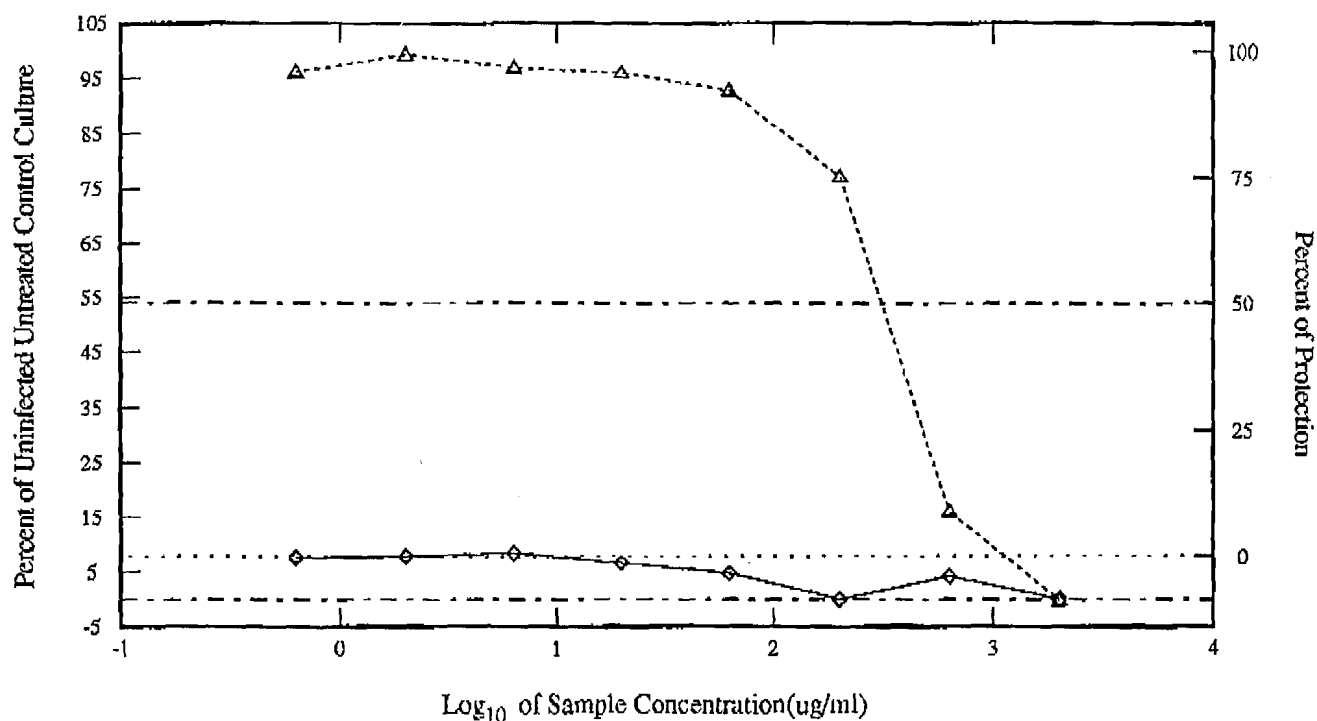
SUMMARY		DOSE	INFECTED RESPONSE	UNINFECTED RESPONSE
Index	Concentration	(ug/ml)	Percent of Control	Percent of Control
IC50 (ug/ml)	3.80×10^{-2}	6.30×10^{-1}	9.72	97.28
EC50 (ug/ml)		2.00×10^0	8.81	99.30
TI50 (IC/EC)		6.30×10^0	12.44	95.64
Conclusion		2.00×10^1	12.78	94.57
		6.30×10^1	6.82	92.80
		2.00×10^2	6.44	74.81
		6.30×10^2	0.00	31.12
		2.00×10^3	0.00	-0.63



National Cancer Institute Developmental Therapeutics Program In-Vitro Testing Results

NSC: N 20846/ 1	Plate: 7416	Lab: 90	Assay:
Test Date: January 17, 1991	SSPL:		COMI:
Report Date: October 11, 1991	Cell Line: CEM-IW		Solvent: Saline < .9% (MEDIA)

IN VITRO ANTI-HIV DRUG SCREENING RESULTS



0%, 50% Reference Lines

Viral Cytopathic Effect

Infected Treated Culture

Uninfected Treated Culture

SUMMARY		DOSE	INFECTED RESPONSE	UNINFECTED RESPONSE
Index	Concentration	(ug/ml)	Percent of Control	Percent of Control
IC50 (ug/ml)	3.30×10^{-22}	6.30×10^{-1}	7.60	96.21
EC50 (ug/ml)		2.00×10^0	7.81	99.30
TI50 (IC/EC)		6.30×10^0	8.45	97.06
Conclusion		$2.00 \times 10^{+1}$	6.63	96.03
		$6.30 \times 10^{+1}$	4.72	92.82
		$2.00 \times 10^{+2}$	0.00	77.20
		$6.30 \times 10^{+2}$	4.12	15.92
		$2.00 \times 10^{+3}$	0.00	-0.24

REDISCOVERY OF THE HUMBOLDT MILK-VETCH

by Ken Berg and Roxanne Bittman

The Humboldt milk-vetch (*Astragalus agnicidus*) historically has been known only from one site, a logged ridge on a ranch owned by the Tosten family south of Miranda in southern Humboldt County. During the early decades of this century the Tostens raised sheep. Everett Tosten was about nine years old when his father became concerned about the sheep-killing properties of the milk-vetch; lambs had been observed to acquire a taste for the milk-vetch and died soon after. The Tostens soon began a concerted effort to eradicate the plant from their ranch. Everett and his brother, Gordon, remember that the milk-vetch grew abundantly on the ridge above their house and that they spent several years pulling up the plants until they appeared to be gone.

Last September we participated in the rediscovery of the Humboldt milk-vetch, which had been presumed extinct. The story of its rediscovery and subsequent protection illustrates how the current conservation network in California works to identify, rank, and protect our rarest plants.

CNPS had placed the Humboldt milk-vetch on List 1A (plants presumed extinct in California) in its *Inventory of Rare and Endangered Vascular Plants of California* because it had not been reported since 1954. CNPS highlights plants that have not been seen or collected in the wild for many years in an attempt to bring them special attention. This strategy seems to be working. Twenty-nine taxa listed as presumed extinct in California have been rediscovered since publication of the first *Inventory* in 1974. The most recent of these were *Sibara filifolia* on San Clemente Island in 1986 and *Arabis hoffmannii* in 1985. Some of these rediscovered taxa are now the focus of active recovery programs, including *Cordylanthus palmatus*, *Dudleya traskiae* and *Lilium occidentale*.

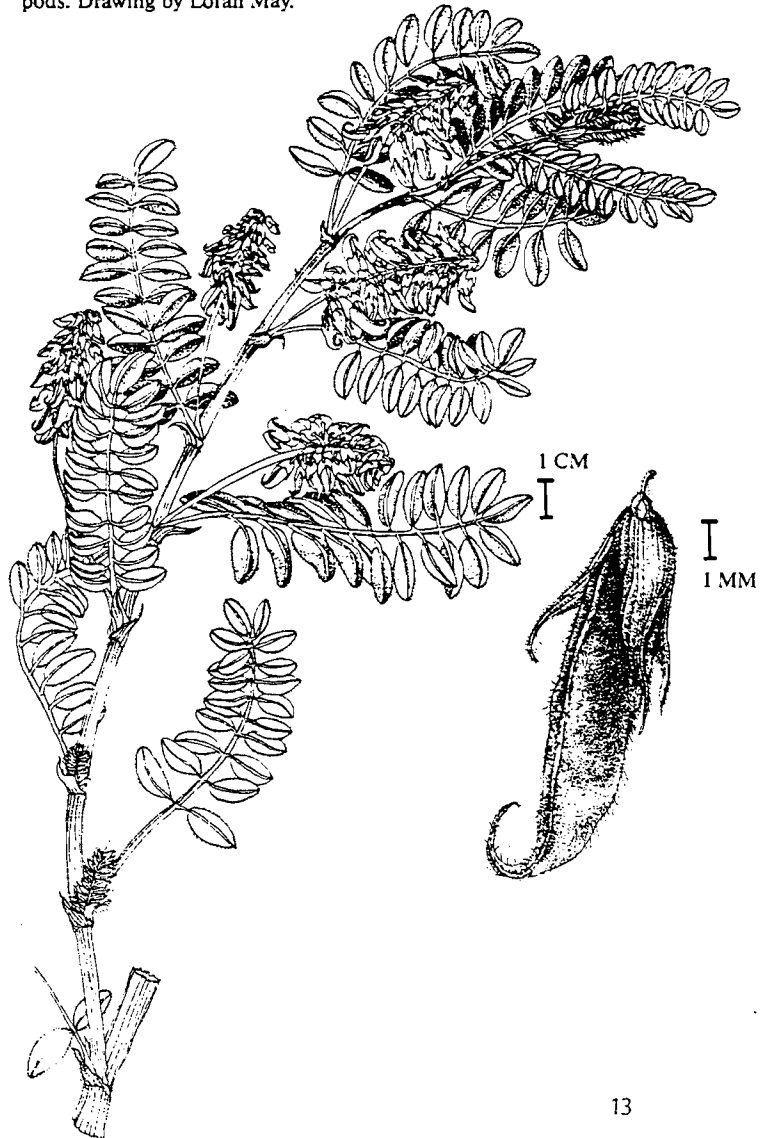
Was The Humboldt Milk-vetch Extinct?

The Natural Diversity Data Base (NDDDB), currently housed in the California Department of Fish and Game's Nongame Heritage Program, provided us with information to locate the Humboldt milk-vetch. The NDDDB is part of a nationwide network of natural heritage programs originally established by The Nature Conservancy (TNC) to inventory the rare biota in each state. Data on endangered plants, animals, and natural communities are available from NDDDB in the form of printouts of text or as map overlays that graphically display the distribution of rare elements for a particular area.

The NDDDB computer printout indicated that the milk-vetch was last seen on a private ranch in Humboldt County. It noted a herbarium collection to which we could refer and a legal description of the site to help us locate it. From the database information on the plant, we learned that the landowners had cooperated with visiting botanists during previous unsuccessful searches. It also noted that Robert Sutherland, a local conservation biologist, had been interested in the milk-vetch for a decade and had searched for it earlier.

We contacted Sutherland, who gained permission from the Tostens for us to visit. They generously invited

The Humboldt milk-vetch has straw-colored stems up to three feet tall and dense racemes of white flowers that develop into characteristic pods. Drawing by Loran May.





Gordon and Everett Tosten sign a voluntary protection agreement with Lynn Lozier (left) of The Nature Conservancy.

us to look anywhere on their ranch. The ridge where they remembered seeing the milk-vetch thirty years ago has grown back to dense mixed evergreen forest, typical of the region. From a cursory examination it didn't appear to be suitable for the milk-vetch, so we investigated other areas on the ranch with more open vegetation.

We knew from the published description that the plant could be nearly three feet tall with straw-colored stems. Although the August blooming period was long past, we were certain that if we found the milk-vetch, its persistent fruits would allow identification.

After searching for a couple of hours we were somewhat discouraged. We had covered a wide area composed of a mosaic of non-native grasslands and second-growth mixed evergreen forest. A history of heavy grazing, the low rainfall that year, and the lateness of the season presented grim prospects.

Toward the end of the day we returned to the historic site in hopes of finding some clearings or persistent openings. Just before dark we found a logging trail leading to an area where the Tostens had removed a Douglas-fir snag several years earlier; this had opened the canopy and scarified the ground. There in the clearings were small herbaceous plants resembling a milk-vetch. We counted fewer than twenty-five plants, and all appeared to be either young or badly browsed; the tallest one was only six inches high.

A trip to the Jepson and University of California herbaria in Berkeley confirmed our find. The plants we had found were almost definitely the elusive *Astragalus agnicidus*.

Protection Efforts Begin

In November, Sutherland and Berg returned to the Tosten Ranch with Lynn Lozier, Director of The Nature Conservancy's Landowner Notification Program in Cal-

ifornia. Lynn contacts private landowners who have significant rare plants or animals on their property and encourages them to protect the sites. These voluntary agreements grant TNC access to the sites for annual monitoring and request that the landowners present notification of any pending change in land use or ownership.

The Tostens were enthusiastic about protecting the site. They signed a voluntary protection agreement and gave us permission to fence a portion. They no longer raise sheep, and their cattle don't need the area where the milk-vetch grows. The ranch is now run primarily for timber production, and the Tostens operate a small sawmill. These activities seem to be compatible with protection of the milk-vetch. Properly managed logging could create new open areas that benefit the rare plant.

During a subsequent visit to the ranch, many additional seedlings and young plants were found in a nearby clearing. The total number of individuals of the rare milk-vetch now exceeds one hundred.

Studies of the milk-vetch can begin after the population is fenced. Its population biology and toxic properties warrant further research. About half of the pharmaceuticals in use today come from wild organisms, primarily plants. Often poisonous plants contain active compounds that, when modified or diluted, have medicinal properties. It is encouraging that we still have the opportunity to survey the rare milk-vetch for its potential uses in medicine.

While the future of the Humboldt milk-vetch appears promising, there are still dozens of California rare plants missing in action. We challenge our colleagues to take to the trail in search of the showy Indian clover (*Trifolium amoenum*), Merced pennyroyal (*Monardella leucocephala*), or the more than thirty other rarities presumed extinct in California.

For information about rare plants contact the CNPS Botanist or NDDB by writing to the Natural Diversity Data Base, California Department of Fish and Game, 1416-9th Street, Room 1225, Sacramento, CA 95814.

Gordon Tosten and Robert Sutherland inspect a seedling of *Astragalus agnicidus* that became established after woodcutting opened a light gap in the second-growth forest.

