A Preliminary Investigation of the Seed Bank of *Astragalus agnicidus*

Prepared by: Robin Bencie
January 4, 1995

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Department of Fish and Game
Endangered Plant Program
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Introduction

In 1987, after 33 years of presumed extinction, Astragalus agnicidus was rediscovered at its only known historical location (Berg & Bittman 1988). The Humboldt milk-vetch had survived via a dormant seed bank which was induced to germinate by logging activities. Since that time, The Nature Conservancy has monitored the population dynamics and studied the reproductive biology of this narrow endemic. The seed bank obviously has a pivotal role in the life cycle of this species, and thus, must be studied in conjunction with other aspects of A. agnicidus's reproductive biology to develop a conservation management plan which mimics its natural life history.

The entire population of A. agnicidus is confined to approximately 8 acres on a ridge just north of Bear Buttes in southern Humboldt county. Currently, there are two major subpopulations and many scattered individuals located in open areas within the Douglas fir/Tan oak forest. Attempts at locating other populations on the Tosten Ranch have been unsuccessful, and it appears that neighboring ranchers have not encountered the milk-vetch either (Berg & Bittman 1988, Barneby 1957). During the late 1930's, the Tosten family had successfully eradicated the milk-vetch to save their sheep from poisoning. Interviews with the Tostens reveal that A. agnicidus once occurred in dense patches which were located underneath the canopy gaps between old-growth Douglas fir trees (1992). Apparently, these large patches formed a nearly contiguous population across the ridge.

A. agnicidus, a member of the family Leguminosae, is a short-lived perennial with an estimated life span of 5-10 years. The plants are suffrutescent and often appear weedy as they produce many long, often decumbent, stems in a basal rosette fashion. Each stem produces from 5-8 densely compacted racemes which arise singly from the leaf axils. These inflorescences may have10-40 flowers each. With an average seed set of 5 per pod, even a small population of mature individuals has the potential to produce an enormous number of seeds throughout its lifetime. These seeds, like many other members in this family, have an impermeable seed coat which allows them to remain viable in the soil for long periods of time (Baker 1989). The historical description of the population, coupled with observations of robust fruit set, suggest that A. agnicidus's seed bank may be extensive.

Previous studies have been aimed at characterizing A. agnicidus's habitat requirements. In germination experiments, Hiss found that scarification followed by stratification resulted in an 88.5% germination rate. A complex germination regime is one adaptation which enables plants to survive long periods of unfavorable environmental conditions (Thompson 1992). The seeds may remain dormant for many years awaiting the correct sequence of environmental cues which trigger germination. Another study showed that A. agnicidus requires maximum light exposure for optimum growth and development (Enberg 1990). The results of these two studies are indicative of an early successional species which survives the temporal gap between disturbances by persisting in the soil in a dormant phase (Thompson 1992). Seed

banks from these types of species tend to have their peak germination period immediately, followed by a gradual decline. However, some seeds may remain dormant, yet viable, for many years (Thompson 1992).

One proposed long-term management plan for A. agnicidus is to create new subpopulations through selective logging activities in areas where a seed bank is known to occur (Pickart et al. 1991). These subpopulations would be transient both temporally and spatially, but always allowed to complete their life cycle and the gaps to revert back to forest. This scenario maintains a continual above-ground population. An alternative management plan is to simply allow the current subpopulations to complete their life cycle and let the species be preserved in the seed bank until naturally regenerated (TNCC 1993). In order to implement and rely on either management plan, the seed bank must be accurately described. As the population matures, there is a noticeable decline in the number of seedlings and reproductive individuals (TNCC 1993). Thus, study into the regeneration strategy of A. agnicidus and implementation of an appropriate conservation management plan are immediate concerns.

Objectives

This study comprises the initial examination of A. agnicidus's seed bank. Therefore, three primary objectives were established and each was met in the following chronological order:

1) Determine an accurate, yet economical, soil core size to use as the sampling unit. This includes the diameter and the depth of the soil core.

2) Establish a methodology for seed extraction that is appropriate for this species.

3) Estimate the seed bank density, any microsite distribution patterns, and the viability of the seeds.

This study was partitioned into two sampling phases in order to efficiently meet these objectives. Potentially, the results could provide sufficient data to meet the ultimate goal of the next stage of seed bank research: to develop a large-scale sampling strategy which can be used to reliably locate areas where the seed bank exists.

Pilot Sampling Phase

The pilot sampling was designed to determine the soil core dimensions that would provide the most precise estimate of the seed bank density for the most efficient output of labor. While sampling the largest volume of soil is the most accurate, the

labor and time required to process each sampling unit necessitates taking the smallest core size possible. The dimensions of these pilot soil cores were selected based on methods and results from many seed bank studies. In a study of mature forest habitats, 67% of the viable seeds were found in the top 5 cm of soil (Kramer 1987).

These initial soil samples were also used to establish the most appropriate method for extracting seeds. The most common method for determining species composition and estimating density of the soil seed bank is the direct greenhouse germination method (Gross 1990). Soil samples are spread thinly in flats and placed in the greenhouse until identification of emerging seedlings can be made. Due to the complex germination requirements of A. agnicidus, and also the greenhouse space and stratification time required, this method was dismissed. This method would have underestimated the seed bank density if some viable seeds did not receive adequate scarification to break dormancy. Instead, a more accurate, but potentially more labor intensive, method of sieving and direct counting under a microscope was employed.

Many possible locations for the sampling sites were identified with the consultation of one of the landowners, Mr. Everett Tosten. These were areas that he remembers being dense with A. agnicidus and are located intermediately between large burned stumps. From these, two sampling sites were chosen and are labeled as Plots 1 & 2 in Figure 1.

Methods

Plot 1 is located approximately 150' north of Exclosure A, adjacent to a rock outcrop at the top of a 50' slope. A 7x7 m ($49m^2$) area was divided into 49 $1m^2$ quadrats. From these, 30 quadrats were randomly selected as sampling points. One sampling unit was taken from the southwest quadrant of each sampling point.

Each sampling unit was composed of a 2" diameter core nested within a 4" diameter core (Figure 2). This cylinder was secondarily divided into three depth intervals: duff layer, 0-3", and 3"-6". From this factorial design, 6 possible diameter x depth classes were created. However, the 2" diameter x 3"-6" depth class was considered the least likely to contain seeds, and therefore, was not examined in order to reduce sampling costs (Table 1). Thus, 5 separate sample cores were obtained from each sampling unit yielding a total of 150 sample cores from Plot 1. Additionally, two control sampling units (totaling 10 sample cores) were taken from Exclosure B in areas where robust plants had died the preceding winter. Seeds from the previous seed rain would provide a gauge for the successfulness of the seed extraction method.

All samples were placed in a plant drier until thoroughly dried before beginning the extraction process. Each sample core was first rolled-out under a heavy glass jar in order to crush soil aggregates and thus facilitate the sieving process. The soil was then sieved to a particle size between 1 and 2 mm with a sieve pan shaker. This sediment was rinsed thoroughly of residual fine silt and then placed in a petri dish for

direct counting of seeds under the microscope. Before processing any of the pilot soil samples, practice cores were created by placing previously collected seeds in soil. These practice cores were sieved and the sediment examined. All seeds were successfully retrieved.

Results

A total of 5 seeds was found in the 150 sample cores which were taken from Plot 1 (Table 1). These seeds were found within the 4" diameter core in the top 3" of soil (LT), with the exception of 1 seed found in the LD size class. The majority of seeds in both the control and pilot samples were captured by the LT size class. Counts for the 4" diameter classes included seeds found within the corresponding 2" diameter class. In a strict statistical analysis, this sampling design could have shown whether there was a significant difference in the number of seeds between size classes. A nonsignificant p-value would have suggested that processing a smaller core is as accurate, but more economical, than processing a larger one. However, with such a few number of seeds found and some classes without any seeds, such an analysis was unnecessary.

The number of seeds retrieved from the control cores illustrates that this method of seed extraction is reliable. Fortunately, A. agnicidus seeds are a much darker brown color than the red color of the soil particles, and so were readily seen during the microscope viewing. The only other type of seed found in the soil samples is also from the family Leguminosae. From the 150 sample cores, 112 of these seeds were retrieved . Initially, these seeds were confused with A. agnicidus seeds, but have been tentatively identified as Lotus micranthus. This has not yet been tested by germination, but the species occurs commonly in disturbed areas nearby.

Table 1. Number of A. agnicidus seeds found per size class in the pilot sampling phase. Class dimensions are given as diameter" x depth". For each class, n=30 for Plot 1 and n=2 for control cores. Symbols: B=bottom, D=duff, L=large, S=small, T=top. '*' = Count includes seeds from the corresponding 2" nested core. '**' = Total number of seeds found in control cores.

Size Class	Symbol	Plot 1	Control
2" x Duff	SD	0	10
4" x Duff *	LD	1	16
2" x 0-3"	ST	0	3
4" x 0-3" *	LT	4	32
4" x 3-6"	LB	0	5
Total		5	53 **

Preliminary Sampling Phase

The preliminary sampling phase was conducted in order to collect additional data for estimating the seed bank density and any microsite distribution patterns. After assessing the results from the pilot sampling phase, the LT size class was chosen as the most appropriate to use as the sampling unit for the second sampling site. Many seed bank studies report that for a given volume of soil, better precision is achieved if a large number of small sampling units is taken rather than a small number of large sampling units. A larger sample size reduces the sampling variance which increases the precision of the estimate of the mean (Benoit et al. 1989).

Methods

Plot 2 was established approximately halfway between Exclosure B and C in a shallow ravine (Figure 1). The same protocol of sampling and seed extraction as followed in the pilot sampling phase was employed. A total of 30 cores, each 4" in diameter from the top 3" of soil (618 cm^3), was taken from Plot 2.

For both sampling phases, the time required to complete each stage of seed retrieval was recorded. This data is to be used to estimate costs for future seed bank studies. The average time per sample core (LT size class) totaled 81 minutes, of which an average of 54 minutes for sieving and 27 minutes for microscope viewing was required. Initially, less time was spent sieving, however, this resulted in an equally greater time (and eye strain) when rinsing and examining the sediment. This data does not include the time required to actually collect the samples from the field.

Seed Bank Density

In Plot 2, a total of 3 seeds was found. The 4 seeds in the LT size class from the pilot sampling phase were pooled with these results to give a total of 7 seeds retrieved from 60 samples. Using the standard formulas for simple random sampling methods, this gives an average of .12 seeds per 81 cm^2 of soil (the surface area of each core) with a standard deviation of .37 and a standard error of .0478 (Scheaffer 1990). For the total area sampled of 98 m^2, there are 12,054 units of 81 cm^2. Therefore, the estimate of the seed bank density is 1,446 seeds $\pm$ 1,205 or [241, 2651] seeds (or equivalently, 723 seeds $\pm$ 603 or [120, 1326] seeds per 49 m^2 plot). These are 95% confidence intervals which signifies that there is a 95% probability that a random sample drawn from this population will generate confidence intervals which include the true population mean (Sokal & Rohlf 1987).

Microsite Distribution Pattern

Due to the small number of seeds found, it was not possible to make any general statements about the microsite distribution of seeds. The seeds were found in cores that were distant from each other and only one core contained two seeds. When A. agnicidus pods are mature, they forcefully open by recoiling the legume valves in an attempt to disperse seeds away from the parent plant. This could act to scatter the seeds within a short radius, but may still result in a clumped pattern when viewed from a larger perspective. There have been observations of caching of pods by rodents which may also contribute to a clumped distribution pattern.

Seed Viability

All 8 seeds found from both sampling phases were subjected to the germination protocol established by Hiss. All seeds germinated and seemed to grow as well as seeds collected in recent years. These seedlings were grown for approximately 4 weeks.

Conclusions

The core size and method of seed extraction established in this study appear to be sufficient for A. agnicidus. However, the amount of processing time needed per sample prohibits taking as many samples as would be required to attain a preferred precision in the estimate of the mean seed density. In this study, the accuracy level is only 83%. Using a standard deviation of .37, a sample size of 447 would be needed to reach an accuracy level of 30%.

General guidelines for a sample size that results in estimates with a precision of 20-30% have been attempted in other seed bank studies (Benoit et al. 1989, Gross 1990). For seed distributions which are expected to be aggregated, sample sizes greater than 100 are typically recommended. For precision estimates near 20%, a sample size ranging between 100 and 200 samples may be needed when the expected mean seed density is between 1 and 5 seeds. Due to all the factors which influence the density estimate, i.e., species abundance, distribution, core size, and standard error of the mean, it is difficult to rely on any one suggested sample size. The primary concern is to balance the greater accuracy gained from increasing the sample size with the substantial increase in sampling effort.

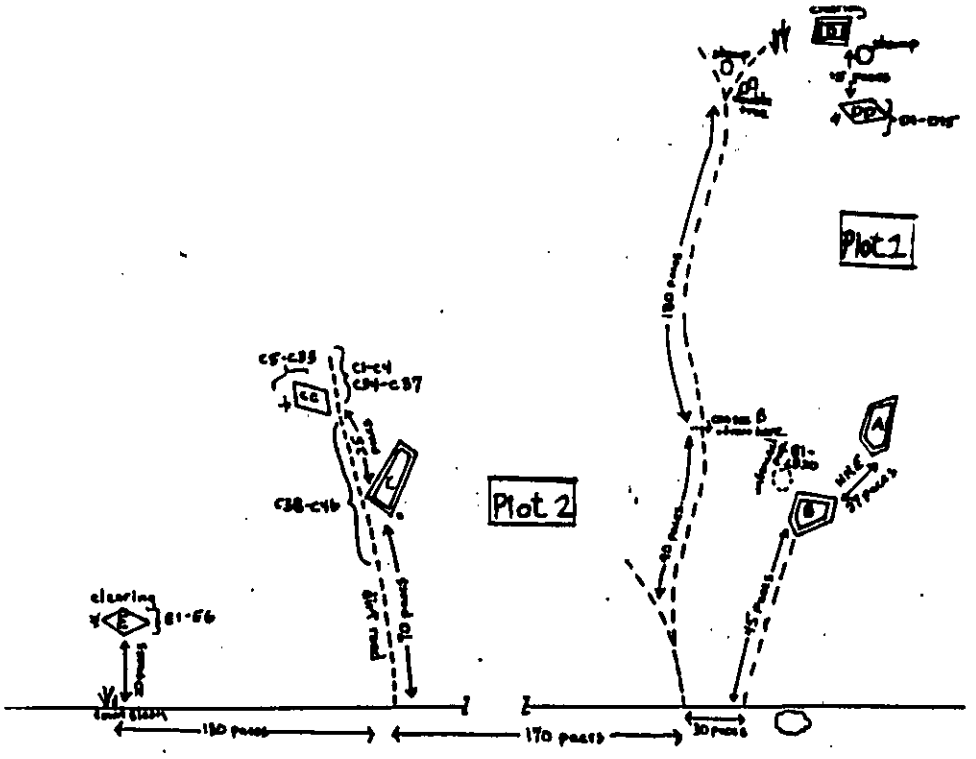

Figure 1. Map of A. agnicidus subpopulation locations (lettered exclosures). Plots 1 & 2 are seed bank sampling sites. (Steele 1991)

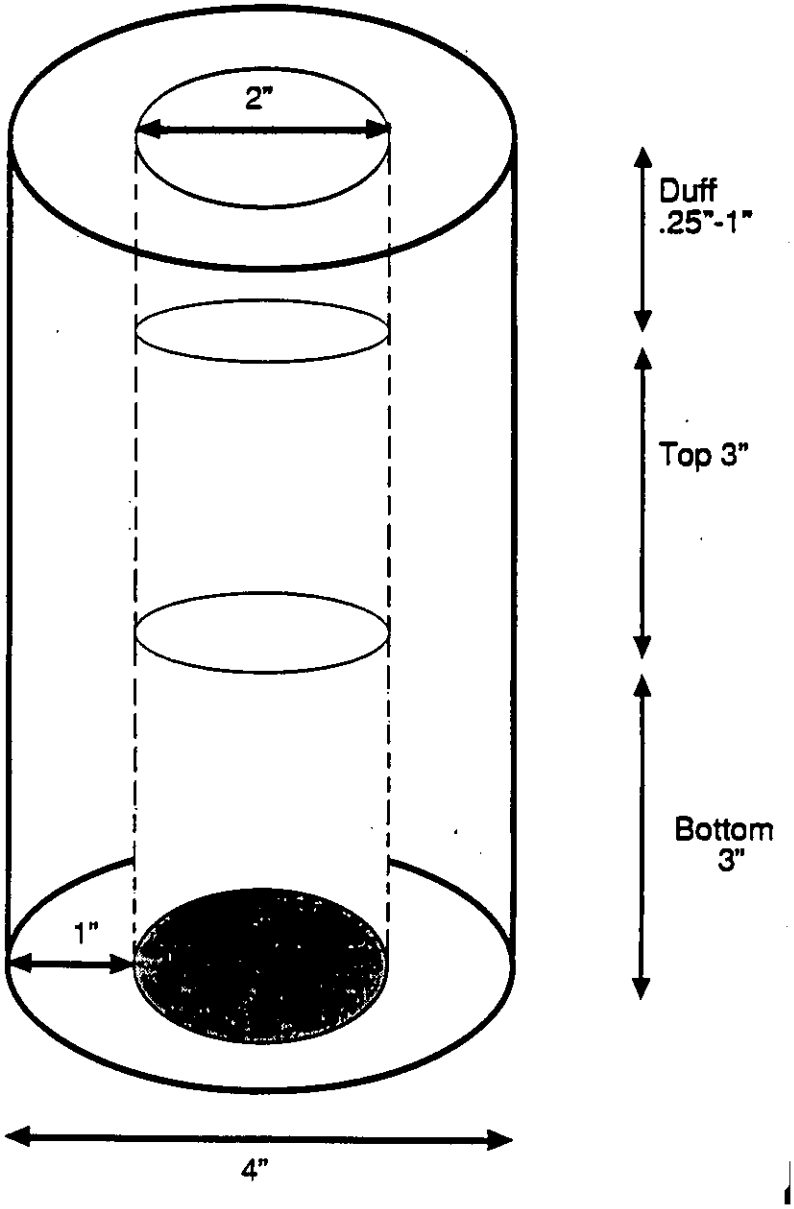

Figure 2. Diagram of soil core sampling unit for the pilot sampling phase.

References

Baker, H.G. 1989. Some aspects of the natural history of seed banks. pp. 9-12. In: M.A. Leck, V.T. Parker and R.L. Simpson, eds., Ecology of Soil Seed Banks. Academic Press, San Diego, CA. 462 pp.

Barneby, R.C. 1957. Astragalus agnicidus, a new locoweed from Humboldt County, California. Madrono 14(2):37-40.

Benoit, D.L., N.C. Kenkel, and P.B. Cavers. 1989. Factors influencing the precision of soil seed bank estimates. Can. J. Bot. 67:2833-2840.

Berg, K. and R. Bittman. 1988. Rediscovery of the Humboldt milk-vetch. Fremontia 16:13-14.

Enberg, A. 1990. The effects of shading on the emergence, growth, and development of the rare and endangered California plant species Astragalus agnicidus. Unpublished report. Humboldt State University, Department of Biology, Arcata, CA. 10 pp.

Gross, K.L. 1990. A comparison of methods for estimating seed numbers in the soil. J. of Ecol. 78:1079-1093.

Hiss, A.E. 1991. A study of the germination requirements of Astragalus agnicidus. Unpublished report. The Nature Conservancy of California, Arcata, CA. 9 pp.

Kramer, N.B. and F.D. Johnson. 1987. Mature forest seed banks of three habitat types in central Idaho. Can. J. Bot. 65:1961-1966.

Pickart, A.E., A.E. Hiss, and A.W. Enberg. 1992. Return from extinction: recovery of the Humboldt milk-vetch. pp.255-261. In: H.M. Kerner, ed., Proceedings of the Symposium on Biodiversity of Northwestern California, October 28-30, 1991, Santa Rosa. University of California, Wildland Resources Center Report No. 29, Berkeley, CA. 283 pp.

Scheaffer, R.L., W. Mendenhall, and L. Ott. 1990. Elementary Survey Sampling. 4th Ed., PWS-Kent Publishing Company, Boston. 390 pp.

Sokal, R.R., and F.J. Rohlf. 1987. Introduction to Biostatistics. 2nd Ed., W.H. Freeman and Company, New York. 363 pp.

Steele, K. 1991. A population survey of the rare Humboldt milk-vetch and recommendations for management. Unpublished report. The Nature Conservancy of California, Arcata, CA. 6 pp.

Thompson, K. 1989. Functional ecology of seed banks. pp. 231-258. In: M. Fenner,

ed., Seeds, the Ecology of Regeneration in Plant Communities. Redwood Press Ltd., Melksham, UK.

TNCC (The Nature Conservancy of California). 1993. Monitoring report for Astragalus agnicidus. Unpublished report. The Nature Conservancy of California, San Francisco, CA. 8 pp.

Tosten, Everett. 1992. Personal communication with R. Bencie on December 18 at population site.