

**Reintroduction of *Amsinckia grandiflora* to Three Sites  
Across its Historic Range**

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### Abstract

Natural populations of *Amsinckia grandiflora* Kleebl. ex Gray are known from three locations in Alameda and San Joaquin counties, California. Two lie within Lawrence Livermore National Laboratory's Site 300 and one was discovered in 1991 at Carnegie Canyon. The Site 300 populations are small and have not been able to grow during the last 5-10 years. The Carnegie Canyon population, however, is large, relatively undisturbed and apparently self-sustaining. The recovery plan, drafted by the U.S. Fish and Wildlife Service, calls for the establishment of three new *Amsinckia* populations (each with 2500 individuals) within historic range and the enhancement of the Droptower population at Site 300 in order to significantly reduce the probability of extinction. The present study is part of an overall recovery effort to create and enhance those populations.

Using methods developed on this and other endangered plants, Pavlik (1990) succeeded in creating a new, vigorous population of *Amsinckia grandiflora* within its historic range. An experimental design with demographic monitoring was used to test the effects of burning, hand clipping and a grass-specific herbicide on the fates of 3,460 *A. grandiflora* nutlets. A total of 1101 plants survived to reproduce at the Lougher Ridge site in Black Diamond Mines Regional Preserve (Contra Costa County). The study concluded that new populations of *Amsinckia grandiflora* could be created in mesic annual grassland if the habitat is treated to minimize competition with annual grasses. The present study employed those methods and results to; 1) reintroduce *Amsinckia grandiflora* to three sites across its historic range, and 2) examine the effects of competition between *Amsinckia* and annual grasses in habitats that differed in annual grass cover.

Regarding the recovery effort in general, the current project resulted in the creation of two new, growing populations of *Amsinckia grandiflora* at the northern and southern extremities of historic range. To the north, at Black Diamond II (BD II), 288 plants survived to produce 11,282 nutlets in May 1991. This new population is predicted to grow by 40% in the coming year. At Connolly Ranch (CR), 580 plants produced 17,302

nutlets and should grow by 20% if protected from grazers during the spring. Although 374 plants survived to reproduce at Los Vaqueros (LV), they produced only 3,202 nutlets. The LV population is expected to decline by 65% or more in the coming year.

The hypothesis that annual grass cover has no effect on the demographic performance of *Amsinckia grandiflora* was accepted with respect to *in situ* germination. It was rejected, however, with respect to survivorship to reproduction, which was significantly reduced by the presence of annual grasses. Therefore, annual grass cover must be controlled in order to promote population growth and stability of this highly endangered plant. These findings were also observed during the 1989-90 reintroduction at Lougher Ridge (Pavlik 1990).

Unlike the 1989-90 reintroduction at Lougher Ridge, annual grasses did not significantly reduce mean plant size, maximum plant size or nutlet production at any of the reintroduction sites. This discrepancy has been ascribed to the unusual pattern of rainfall and temperature in 1990-91. The winter drought and heavy rains of March, in particular, seem to have prevented intense competition during the period of maximum growth and reproduction. These observations have direct management implications. Years with below-normal rainfall in October, November, December, and January (such as 1990-91) would not require the manipulation of *Amsinckia* populations with fire or herbicide treatments. However, years with near- or above normal rain in late fall (such as 1989-90) would require the manager to manipulate the population by treating with an appropriate herbicide a few weeks after grass emergence (early winter).

The internal water status and growth of *Amsinckia* plants were significantly reduced by annual grass cover, but not to levels that impaired nutlet production. In years without heavy spring rains, however, it is likely that competition for water is an important determinant of *Amsinckia* performance in mesic annual grassland.

The hypothesis that demographic and physiological performance will not vary with annual grass cover between reintroduction sites was rejected. Annual grass cover was highest at BD II, intermediate at LV, and lowest at CR. Correspondingly, reductions in survivorship occurred at BD II and LV, but not at CR. In addition, *Amsinckia* plants in control plots at BD II had significantly lower xylem water potentials when compared to treated plots, demonstrating the effectiveness of the dense grass canopy in reducing soil water availability at that site. With regards to future reintroductions these data indicate that choosing sites with low grass cover is more important than choosing sites based on

annual rainfall or slope.

The hypothesis that grazing mammals will not have significant impacts on *Amsinckia* during reintroduction was rejected. Both deer and cattle were shown to significantly decrease the density and survivorship of *Amsinckia*, either by trampling or browsing the plants directly. Cattle, however, were especially detrimental because they reduced plant size and nutlet production during the critical April to May period of reproduction. It was clear from the results at CR (where the fenced population was predicted to grow by 20% in the next year while the exposed population would decrease by 53%) that livestock and *Amsinckia* were incompatible despite the abundance of alternate feed and the presence of fiddleneck alkaloids. If the fenced population does grow in the coming years (and it should), a conservation easement or the development of post-dispersal stocking schedules need to be explored.

### Acknowledgements

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## Reintroduction of *Amsinckia grandiflora* to Three Sites Across its Historic Range

Bruce M. Pavlik

### Introduction

Natural populations of *Amsinckia grandiflora*<sup>1</sup> Kleeb. ex Gray are known from three locations in Alameda and San Joaquin counties, California. Two lie within Site 300 (~ 24 km east of Livermore) and one was discovered in 1991 at Carnegie Canyon (~2 km SE of Site 300). The Site 300 populations are small (92 plants at the Droptower site in 1991, 29 at the Draney Canyon site) and have not been able to grow during the last 5-10 years. The Carnegie Canyon population, however, is large (~3200 in 1991), relatively undisturbed and apparently self-sustaining. The recovery plan, drafted by the U.S. Fish and Wildlife Service, requires the establishment of three new *Amsinckia* populations within historic range, and the enhancement of the Droptower population at Site 300 in order to significantly reduce the probability of extinction. The present study is part of an overall recovery effort to create and enhance those populations.

Prior to 1990, there had been no successful, scientific attempts to recover an endangered plant species in California and perhaps the nation. Recovery requires the creation of new, self-sustaining populations and the enhancement of existing natural populations. Self-sustaining populations are those which are able to complete all life history phases (seed (or propagule), seedling, juvenile, adult, parent) and, therefore, have the potential for growth and long-term persistence. Havlik's project on *Holocarpha macradenia* (1987) was a relocation of an existing population to an adjacent, protected site and cannot be considered recovery. Furthermore, no quantitative monitoring was done and so no evaluation of the project's efficiency or long-term effectiveness is possible. Olwell et al. (1987, 1990) outplanted 150 greenhouse-grown *Pediocactus knowltonii* raised from cuttings, but new juvenile plants have not been produced. Although outplanting creates an "instant population", it does not provide crucial information on a major demographic hurdle - the transition from

<sup>1</sup> *Amsinckia grandiflora* will often be referred to by its generic epithet.

seed to established juvenile. Creating populations by sowing seeds does carry the risk that valuable propagules and genes may be lost to intense and often unpredictable sources of natural mortality. Such an approach must be taken, however, if recovery efforts are going to produce long-lived, self-sustaining populations instead of short-lived, rare plant gardens. At the same time, the reintroduction must have an experimental design, with demographic monitoring to generate data for evaluating failure or success.

Using methods developed on this and other endangered plants, Pavlik (1990) succeeded in creating a new, vigorous population of *Amsinckia grandiflora* within its historic range. That study documented the genetic structure of nutlet source populations, conducted experiments to determine the demographic effects of fire, grass clipping and a grass-specific herbicide, and developed techniques for closely monitoring the new population. After sowing 3,460 nutlets in a total of 20 experimental plots, the number of germinules produced during the 1989-90 growing season (November to April) was large (1774) and many (1101) survived to reproduce. From these plants, an estimated 35,800 nutlets were produced, indicating that the population had a high potential for growth during the next year. The study concluded that new populations of *Amsinckia grandiflora* could be created in mesic annual grassland if the habitat is treated to minimize competition with annual grasses.

Given these results, the present study attempted to; 1) reintroduce *Amsinckia grandiflora* to three sites across its historic range, and 2) examine the effects of competition between *Amsinckia* and annual grasses in habitats that differed in annual grass cover. The hypotheses to be tested are presented in Table 1.

Table 1. Statement of the basic hypotheses to be tested during the reintroduction of *Amsinckia grandiflora* to three sites across its historic range.

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- a) Annual grass competition has no effect on the demographic or physiological performance of *Amsinckia grandiflora*.
  - b) Demographic and physiological performance will not vary with annual grass cover between reintroduction sites.
  - c) Grazing mammals, including livestock, have no significant impact on *Amsinckia grandiflora* during reintroduction.
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## Methods and Materials

### Site Selection

The process of selecting reintroduction sites for new *Amsinckia* populations was described in detail by Pavlik and Heisler (1988). Many factors were taken into consideration, some ecological (macroclimate, soil, exposure, community associates, habitat size and degree of disturbance), and others logistic (land use history, road access, property ownership). The selection of Stewartville 1 for the 1989-90 project was based on its high potential as habitat (mesic grassland climate on or near soils of the Altamont-Fontana complex), its public status as part of the East Bay Regional Park system (it lies within Black Diamond Mines Regional Preserve), and the fact that it is within the historic range of *Amsinckia grandiflora*. The exact location of the reintroduction plot (the microsite) was determined from additional field and laboratory studies (Pavlik 1990). This microsite was located on Lougher Ridge (Figure 1), which came to support a population of 1101 reproductive plants in March 1990.

The successful reintroduction at Lougher Ridge (LR) validated the criteria for selecting reintroduction sites. Using the survey of Pavlik and Heisler (1988), only four additional field days were required to select three locations for the 1990-91 project (Figure 1). Verbal and written inquiries (see Appendix A for sample letter) were made to the landowners in order to secure permission to conduct the reintroductions on their respective lands. One new site, at the north end of historic range of *Amsinckia*, was to be located within Black Diamond Mines Regional Preserve east of Markley Canyon (Contra Costa County). That site (Black Diamond II, BD II), some 1.4 km east-southeast of LR, is under the jurisdiction of the East Bay Regional Park District. The successful 1989-90 project allowed the 1990-91 project to proceed with verbal permission from Kevin Shea (Chief, Land Stewardship) and Roger Epperson (Park Ranger) of the District. A verbal agreement regarding the southernmost of the three new sites was also reached with Pat Connolly, owner and principal operator of Connolly Ranch (CR). Finally, a formal written agreement (see Appendix B) was reached regarding the middle site (Los Vaqueros, LV) with the Contra Costa Water District (CCWD). It was negotiated by Ann Howald (CDFG Endangered Plant Program) with John Gregg and Terry Cox of the CCWD and approved by the Board of Directors on 7 November, 1990.

Long-term management needs for the reintroduction sites will be determined after an evaluation of the performance of the new populations over several years. The possible use of a conservation easement to protect the CR site was discussed with Mr. Connolly, who indicated he would like to have more information. Provisions to consider long-term protection of the site on Contra Costa Water District land were contained within the letter of agreement.

### Acquisition of Propagules

All of the propagules (= nutlets, seeds) of *Amsinckia* used in this reintroduction effort were ultimately derived from collections made by Dr. Robert Ornduff at Site 300 in the mid-1960's (see Pavlik 1990 for a complete history and genetic characterization of the propagules). Some of those nutlets were used to propagate the species in a UC Davis experimental garden. A portion of the 1988 crop, about 5000 nutlets (referred to as the Davis source), was donated to the reintroduction project. They were used in the 1989-90 project at LR and constituted the main source for the present project at BD II, LV, and CR. The Davis source nutlets had 59% germination in the lab and 70% germination *in situ* during the 1989-90 project (Pavlik 1990).

Another small portion of the Ornduff nutlets had been stored in paper pouches in a freezer at UC Berkeley since its collection 25 years ago. These nutlets (subsequently called the Site 300 source) were found to contain more genetic (electrophoretic) variation than those from propagation in Davis, and were, therefore, regarded as important components in the reintroduction effort. Consequently, their germination, survivorship, and reproductive output during the 1989-90 project were monitored separately from the Davis nutlets in order to conserve the largest possible gene pool. Only 300 high-quality Site 300 nutlets remained for the 1990-91 project, so these were evenly divided between the BD II, LV, and CR sites. The Site 300 source nutlets had 31% germination in the lab and 43% germination *in situ* during the 1989-90 project (Pavlik 1990).

### Plot Design and Treatments

After selection of the microsites during the September to November 1990 period, a large area (7.6 X 7.6 m) was fenced at each of the BDII, LV, and CR locations (Figure 2).

At BD II the area was fenced with three strands of barbed wire to exclude cattle and deer. The LV site had not been grazed by livestock in three years and was not scheduled for grazing in 1990-91. Therefore, only two strands of wire were required to exclude deer and to facilitate their escape from the enclosure in the unlikely event they jumped the fence. At CR, it was necessary to use three strands of barbed wire to exclude cattle and deer and 36" wire mesh fence to exclude wild pigs. The bottom of the wire mesh was buried in order to discourage entry by digging. Each of the enclosures had a small gate and a post for supporting a rain gauge and a max/min thermometer.

The three new areas were prepared to receive plots, treatments and weather equipment during the fall of 1990. A cross design (Figure 2) was used to determine the positions of 12 plots, nine of which would be treated to minimize annual grass cover (burn + grass-specific herbicide) and three were left as is (with cover intact) to serve as controls. Each plot was 1.25 X 1.25 m in area. The inner four plots (#s 4,5, 8, 9) were sown with 25 Site 300 nutlets and 75 Davis nutlets each (see below). The outer eight plots received 100 Davis nutlets. Control plots were randomly selected (using a random numbers table) from among the eight outer plots so that the few remaining Site 300 nutlets would not be subjected to grass competition. Designated paths between plots ensured that no human impact occurred where nutlets were sown. In addition, the position of each native bunchgrass plant in the fenced areas (usually *Stipa pulchra* or *Poa scabrella*) was marked with a survey flag in order to prevent it from being sprayed with herbicide later in the season.

In the center of each 1.25 X 1.25 plot a 1 x 1 m frame was located, allowing a 0.125 m border to minimize edge effects. These were permanently marked with two, 35 cm long stainless steel rods driven into the soil so that 8 cm protruded above the surface. The rods positioned a removable wooden frame, 100 cm x 105 cm, into which a grid of 100 holes (10 holes x 10 holes, each 2.5 cm diameter) had been drilled (Pavlik 1990). The holes allowed exact placement of nutlets within the plot and subsequent monitoring of germinules and juvenile plants.

Treated plots were burned after sowing using the burn box technique for containing small fires in grassland habitats (Pavlik 1990). BD II was burned on 24 October, CR on 28 October, and LV on 16 November, 1990, before any significant rainfall (> 6 mm per storm) was received (see Appendix D). In addition to burning, each of the treated plots received 370 ml of a dilute (1/10th strength) solution of a grass-specific herbicide

(fluazifop-p-butyl or "Fusilade®" from ICI Corp). For each of the three areas this consisted of 4.1 ml of Fusilade® and 10.2 ml of a non-ionic surfactant (Monterey Herbicide Helper, Lawn and Garden Products Inc., Fresno CA) in 4.1 l of water (the remaining 740 ml was used to treat two additional, exposed plots at each area, see below). The herbicide was applied to all treatment plots at all three areas on 15 February, 1991. A hand sprayer was used to produce a fine mist that wetted the grass blades and culms. Care was taken to prevent overspray and very little (if any) of the liquid dripped onto the soil surface within the plots. Native bunchgrasses that grew within the plots were shielded using one gallon plastic milk containers that had the bottoms removed. Dieback of the non-native grasses was obvious by 7 March 1991. Native forbs, including monocots such as *Allium serratum* and *Dichelostemma pulchellum*, were not noticeably affected by this dilute application of Fusilade®.

Three plots within each enclosure were left untreated as controls and contained high cover by introduced annual grasses (mostly *Avena fatua*, *Bromus diandrus*, *B. mollis*, *B. rubens*) and a few native and introduced forbs (*Montia perfoliata*, *Dichelostemma pulchellum*, *Microsteris gracilis*, *Brassica geniculata*, *Erodium cicutarium*). The use of control plots allowed an assessment of the effects of grass competition on *Amsinckia* throughout its historic range, where rainfall and annual grass cover varied greatly (Table 3). The assessment was made using demographic data collected the seedling and juvenile phases of the life cycle (see below).

Plant cover in control and treatment plots was recorded using a circular, 0.125 m<sup>2</sup> quadrat. The quadrat was dropped in the center of each control plot on 6 May (CR) and 9 May (BDII and LV), 1991, and estimates of absolute cover (% by each dominant grass species) were made.

Outside of each enclosure, approximately 15 m downhill, two additional plots were treated with fire and herbicide in the same manner as the nine treatment plots within the fence. Because they were not protected by a fence, however, the exposed plots were subjected to the actions of large, grazing mammals. At BDII those mammals were cattle and deer (a deer trail was within 2 m of these unfenced plots). Only deer were present at LV (a trail was within 2 m), but at CR the unfenced plots were exposed to deer, cattle and wild pigs. Comparison of these exposed plots with treated plots inside the fence allowed some assessment of the effects of such mammals on *Amsinckia* during reintroduction.

### Sowing the Nutlets

A total of 1,400 nutlets of *Amsinckia grandiflora*, 1300 from the Davis source and 100 from the Site 300 source, were sown at each of the three reintroduction sites in late October and early November, 1990, just prior to burning. All of the nutlets were of high quality, with a 1.5-3.0 mg range of weight per nutlet (see Pavlik 1988).

Using the wooden frames, each plot was planted with 100 nutlets. The inner four plots (#'s 4,5, 8, 9) were sown with 25 Site 300 nutlets and 75 Davis nutlets each. The Site 300 nutlets were sown in a pattern that would distribute the germinules to better insure crossing of the two sources. The outer eight plots within the fence and the two plots outside the fence received 100 Davis nutlets each. Nutlets were pressed into shallow depressions in the mineral soil made with a blunt nail, covered with about 20 cc of loose, native soil (a depth of one cm to ensure good moisture retention) and tamped down uniformly. Each plot took 45 to 60 minutes to sow.

No supplements of nutrients were applied during the experiment. On 30 and 31 January, 1991, however, the region had received only about 27% of the average precipitation for that date. In an effort to conserve the existing seedlings, each fenced area was treated with 20 mm of supplemental precipitation delivered by means of a field-portable sprinkler system.

### Demographic Monitoring of the New Populations

Intensive demographic monitoring of all plots was conducted in order to identify those factors that might limit the establishment or growth of the new populations (Pavlik 1987, Pavlik 1990). The monitored parameters included field germination, stress factors (desiccation, etiolation, grazing), mortality, phenology, reproductive survivorship, pin-thrum ratio, and nutlet output per plant and per plot. Individuals from different source populations (Site 300 and Davis) were analyzed separately so that the effects of electrophoretically-detectable genetic differences could also be assessed.

Monitoring the fates of nutlets, germinules, seedlings and established plants was made possible by the repeated use of the planting frame to locate and identify individuals. Encoded data sheets (Appendix C) that duplicated the plot frame design greatly facilitated the arduous task of recording detailed information about each plant (Pavlik 1990). After

all nutlets were sown, plots were censused without the frames on 30 November 1990, and with the frames on 3 January (BDII), 4 January (LV, CR), 30 January (LV), 31 January (BDII, CR), 7 March (BDII), 13 March (CR), 14 March (LV), 5 April (CR), 10 April (BDII), 26 April (LV), and 29 April (BDII). These techniques have been developed and described in detail by Pavlik and Barbour (1988), Pavlik et al. (1988), and Pavlik (1990).

Estimates of nutlet production per plant and per plot were based on the correlation technique developed by Pavlik and Barbour (1988) and applied to garden-grown (Pavlik 1988) and field-grown (Pavlik 1990) plants of *Amsinckia grandiflora*. The technique allows for estimates of nutlet output based on the sum of the inflorescence lengths or shoot length of an *Amsinckia* plant. For plants in the field, the latter was easiest to apply since shoot length (equivalent to maximum plant height above the soil) was readily measured for each plant in the plots at the time of maximum nutlet production (early April for CR, late April for BDII and LV). Such measures of plant size have been shown to be the best indicators of reproductive yield in herbaceous annuals and perennials (Winn and Werner 1987, Lee and Bazzaz 1982), including *Amsinckia* (Pantone et al. 1989).

The relationship between shoot length and nutlet output per plant used in this study was developed by harvesting ten individuals from each of the three sites, chosen to vary in size from among all of the plots (treatment and control and Site 300 and Davis plants were pooled). Plants were selected in April after growth and nutlet production had slowed or ceased. Maximum shoot length was measured and the plants were clipped at soil level, sealed in separate polyethylene bags and kept refrigerated until the remaining data were obtained one week later. Measurements of total inflorescence length and counts of the number of branches, inflorescences, flowers and nutlets were made in the lab. Inflorescences were removed from the vegetative portions of the plant by clipping immediately below the first flower. Each flower was examined for the presence of filled (good quality) nutlets which were then counted, removed, and placed in an envelope assigned to that individual plant. The number of ovules was estimated by multiplying flower number by four since each flower produces four single-ovuled nutlets (Ornduff 1976).

Linear and non-linear regressions were made using total shoot length and total inflorescence length (the sum of inflorescence lengths from a single plant) as the independent variable and nutlet output per plant as the dependent variable. The equation derived from a site was used to convert the height of each plant at that site (=

maximum shoot length) to nutlet output at the peak of fruit set (April 1991). Plot analyses were made by summing the nutlet output of all plants in treatment or control plots.

Evaluation of the treatment was made by comparing field germination, survivorship to reproduction and nutlet output per plot between replicate plots and the control plots. Statistical analysis of differences was made using analysis of variance (ANOVA) with arcsine transformation where appropriate.

### Physiological Monitoring of the New Populations

Physiological monitoring can indicate the specific effects of an experimental treatment and identify environmental factors which restrict the growth and reproduction of an endangered species (Pavlik 1987). Both are relevant to the present study. In order to assess competition between *Amsinckia* and annual grasses for water, the water status of reproductive plants in control and treated plots was compared using a Scholander-type pressure bomb. Stems from six control and six treated plants were used at midday (1200 to 1500 hours, the time of maximum stress levels) on 6 May (CR) and 9 May (BDII and LV), 1991. Under these conditions, differences in xylem water potential between control and treated plots would be due to competition from annual grasses (Fonteyn and Mahall 1981, Robberecht et al. 1983).

## **Results and Discussion**

### Characteristics of the Reintroduction Sites

The reintroduction sites were located along a north-south axis that spanned the known historic range of the species (Figure 1), and are herein referred to as Black Diamond II (BDII), Los Vaqueros (LV) and Connolly Ranch (CR). BDII, LV and CR met the essential criteria for a reintroduction site and had several important characteristics in common (Tables 2 and 3). All sites occurred on north/northwest-facing slopes with loamy or clay-loamy soils derived from sandstone bedrock. Soil thickness was at least 60 cm as revealed by replicate core samples. The predominant vegetation type was annual grassland bordering on oak savanna or woodland. In general, non-native annual

Table 2. General characteristics of the reintroduction sites, 1990-1991.

name	ownership	lat	long	elev	slope	aspect	est ann ppt (cm)	soil	dominant plants	
									herbaceous	woody
Black Diamond II (BD II)	public1	37°57'35"	121°51'48"	1150'	35%	3180	347	AcF4	Avena fatua	Quercus wislizenii
									Bromus diandrus	Aesculus californica
									Stipa pulchra	Lupinus albus
									Dichelostemma	Q. douglasii
Los Vaqueros (LV)	public2	37°48'35"	121°46'24"	1800'	30%	3100	456	MeF5	Bromus mollis	Quercus wislizenii
									Avena fatua	Q. douglasii
									Poa scabrella	Umbellularia californica
									Delphinium patens	
Connolly Ranch (CR)	private3	37°35'35"	121°29'17"	1250'	25%	3040	302	VaF6	Microsteris gracilis	Quercus douglasii
									Bromus mollis	Juniperus californica
									Allium serratum	
									Avena fatua	

- 1 Black Diamond Mines Regional Preserve, East Bay Regional Park District
- 2 Contra Costa Water District, Los Vaqueros Reservoir Project
- 3 Pat Connolly, San Joaquin County, CA
- 4 Altamont-Fontana complex, 30-50% slope, silty clay loam from sandstone, 1.5-3 feet deep, pH 6.1-8.4
- 5 Millisholm loam, 30-50% slope, loam from sandstone, 1-1.5 feet deep, pH 5.1-6.5
- 6 Honker-Vallecitos-Honker eroded complex, 30-50% slope, clay loam from sandstone, 1.5-3 feet deep, pH 5.6-7.3

Table 3. Plot-specific characteristics of the reintroduction sites, 1990-91

	actual precipitation (mm) / (% of ann)		actual Nov 1-May 30 min temp (°C)		maximum grass canopy height range (cm)		total abs. grass cov. (%)	Avena fatua (%)	absolute cover by		
	239 / 69%	30.5	50-60	86.7	Bromus diandrus (%)	B. mollis (%)			B. rubens (%)	Stipa pulchra (%)	
BD II	239 / 69%	30.5	50-60	86.7	53.3	31.7	0	0	0	0	0
LV	374 / 82%	25.0	20-30	51.7	16.7	0	21.6	11.7	1.7		
CR	203 / 63%	27.5	30-40	31.6	8.3	0	23.3	0	0	0	0

grasses dominated the cover, but native perennial grasses (*Stipa pulchra* and *Poa scabrella*) and native forbs were also common. One or more species of *Amsinckia* were found at each of the sites, including *A. intermedia* (BDII, LV, CR) and *A. retrorsa* (CR).

There were, however, some important differences between the sites (Table 3). LV occurred at the highest elevation and received the most rainfall during the growing season. It also had the coolest maximum and minimum temperatures during this time. BD II was warmer and drier, but the large ridge which rises above the site produced cold air drainage not found at any of the other sites. As a result, BD II was cooler than might otherwise be expected, and this may have been a factor in delaying the growth of *Amsinckia* plants during the early spring. CR was the warmest and driest site, and had relatively low cover by annual grasses. The grass canopy at CR was sparse (30-35% absolute cover) and short (30-40 cm high), especially when compared to BD II. This was due to the predominance of *Bromus mollis* at CR, rather than *Avena fatua* or *B. diandrus*. Based upon the structure and composition of the grass canopies, competition would be most intense at BD II and least at CR.

#### Comparison of Weather Patterns and Phenology during 1990-1991 and 1989-1990

In northern California, the 1 November to 30 May growing season of 1990-1991 had below-average precipitation, as did the previous four growing seasons. Records for San Francisco, Oakland, and Sacramento indicate that rainfall was 73-80% of normal during the 1 Nov to 30 May period of 1990-1991, a deficit of about 25%. A similar regional deficit (about 20%) occurred during the same period in 1989-90. The total precipitation received at Lougher Ridge during the October to May period of *Amsinckia* activity was nearly the same in both years - 280 mm in 1989-90 vs. 266 in 1990-91. Rainfall amounts received by BD II, LV, and CR during 1990-91 are given in Table 3.

The significant differences between 1990-1991 and 1989-1990 were not in the amounts of rain received, but in the temporal patterns of rainfall (Figure 3) and temperature. Using data collected at Lougher Ridge (representative of all reintroduction sites), regional rainfall during 1990-91 began later, occurred less regularly, and came from fewer major storms than in 1989-90. The first significant storm of 1989-90 dropped 38 mm of rain during three days in October (22-24) and was accompanied by relatively warm air temperatures (daily means above 12 C). The first significant storm of 1990-91

did not occur until 25 mm fell over a seven day period in December (10-16). Daily air temperatures were just above freezing while nighttime temperatures were at -9.5 C or lower. This period of rain and sustained sub-zero temperatures was subsequently followed by a cold, dry mass of Arctic air. Although a few small storms occurred in mid-January, the next hard rain did not occur until 40 mm fell in early February 1991 (2-5). Most of the 1990-91 rainfall was delivered by a series of consecutive storms that began on 27 February and lasted until 27 March. The "March miracle," as it was dubbed by the press, decreased the seasonal rainfall deficit from 55% (mid-February) to 20% (late March). In contrast, the major storms of 1989-90 came in near-monthly intervals, each one delivering more than 30 mm until mid-March.

These distinctive annual patterns had profound effects on the phenology of *Amsinckia* at the field sites (Figure 4). Whereas 90% of the 1989-90 germination happened before 6 November, comparable levels in 1990-91 did not occur until 31 January. The cold December storm did not stimulate much germination (less than 5% at all sites by 4 January), but a lesser, warmer storm of 7, 8, and 15 January (a total of 7-11 mm at all sites) apparently did. The onset of floral anthesis, peak floral display, nutlet set, and death were also delayed, especially at BD II and LV. Open flowers and unripened nutlets persisted until late May at BD II and LV, compared to mid-April at LR in 1990. Many live, green plants were still found in early June at BD II, perhaps sustained by cooler temperatures from cold air drainage. CR had the shortest growing season, with peak germination in late January and death by mid-May. Nevertheless, *Amsinckia grandiflora* responded favorably to the weather in 1990-91, with robust growth and showy floral displays at LR, BD II, Site 300, and CR (see below).

The storm pattern of 1990-91 benefited other *Amsinckia* species as well. Roger Epperson of Black Diamond Mines and Pat Connolly independently volunteered the same springtime observation: neither had ever recalled seeing so much *Amsinckia intermedia* in bloom. Indeed, hillsides from Antioch to Mount Hamilton were golden with dense stands of common fiddleneck. At Draney Canyon on Site 300, *A. vernicosa*, *A. gloriosa*, and *A. lycopsoides* were robust and showy. Other native plants in the region also responded well, including *Phacelia ciliata*, *Lupinus albifrons*, *Microsteris gracilis*, *Monolopia major*, *Collinsia heterophylla*, *Dephinium patens*, and *Dichelostemma pulchellum*. The annual grasses did not appear to be any more or less abundant than in previous years, except in areas with particularly dense stands of *A. intermedia*.

## Demographic Monitoring of the New Populations

### Germination

Low rates of germination (less than 5% of the sown nutlets at all sites) were detected in the first few days of January 1991, at least 45 days after the last nutlets were sown. In the control plots of BD II and LV, which had dense cover by last year's grasses, germination was significantly higher (average of 15%) than in the exposed treatment plots (1-3%). This early germination extended the growing season of some control plants by as much as one month. Control plots at CR, however, had almost no grass cover at this time (due to previous grazing) and did not have significantly higher germination than the treatment plots (both around 2% on 4 January). The majority of nutlets at all sites germinated between 7 and 30 January in response to the mid-month rains. Nutlets continued to germinate sporadically throughout the growing season, with the last germinules recorded on 13 March at CR, 26 April at LV, and 29 April at BD II.

Total *in situ* germination (% of nutlets sown) during the December to April period was lower than expected at BD II and LV, with an average of about 30% for control and treatment plots (Tables 3 and 4). Based on a lab germination of about 45% (both Site 300 and Davis sources) and a previous *in situ* figure of 55% (LR in 1989-90), it may be concluded that the conditions for germination were less than optimal at these two sites in this particular year. At CR, however, germination averaged 50% in control and treatment plots, thus comparing well to previous observations in the lab and field (Pavlik 1990).

There were no significant differences in germination between control and treatment plots at any of the reintroduction sites. Although germination tended to be higher by 3-6% in control plots, the variability among treatment plots was always high. Also, because most germination occurred prior to the water application of 30-31 January, it could not have been stimulated by the 20 mm of supplemental rainfall.

### Population Growth and Mortality

Low germination rates constrained population growth at BD II and LV, resulting in smaller total populations (Table 4) and fewer reproductive plants per plot (Table 5) than at CR. The fenced, reproductive population at CR (561 individuals) was about twice the size

of the other new populations. The highest proportion of Site 300 plants was at BD II (8.7%), but the largest number was at CR. The highest number of plants exposed to grazing mammals outside the fence was at LR, even though more had germinated at CR (see below). There were no significant differences in plant density between control and treatment plots at any of the sites (Table 5).

The first seedling deaths were detected by the end of January 1991, a little more than one month after the first germinations had occurred. Peak mortality occurred by early March and declined after that at all sites. The decline in mortality came, for the most part, after the heavy rains of mid- to late March. The causes of seedling mortality were

Table 4. Number of plants in all plots (control, treatment, exposed) at the three reintroduction sites, 1991. Census dates for reproductive plants were 5 April (CR), 26 April (LV) and 29 April (BD II). The sum ( $\Sigma$ ) of all reproductive plants from 12 plots within the fence (3 control, 9 treatment) represents the protected, fenced population. The exposed population consisted of 2 unfenced plots.

	total # sown	total # germinated	total # repro plants, Davis + Site 300	total # repro Site 300 plants
<b>BD II</b>				
control	300	92	70	---
treatment	900	221	<u>194</u>	23
			$\Sigma = 264$ (fenced population)	(8.7 % of $\Sigma$ )
exposed	200	36	24	---
<b>LV</b>				
control	300	100	74	---
treatment	900	271	<u>242</u>	13
			$\Sigma = 316$ (fenced population)	(4.1 % of $\Sigma$ )
exposed	200	75	58	---
<b>CR</b>				
control	300	156	148	---
treatment	900	431	<u>413</u>	27
			$\Sigma = 561$ (fenced population)	(4.8% of $\Sigma$ )
exposed	200	95	19	---

not always clear, but grazing by insects was common at LV and CR, while wilting was observed at all sites prior to the February rains.

There were significant differences in *Amsinckia* mortality between control and treatment plots at BD II and LV, resulting in differential reproductive survivorship (Table 5). Survivorship was close to 90% in treated plots where the grass cover had been eliminated by fire and Fusilade. Control plots had lower *Amsinckia* survivorship (~75%), corresponding with the rapid development of the annual grass canopy during the February to March period. In contrast, all plots at CR had high survivorship (~95%) and relatively low grass cover regardless of treatment. These results are the same as observed at LR in 1989-90, although the range of *Amsinckia* survivorship in this set of experiments (75-95%) was higher than in the previous year (43-75%). It is possible that 1) survivorship in treated plots was elevated by the burn-herbicide combination

Table 5. Treatment effects on germination, population size, survivorship to reproduction and pin/thrum ratio of *Amsinckia* at three fenced reintroduction sites, 1991. Control and treatment values for a single site (means  $\pm$  SD,  $n = 3$  for controls,  $n = 9$  for treatment) followed by the same number of asterices are statistically different (ANOVA, arcsine transformed % and ratios) at the indicated probabilities.

	<i>in situ</i> germination (%)	mean # of repro plants per plot	survivorship to reproduction (% of germ)	pin / thrum ratio
<b>BD II</b>				
control	30.7 $\pm$ 1.7	23.3 $\pm$ 0.5	76.3 $\pm$ 4.7**	2.25 $\pm$ 1.78
treatment	24.6 $\pm$ 5.2	21.6 $\pm$ 4.0	88.5 $\pm$ 6.5**	1.49 $\pm$ 0.39
<b>LV</b>				
control	33.3 $\pm$ 11.1	20.5 $\pm$ 9.9	72.9 $\pm$ 3.5***	1.31 $\pm$ 0.19
treatment	30.1 $\pm$ 13.0	26.7 $\pm$ 12.1	89.1 $\pm$ 5.6***	1.33 $\pm$ 0.50
<b>CR</b>				
control	52.0 $\pm$ 0.8	49.0 $\pm$ 0.8	94.9 $\pm$ 2.4	1.52 $\pm$ 0.61
treatment	47.9 $\pm$ 9.9	46.0 $\pm$ 9.7	95.7 $\pm$ 2.4	1.40 $\pm$ 0.23

\*\* =  $P < 0.025$  \*\*\* =  $P < 0.005$

and that 2) survivorship in control plots was elevated by a particularly favorable weather pattern in 1990-91. Nevertheless, these results strongly reinforce the conclusion (Pavlik 1990) that variations in annual grass cover effect the survival of *Amsinckia grandiflora*.

### Flowering and Nutlet Output

Inflorescences of *Amsinckia grandiflora* were first observed on 2 February, 1991 at CR, 7 March at BD II and 14 March at LV. Correspondingly, CR was the first to reach 100% anthesis (each individual with at least one open flower) on 5 April, followed by BD II (April 10) and LV (26 April). Plants in control plots tended to flower earlier than those in treatment plots except at CR. There was no treatment or site effect on pin/thrum ratio (Table 5), which varied between 1.3 and 2.2 among all plots. Over several years at Site 300, Ornduff (1976) reported a range of 1.0 to 2.0, while Taylor (1987) found 0.75 to 1.2. During its first survey in April 1991, the Carnegie Canyon population had a ratio of 1.72 ( $n = 543$ ). The ratios of the new populations are, therefore, similar to those seen in natural populations.

The output of nutlets by individual plants at the three new sites was linearly related (Table 6) to the sum of the inflorescence lengths and shoot length (Figure 5). The relationships at BD II and CR were statistically the same as those found at LR (Pavlik 1990). The plants at LV, however, were much less fecund per unit of shoot or inflorescence, having a slope that was a fourth of those observed at the other reintroduction sites. There could be several explanations for reduced fecundity at LV, including cold temperature stress, nutrient deficient soil, or lack of appropriate pollinators at the right time. Plants at LV were significantly smaller than those at BD II and CR (Table 7 and Appendix D), indicating that either of the first two explanations are more likely than the third. As a result, nutlet output per plant and per plot were very low at LV, especially when compared to those parameters at CR.

Ovule output was also related to the sum of the inflorescence lengths, but larger plants were not more efficient than smaller ones in converting ovules into nutlets (i.e. the slope of the sum of inflorescence lengths vs. reproductive efficiency  $\sim 0$  and  $P = n.s.$ , Table 6). Typically, medium-sized plants had reproductive efficiencies (nutlet/ovule ratios) around 0.20, but the means at all sites ranged between 0.117 (CR) and 0.147 (BD II). Maximum reproductive efficiency was 0.24 (at LV), which compares well to the 0.30

Table 6. Linear correlations between various measures of plant size and nutlet output, ovule output or reproductive efficiency per individual *Amsinckia grandiflora* from 3 sites, April-May 1991. **Bold type** indicates the relationship shown in Figure 5. Data on 1990 Lougher Ridge plants provided for comparative purposes. ns = not significant,  $\Sigma$  inflor lgth = sum of the lengths of all inflorescences, repro eff = reproductive efficiency

n	X	Y	slope	intercept	r	P
<b>Black Diamond II 1991</b>						
10	<b>shoot length (cm)</b>	<b># nutlets</b>	<b>5.61</b>	<b>-93.14</b>	<b>.85</b>	<b>&lt;0.01</b>
10	$\Sigma$ inflor lgth (cm)	# nutlets	1.48	3.05	.97	<0.01
10	$\Sigma$ inflor lgth (cm)	# ovules	12.65	-34.55	.97	<0.01
10	$\Sigma$ inflor lgth (cm)	repro eff	-0.0004	0.162	.32	ns
<b>Los Vaqueros 1991</b>						
10	<b>shoot length (cm)</b>	<b># nutlets</b>	<b>0.92</b>	<b>-3.64</b>	<b>.64</b>	<b>&lt;0.05</b>
10	$\Sigma$ inflor lgth (cm)	# nutlets	0.94	4.97	.48	ns
10	$\Sigma$ inflor lgth (cm)	# ovules	14.01	-7.72	.95	<0.01
10	$\Sigma$ inflor lgth (cm)	repro eff	-0.006	0.178	.36	ns
<b>Connolly Ranch 1991</b>						
10	<b>shoot length (cm)</b>	<b># nutlets</b>	<b>3.42</b>	<b>-65.46</b>	<b>.86</b>	<b>&lt;0.01</b>
10	$\Sigma$ inflor lgth (cm)	# nutlets	2.32	-12.62	.92	<0.01
10	$\Sigma$ inflor lgth (cm)	# ovules	11.17	-10.50	.98	<0.01
10	$\Sigma$ inflor lgth (cm)	repro eff	0.003	0.07	.55	ns
<b>Lougher Ridge 1990</b>						
18	shoot length (cm)	# nutlets	4.60	-79.25	.77	<0.01
18	$\Sigma$ inflor lgth (cm)	# nutlets	2.51	-5.93	.95	<0.01
18	$\Sigma$ inflor lgth (cm)	# ovules	10.95	7.23	.99	<0.01
18	$\Sigma$ inflor lgth (cm)	repro eff	0.001	0.144	.43	ns

reported for plants at Site 300 (Ornduff 1976) and exceeds the 0.22 reported for greenhouse-grown plants (Pavlik 1988).

Even though plots with high annual grass cover were shown to decrease the survivorship of *Amsinckia* juveniles and lower the xylem water potential of *Amsinckia* adults (see below), there were no significant differences in plant size or nutlet production between control and treatment plots at any of the three sites (Table 7). It seems likely that the 80-100 mm of rain which fell in mid- to late March diminished the intensity of spring competition between grasses and *Amsinckia* that was so pronounced at Lougher Ridge during the previous year (Pavlik 1990). This would also explain the vigorous growth and profuse flowering of other *Amsinckia* species throughout the region in this unusual rainfall year.

The new population at Connolly Ranch was the most prolific of the three (Table 7). A total of 16,813 nutlets were produced by the 561 reproductive individuals of *Amsinckia grandiflora* at CR in early April, 1991. This estimate was obtained by calculating the nutlet output of each and every plant in all plots using its measured shoot length (5 April) and the CR equation shown in Table 6. Early-dispersing (before 5 April) and late-forming (after 5 April) nutlets could not be included in the sample, so it is likely that nutlet output has been slightly underestimated. Because a total of 1200 founder nutlets were input to the site, the seed bank population of *Amsinckia grandiflora* at CR was amplified by about a factor of 14.0. Approximately 5% of the resident CR nutlets (those produced on site) were derived from the Site 300 source. Resident nutlets were left to disperse on their own. Using the 4% yield of reproductive plants observed at Lougher Ridge between 1990 and 1991 (% yield =  $1301 \text{ 1991 plants} / 35,800 \text{ 1990 nutlets} \times 100$ ), I can predict (with many assumptions) that next year's CR population should be about 672 individuals, a 20% rate of population growth.

Total nutlet output by the 264 plants at Black Diamond II was estimated at 10,446 by late April, 1991. The seed bank amplification factor was, therefore, 8.7 (similar to the 10.2 observed at LR in 1990), with 8% of the resident nutlets derived from the Site 300 source. Again applying the 4% yield figure, next year's BD II population should be about 418 plants, a 58% rate of population growth.

The estimates of nutlet output at BD II and CR predicted positive population growth. This was not the case at Los Vaqueros. Total nutlet output by the LV plants was estimated at 2,801 by late April, 1991. The seed bank amplification factor was only 2.3,

Table 7. Treatment effects on plant size (length of main shoot) and nutlet production (per plant and per plot) of *Amsinckia* (both sources) during the period following maximum flowering (April, 1991) at three fenced reintroduction sites. Mean maximum plant size calculated from the 10 largest individuals in each plot. Control (n = 3 plots) and treatment (n = 9 plots) values for a single site (mean  $\pm$  SD) did not statistically differ ( $P < 0.05$ , ANOVA).

	plant size		nutlet production		total nutlets ( $\Sigma$ plots)	total Site 300 nutlets ( $\Sigma$ plots)
	mean maximum (cm)	mean (cm)	mean (# / plant)	mean (# / plot)		
<b>BD II</b>						
control	25.7 $\pm$ 4.2	20.8 $\pm$ 2.0	27.2 $\pm$ 12.4	631 $\pm$ 280	1893	---
treatment	29.8 $\pm$ 5.1	23.6 $\pm$ 4.7	44.5 $\pm$ 22.1	950 $\pm$ 523	<u>8553</u>	831
					10,446	
<b>LV</b>						
control	17.9 $\pm$ 1.2	14.5 $\pm$ 2.0	9.6 $\pm$ 1.8	224 $\pm$ 39	672	---
treatment	16.7 $\pm$ 2.6	13.5 $\pm$ 1.4	8.7 $\pm$ 1.4	236 $\pm$ 113	<u>2129</u>	127
					2,801	
<b>CR</b>						
control	39.6 $\pm$ 2.7	28.6 $\pm$ 0.4	33.3 $\pm$ 1.3	1634 $\pm$ 50	4902	---
treatment	35.4 $\pm$ 2.6	27.1 $\pm$ 2.5	29.0 $\pm$ 7.5	1323 $\pm$ 415	<u>11,911</u>	799
					16,813	

with 4% of the resident nutlets derived from the Site 300 source. Applying the 4% yield figure (which is probably very site and year specific), next year's LV population would be about 112 plants - a decline of 65 % rate in the size of the population.

The overall showiness of the floral displays at the three sites varied greatly. By far the population at CR was the most impressive, with 12 bright orange patches that were visible from quite a distance away (see Appendix D). Sparse grass cover provided minimal "green dilution" and the dense understory of *Microsteris gracilis* was unique. Although floral anthesis at BD II was slower to develop and less synchronized, there were seven very showy patches by late April (see Appendix D). Tall annual grasses obscured the plants from most vantage points, and even *Stipa pulchra* grew lush enough in the treatment plots to

hinder the display. In contrast, plants at LV were so small and had so few inflorescences that they barely stood out among the *Ranunculus* and *Lasthenia*.

### Physiological Monitoring of the New Populations

Measurements of xylem water potential during the period of maximum fruit set (early to late May) showed that *Amsinckia* plants were competing with annual grasses for water (Table 8) at BD II. *Amsinckia* plants in plots with no annual grasses had significantly higher water potentials than those in control plots. Such plants were also active into late May, with less senescence and more open flowers than control plants. But control plants may have had a growing season of similar length since many germinated in late December instead of late January (see above). Furthermore, the water potentials of control plants at BD II did not fall within the stressful range of -1.5 to -2.0 MPa associated with herbaceous dicots in annual grassland (Gulmon et al. 1983). It is unlikely, therefore, that this level of competition significantly impaired leaf gas exchange, plant growth or nutlet production during the critical April to May period at BD II. In a rainfall year like 1989-90, however, water competition during spring is likely to be a major factor in reducing *Amsinckia* reproduction where annual grass cover is high.

Competition for water appeared to be less pronounced at the other two sites, either because annual grass cover was low or spring precipitation was high or both. Significantly more plants maintained open flowers in the treatment plots at LV, but control plants germinated up to a month earlier and may have had a growing season of similar length. No differences between control and treatment plants could be detected in water status or phenology at CR.

### Effects of Mammalian Grazers

The impacts of grazing mammals on *Amsinckia* were detected to different degrees at all three reintroduction sites. At BD II, the passing of a single, browsing deer significantly reduced *Amsinckia* density and survivorship and increased the pin/thrum ratio, but had no significant impact on plant size or nutlet production (Tables 9 and 10). Between 10 April and

Table 8. Effects of treatment on plant water status at midday (1200-1300 hr), and the proportions of plants which were senescent or had open flowers at three fenced reintroduction sites in May, 1991. Control and treatment water potential values for a single site (means  $\pm$  SD, n = 6 control and 6 treatment plants) followed by the same number of asterices are statistically different (ANOVA,  $P < 0.005$ ). Senescence or flowering proportions for a single site (means  $\pm$  SD, n = 3 control plots, 9 treatment plots) followed by the same number of asterices are statistically different (ANOVA,  $P < 0.005$ , arcsine transformed data).

	midday water potential (MPa)	dried, brown senescent plants (% of total)	plants w/ open flowers (% of total)
<b>BD II</b>			
control	$-1.09 \pm 0.26^{***}$	$85.6 \pm 8.3^{***}$	$2.9 \pm 2.1^{***}$
treatment	$-0.62 \pm 0.10^{***}$	$2.5 \pm 5.9^{***}$	$96.8 \pm 5.2^{***}$
<b>LV</b>			
control	$-0.80 \pm 0.16$	$21.7 \pm 11.7$	$7.3 \pm 2.7^{***}$
treatment	$-0.72 \pm 0.14$	$10.7 \pm 8.8$	$41.2 \pm 14.3^{***}$
<b>CR</b>			
control	$-0.84 \pm 0.10$	$89.4 \pm 6.2$	$16.2 \pm 4.4$
treatment	$-0.83 \pm 0.13$	$82.1 \pm 8.7$	$13.1 \pm 3.8$

\*\*\* =  $P < 0.005$

29 April, six cleft tracks (oval, 6 -6.5 cm long) had been made across exposed plot #14. Seven plants that were in the path of those tracks had completely disappeared, apparently pulled up or nipped at the soil surface by the passing animal. None of the remaining plants (which were flowering at the time) had shoots removed or looked disturbed in anyway. Although nutlet production was reduced relative to the fenced treatment plots, the differences were not statistically significant. The 24 surviving, exposed plants produced an estimated 836 nutlets. Applying the 4% Lougher Ridge yield, this means that next year's exposed population would be 33 plants, an increase of 38%.

It is likely that deer, or possibly rabbits, were responsible for a small amount of shoot grazing detected at LV during late April. Five out of 30 plants (8.4%) in exposed plot #13 had shoots removed, but there were no tracks, no missing plants, and no other apparent

Table 9. Effects of exposure to mammalian grazers on germination, population size, survivorship to reproduction and pin/thrum ratio of *Amsinckia* at 3 reintroduction sites, 1991. Fenced-t (= treatment means  $\pm$  SD, n = 9) and exposed (n = 2) values for a single site followed by the same number of asterics are statistically different (ANOVA, arcsine transformed % and ratios) at the indicated probabilities.

	<i>in situ</i> germination (%)	mean # of repro plants per plot	survivorship to reproduction (% of germ)	pin / thrum ratio
<b>BD II</b>				
fenced-t	24.6 $\pm$ 5.2	21.6 $\pm$ 4.0**	88.5 $\pm$ 6.5**	1.49 $\pm$ 0.39*
exposed	18.0 $\pm$ 0.0	12.0 $\pm$ 3.0**	66.6 $\pm$ 16.6**	2.38 $\pm$ 0.38*
<b>LV</b>				
fenced-t	30.1 $\pm$ 13.0	26.7 $\pm$ 12.1	89.1 $\pm$ 5.6	1.33 $\pm$ 0.50
exposed	37.5 $\pm$ 3.5	30.0 $\pm$ 2.0	80.2 $\pm$ 2.2	1.00 $\pm$ 0.00
<b>CR</b>				
fenced-t	47.9 $\pm$ 9.9	46.0 $\pm$ 9.7***	95.7 $\pm$ 2.4***	1.40 $\pm$ 0.23**
exposed	47.5 $\pm$ 0.5	9.5 $\pm$ 0.5***	20.1 $\pm$ 1.2***	2.35 $\pm$ 0.65**

\* = P<0.05    \*\* = P<0.025    \*\*\* = P<0.005

Table 10. Effects of exposure to mammalian grazers on plant size (length of main shoot) and nutlet production (per plant and per plot) of *Amsinckia* (both sources) at 3 reintroduction sites, 1991. Fenced-t (= treatment means  $\pm$  SD, n = 9) and exposed (n = 2) values for a single site followed by the same number of asterics are statistically different (ANOVA) at the indicated probabilities.

	shoots grazed (% of plants w/)	mean plant size (cm)	nutlet production		total nutlets ( $\Sigma$ plots)
			mean (# / plant)	mean (# / plot)	
<b>BD II</b>					
fenced-t	0.0	23.6 $\pm$ 4.7	44.5 $\pm$ 22.1	950 $\pm$ 523	
exposed	3.4 $\pm$ 3.4	22.1 $\pm$ 1.3	32.7 $\pm$ 8.5	418 $\pm$ 200	83
<b>LV</b>					
fenced-t	0.0	13.5 $\pm$ 1.4	8.7 $\pm$ 1.4	236 $\pm$ 113	
exposed	8.4 $\pm$ 8.4	11.8 $\pm$ 2.6	7.2 $\pm$ 2.4	202 $\pm$ 46	403
<b>CR</b>					
fenced-t	0.0***	27.1 $\pm$ 2.5*	29.0 $\pm$ 7.5*	1323 $\pm$ 415***	
exposed	66.4 $\pm$ 10.4***	21.4 $\pm$ 2.6*	13.7 $\pm$ 5.3*	110 $\pm$ 42***	219

\* = P<0.05    \*\* = P<0.025    \*\*\* = P<0.005

damage. As a result, there were no significant impacts of exposure when these unfenced plots were compared to the fenced treatment plots. Nutlet production was low for reasons related to poor *Amsinckia* performance at the LV site discussed previously. The 4% yield model predicts that next year's exposed population at LV would be 16 plants, a decrease of 72%.

Cattle significantly impacted *Amsinckia* in the exposed plots at CR. Plant density, survivorship, mean plant size, and nutlet production were reduced during the four month period when growing plants and grazing cattle coincided. The first mortality due to livestock (a crushed seedling) occurred when large, circular hoofprints, (8-10 cm in diameter) were found in exposed plots #13 and #14 on 31 January. On 13 March, 7 new prints (smaller than the first, 6-8 cm diameter, circular) were found on the 2 plots and a total of 29 plants disappeared completely or had been crushed. Another 13 plants had shoots removed but were still alive. Although 55 plants were alive at the time of peak flowering (5 April), only 16 had open flowers and developing nutlets (see Appendix D). Most of the non-reproductive plants had been previously grazed and were responding by sending out 2-5 axillary branches. Those branches were leafy and some had small inflorescences. It is possible that these new inflorescences could have borne nutlets later in the month, although the soil was drying rapidly. Otherwise, the estimate of total nutlet production by exposed plants at CR was 219. This means that next year's exposed population could be as few as 9 plants, indicating a 53% decline (compared to a 20% increase for the fenced CR population).

### Conclusions and Management Recommendations

1) Regarding the hypotheses in Table 1:

a) The hypothesis that annual grass cover has no effect on the demographic performance of *Amsinckia grandiflora* is accepted with respect to *in situ* germination. It is rejected, however, with respect to survivorship to reproduction, which was significantly reduced by the presence of annual grasses. Therefore, annual grass cover must be controlled in order to promote population growth and stability of this highly endangered plant. These findings were also observed during the 1989-90 reintroduction at Lougher

Ridge (Pavlik 1990).

Unlike the 1989-90 reintroduction at Lougher Ridge, annual grasses did not significantly reduce mean plant size, maximum plant size or nutlet production at any of the reintroduction sites. This discrepancy has been ascribed to the unusual pattern of rainfall and temperature in 1990-91. The rains of March in particular seem to have prevented intense competition during the period of maximum growth and reproduction. These observations have direct management implications. Years with below-normal rainfall in October, November, December, and January (such as 1990-91) would not require the manipulation of *Amsinckia* populations with fire or herbicide treatments. Years with near- or above normal rain in late fall (such as 1989-90), however, would require the manager to manipulate the population by treating with an appropriate herbicide a few weeks after grass emergence.

The physiological performance of *Amsinckia* was significantly reduced by annual grass cover, but not to levels that impaired nutlet production. In years without heavy spring rains, however, it is likely that competition for water is an important determinant of *Amsinckia* performance in mesic annual grassland.

b) The hypothesis that demographic and physiological performance will not vary with annual grass cover between reintroduction sites is rejected. Annual grass cover was highest at Black Diamond II (BD II), intermediate at Los Vaqueros (LV), and lowest at Connolly Ranch (CR). Correspondingly, reductions in survivorship occurred at BD II and LV, but not at CR. In addition, *Amsinckia* plants in control plots at BD II had significantly lower internal water status compared to treated plots, demonstrating the effectiveness of the dense grass canopy in reducing soil water availability at that site. With regards to future reintroductions these data indicate that choosing sites with low grass cover is more important than choosing sites based on annual rainfall or slope.

c) The hypothesis that grazing mammals will not have significant impacts on *Amsinckia* during reintroduction is rejected. Both deer and cattle were shown to significantly decrease the density and survivorship of *Amsinckia*, either by trampling or browsing the plants directly. Cattle, however, were especially detrimental because they reduced plant size and nutlet production during the critical April to May period of reproduction. It was clear from the results at CR (where the fenced population was

predicted to grow by 20% in the next year while the exposed population would decrease by 53%) that livestock and *Amsinckia* were incompatible despite the abundance of alternate feed and the presence of fiddleneck alkaloids. If the fenced population does grow in the coming years (and it should), a conservation easement or the development of post-dispersal stocking schedules (in which cattle are allowed access to the site only during the May to September period) need to be explored.

2) Regarding the recovery effort in general, two new, growing populations of *Amsinckia grandiflora* were created at the northern and southern extremities of historic range (Table 11). At Black Diamond II (BD II), 288 plants survived to produce 11,282 nutlets in May 1991. This new population is predicted to growth by 40% in the coming year. At Connolly Ranch (CR), 580 plants produced 17,302 nutlets and should grow by 20% if protected from grazers during the spring. Although 374 plants survived to reproduce at Los Vaqueros (LV), they produced only 3,202 nutlets. The LV population is expected to decline by 65% or more in the coming year.

The growing population at BD II will consist of plants from both the fenced and the exposed subpopulations. At CR only the fenced subpopulation will grow under the current circumstances, but it is expected do so vigorously. Neither subpopulations at LV are expected to grow due to poor conditions primarily related to physical environmental factors (e.g. cold site temperatures) rather than grazing.

3) The question of long-term management of the populations has been somewhat clarified by the contrasting precipitation patterns of 1989-90 and 1990-91. In years with heavy (i.e. normal or above-normal) rainfall in October or November, a manager should plan an early winter effort to control competition from annual grasses. Minimally, this would involve treatment with a dilute solution of a grass-specific herbicide. In years with droughted fall and early winter months, treatment does not appear necessary.

The frequency of site burning to improve the community composition has yet to be established. A combination of burning early in the fall and the use of a dilute, grass-specific herbicide in the late winter would manage overall for the native perennials and forbs (including *Amsinckia*), even though the subsequent populations of *Amsinckia* might not be significantly enhanced (see Second Year Management of the Lougher Ridge Population). Intensive treatment of habitat on a large scale and concurrent restoration of

perennial grass cover are required in order to ensure population stability of *Amsinckia grandiflora* within the community.

Table 11. Summary of the 1990-91 reintroductions. Amplif. = amplification, the ratio of nutlets produced by the population to the number of nutlets input (1200 for fenced, 200 for exposed subpopulations). Predicted population growth estimated by assuming the 4% yield measured at Lougher Ridge, 1990-91.

	total # repro plants	# of repro Site 300 plants	mean plant size (cm)	total # nutlets produced	total # Site 300 nutlets	nutlet amplif. factor	predic. pop. growth (%)
<b>BD II</b>							
fenced	264	23	22.2	10,446	831	8.7	+58
exposed	<u>24</u>	--	22.8	<u>836</u>	--	4.2	+38
$\Sigma$	288			11,282			
<b>LV</b>							
fenced	316	13	14.0	2,801	127	2.3	- 65
exposed	<u>58</u>	--	12.6	<u>401</u>	--	2.0	- 72
$\Sigma$	374			3,202			
<b>CR</b>							
fenced	561	27	27.8	16,813	799	14.0	+20
exposed	<u>19</u>	--	24.2	<u>219</u>	--	1.1	- 53
$\Sigma$	580			17,032			

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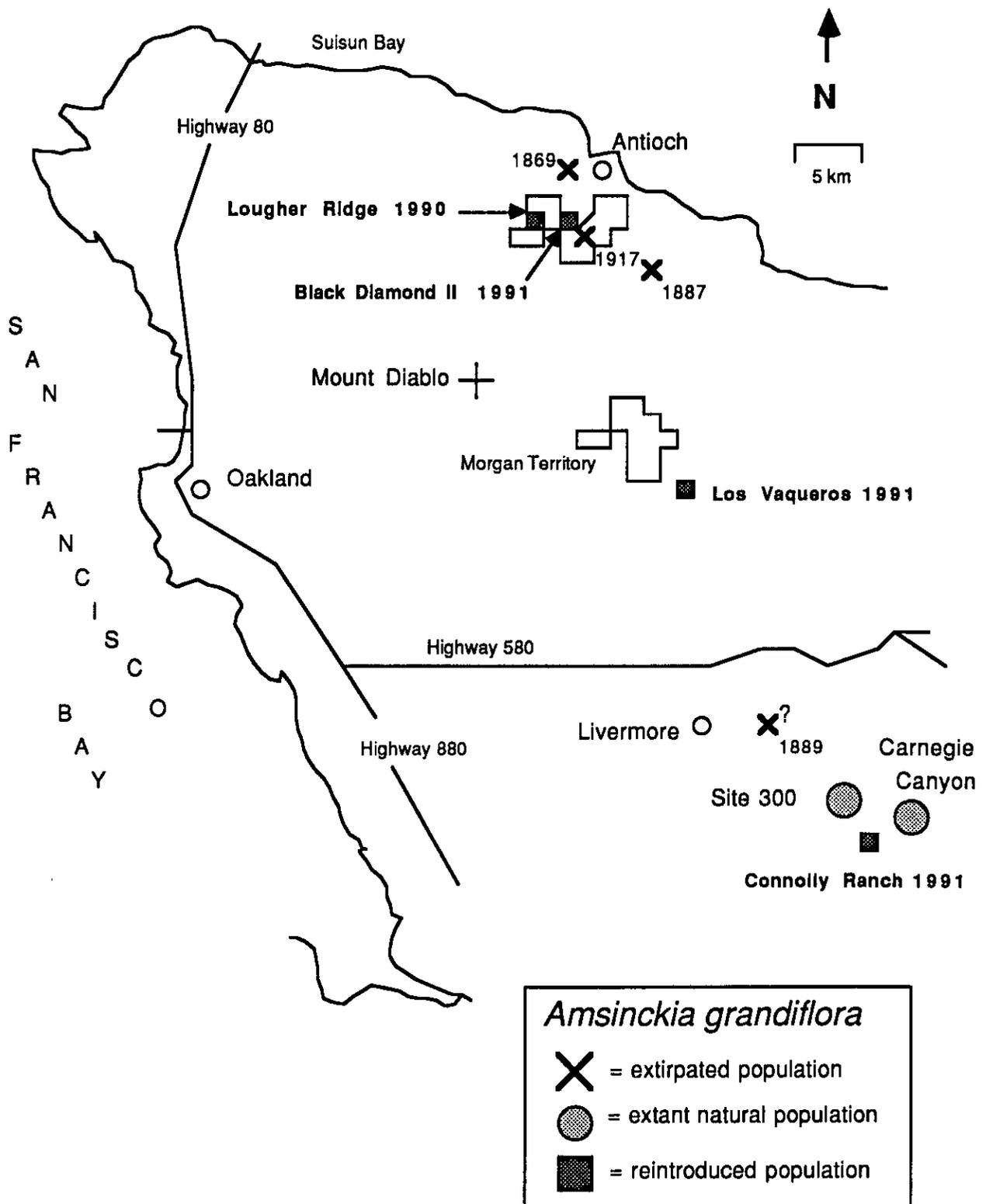


Figure 1. Map of extirpated, extant natural, and reintroduced populations of *Amsinckia grandiflora*, June 1991. Data on extirpated populations from the California Natural Diversity Data Base, Sacramento. Scale is approximate.

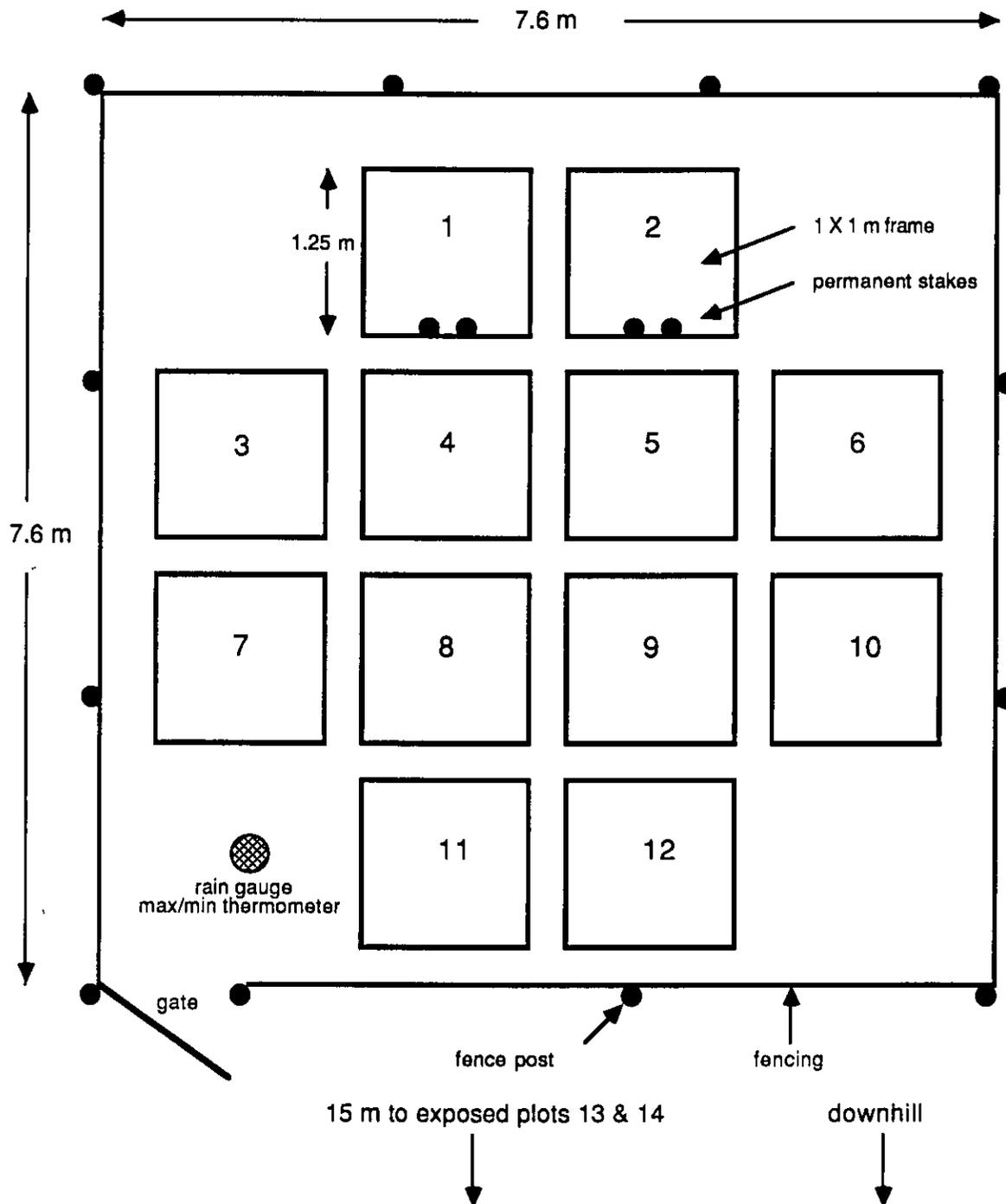


Figure 2. Layout of plots used at the three reintroduction sites, 1990-1991. Control (untreated) plots were randomly selected from plots 1, 2, 3, 6, 7, 10, 11, 12. Plots 4, 5, 8, and 9 each contained 25 Site 300 outlets.

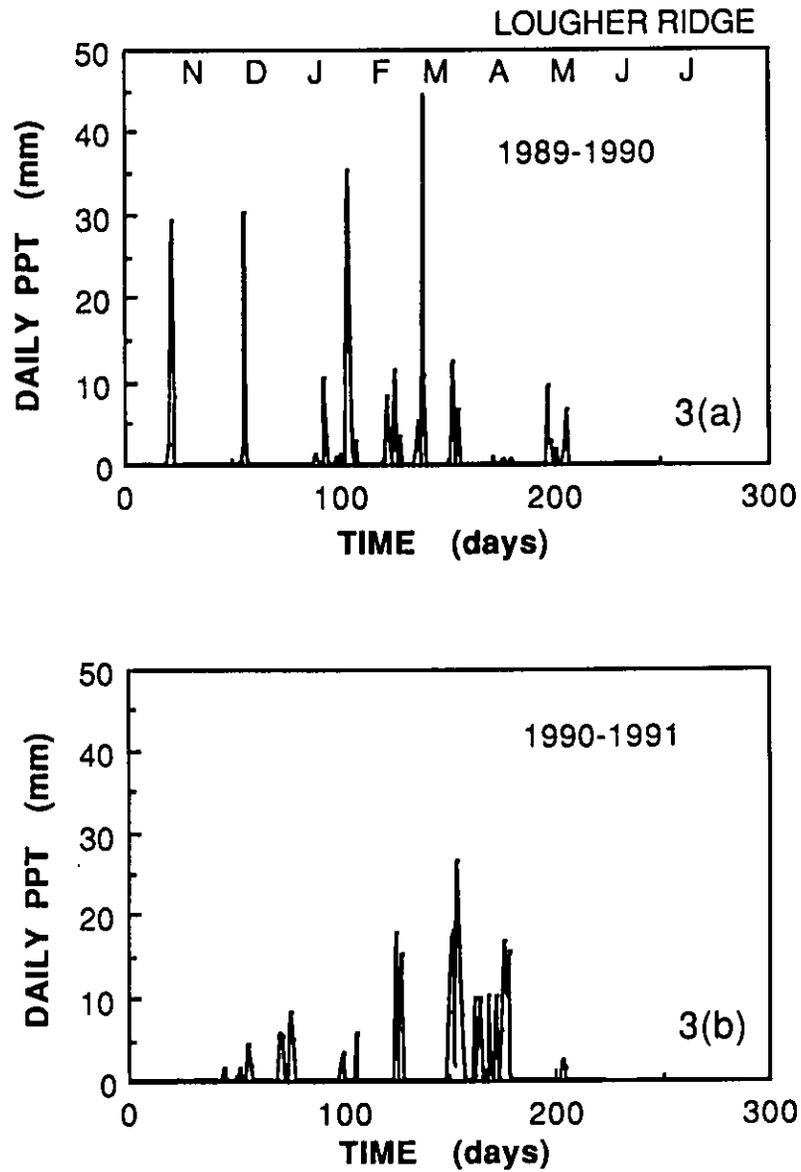


Figure 3. Comparison of precipitation patterns at Lougher Ridge; a) 1989-1990, b) 1990-1991. Day 0 = October 1.

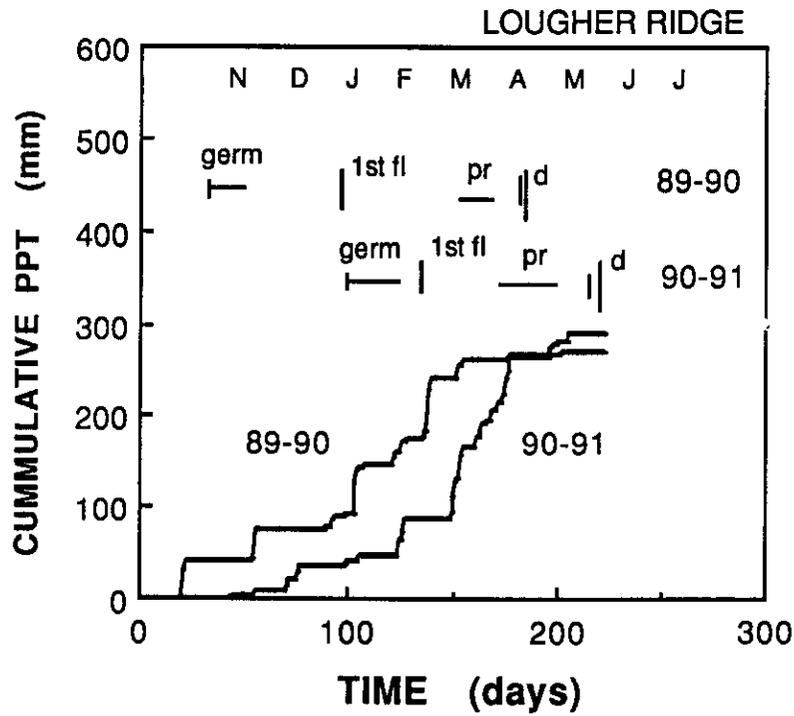


Figure 4. Comparison of cumulative precipitation and phenology of *Amsinckia grandiflora* at Lougher Ridge, 1989-1990 and 1990-1991. Germ = germination, 1st fl = date of first floral anthesis, pr = peak reproduction (floral display and nutlet formation, d = death.

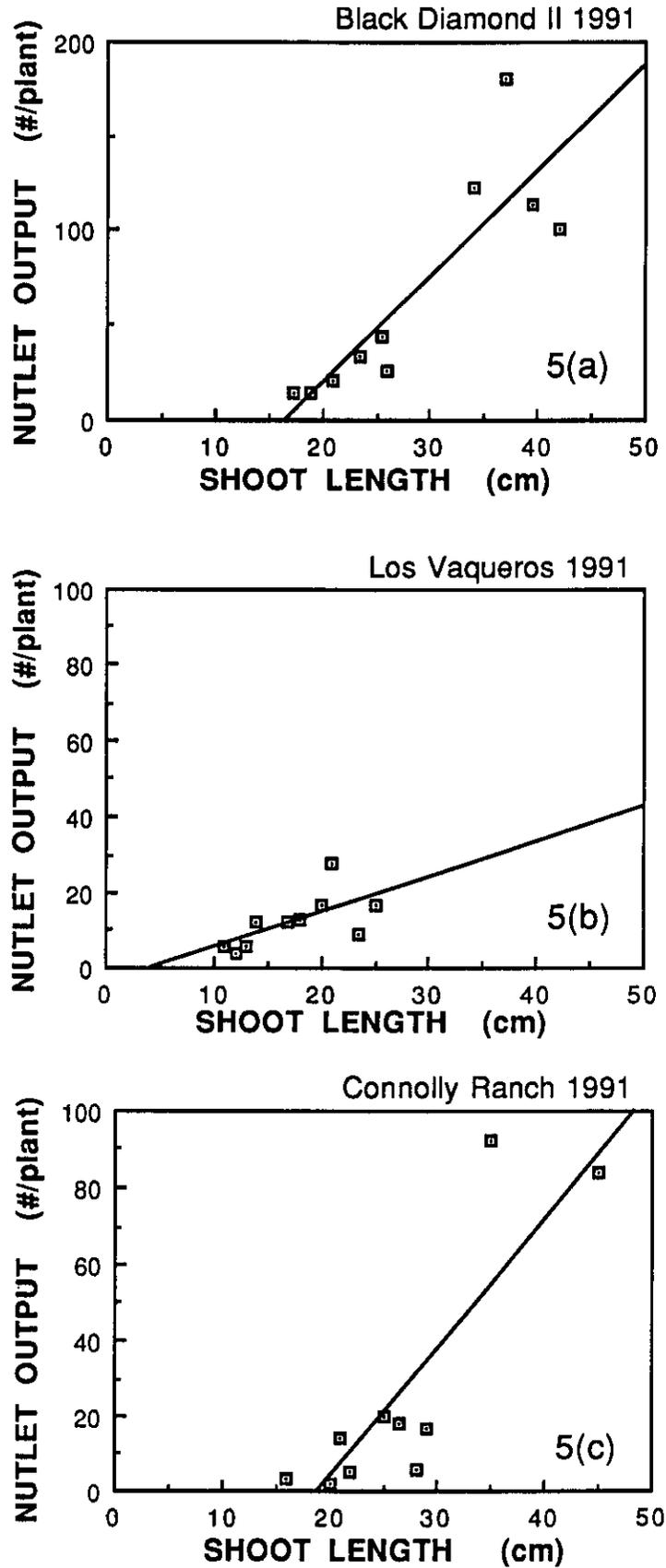


Figure 5. Nutlet output as a function of shoot length for *Amsinckia grandiflora* at three reintroduction sites, May 1991. See Table 6 for line equations.

Appendix A

Sample of a landowner contact letter used during the 1990-1991 reintroduction project.

August 1, 1990

39

Pat Connolly  
P.O. Box 811  
Tracy, CA 95376

Dear Mr. Connolly,

Efforts are currently underway to reintroduce endangered plant species throughout their former ranges. As a part of that effort, we are working with Amsinckia grandiflora, commonly called the Large-flowered Fiddleneck, which is one of the rarest and most endangered plants within the United States. A 1988 study by the Center for Plant Conservation identified it as a species likely to go extinct within five years if recovery actions are not taken. Although it was never a common or widespread species, it was known to occur from Antioch in Contra Costa County to Corral Hollow just east of Livermore in San Joaquin County. So far we have succeeded in establishing a new population in the northern portion of this historic range at Black Diamond Mines Regional Preserve (East Bay Regional Park District) and are now seeking sites further south.

We are contacting you because portions of your land were probably home to this plant a century ago. We would like to know if you would be interested in again hosting this species. We are hoping the project could begin in late September 1990. To facilitate this, we are enclosing; 1) a map identifying the site on the property which would be suitable for the project, 2) a summary of information describing our activities during the project, 3) a description of the initial project at Black Diamond Mines, and 4) photos of the plant and the reintroduction project.

California has been blessed with a great diversity of native wildlife and plants, but the increasing pressures of a growing human population threaten this. Scientists recognize the vital role that genes from wild plants are playing in genetic experiments and remind us that the loss of any single species is irreversible. Our work to recover the large-flowered fiddleneck represents the most carefully planned and executed attempt of its kind in California. The preservation of our natural heritage cannot be accomplished without the cooperation of both public land managers and private landholders. Please consider this opportunity to support the protection of biological diversity and the infinite possibilities for the future that it holds in store.

Questions regarding the specifics of the project can be directed to Dr. Bruce Pavlik c/o Biology Department, Mills College, 5000 MacArthur Blvd., Oakland Ca., 94613, (415)430-2158 or to Karen Heisler at (415)386-6026. The lead agency is California Fish and Game Endangered Plant Project and contact can be made through Ann Howald at (916)323-6201.

Thank you for your consideration,

Bruce Pavlik  
Karen Heisler

A New Population of Amsinckia grandiflora  
at Black Diamond Mines Regional Preserve

The recovery plan for Amsinckia grandiflora calls for the establishment of four new Amsinckia populations within historic range in order to reduce the probability of extinction. Beginning in 1988, we used existing data on the distribution and ecology of the species to characterize and evaluate the most important biological factors affecting Amsinckia and to locate and rank potential sites for new populations based on biologic, land use and logistic criteria. Out of a total 55 candidate sites, 12 were ranked as finalists with respect to the re-introduction effort. The first site was Stewartville 1, within Black Diamond Mines Regional Preserve.

The first year results of the re-introduction are encouraging; controlled experiments identified habitat treatments most effective in fostering seedlings of the large-flowered fiddleneck. As a result more than one third of the 3000 seeds planted last October grew to survive and produce an estimated 35,000 seeds this spring. We are continuing to monitor this population in order to insure that it will be self-sustaining with a minimum of management in the future. However, in order to achieve the goal of removing A. grandiflora from the endangered species list, three additional re-introduction sites must be established. Two years of research were committed to studying the flowering plant's environmental requirements and forces responsible for its decline. We now turn to other localities that were identified as potentially supportive and accomodating to a re-introduced plant community

The planning of this long-term recovery project included careful consideration of any risks to the public. The toxicity of Amsinckia species to horses was taken into account and it was determined that the experimental re-establishment of Large-flowered Fiddleneck does not pose a significant additional threat to horses for a number of reasons. Mr. Ron Kelley, a Ph.D. candidate at the University of California at Davis, who has studied the chemistry of all ten species of fiddleneck, has demonstrated with his work that Amsinckia grandiflora contains fewer toxic chemicals in lower amounts than the more widespread species of this genus. Furthermore, observations of the one remaining natural population of large-flowered fiddleneck, at Lawrence Livermore Laboratory's Site 300, show that the population has not spread beyond its original area of distribution for at least 15 years. Another important factor in assessing the degree of additional risk posed by the project is the fact that the common fiddleneck (Amsinckia intermedia) is already widespread throughout the Diablo Range and much of California.

The new re-introduction sites should be about 25 by 25 feet, fenced if currently grazed, and permit access by vehicle several times a year. There are no costs to the landowner; agreements regarding site location and access can be reached ahead of time so that all parties are comfortable with the arrangements.

Questions regarding the specifics of the project can be directed to Dr. Bruce Pavlik c/o Biology Department, Mills College, 5000 MacArthur Blvd., Oakland Ca., 94613, (415)430-2158 or to Karen Heisler at (415)386-6026. The lead agency is the California Fish and Game Endangered Plant Project and contact can be made through Ann Howald at (916)323-6201.

Appendix B

Letter of agreement between the Contra Costa Water District and the Endangered Plant Program for access to the Los Vaqueros reintroduction site, 1990-1991.

## DEPARTMENT OF FISH AND GAME

P.O. BOX 944209

SACRAMENTO, CALIFORNIA 94244-2090



(916) 445-3531

November 14, 1990

Mr. Ed Seegmiller  
General Manager  
Contra Costa Water District  
PO Box 4121  
Concord, CA 94524

Dear Mr. Seegmiller:

The Department of Fish and Game (DFG) has requested the assistance of the Contra Costa Water District (CCWD) in a project to recover a rare plant, the State and federally endangered large-flowered fiddleneck (*Amsinckia grandiflora*), by establishing it in a portion of its historic range at a site in the Kellogg Creek watershed, within the Los Vaqueros Project area. This letter describes the responsibilities of the DFG, CCWD, and the Principal Investigator with regard to this cooperative effort.

Establishment of new populations of large-flowered fiddleneck within its historic range is called for in the U.S. Fish and Wildlife Service's Draft Recovery Plan for the species. Recovery, as defined by the Plan, calls for the establishment of four new populations, each one consisting of 2,500 individuals, and the enhancement of the natural population at Lawrence Livermore National Laboratory's Site 300 to a population size of 2,500 individuals. DFG is named as the agency responsible for carrying out the majority of the Recovery Plan objectives, including the establishment of new populations.

Since 1987 DFG has contracted with Dr. Bruce Pavlik of Mills College in Oakland to carry out the recovery project for large-flowered fiddleneck. In 1989 Dr. Pavlik completed the first experimental reintroduction at Black Diamond Mines Regional Preserve. The results of this project have been presented in a 1990 report furnished to CCWD. Dr. Pavlik is the Principal Investigator for the CCWD Los Vaqueros large-flowered fiddleneck establishment project and will complete the proposed studies under contract to DFG.

Both DFG and Dr. Pavlik agree to comply with the conditions of CCWD's policy documents for the Los Vaqueros Project, including (1) the Los Vaqueros Project Interim Fire Management Plan, (2) grazing leases on Los Vaqueros Project lands, (3) CCWD Resolution 86034, which makes findings of fact to support adoption of the Stage 1 Environmental Impact Report on the Los Vaqueros/Kellogg Reservoir Project, and (4) CCWD Resolution 88-45, which affirms the CCWD's goals and objectives for the Los Vaqueros Reservoir Project.

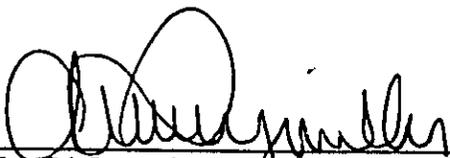
In addition, both DFG and Dr. Pavlik agree to travel only on established roads and to conform to any additional restrictions communicated to us in writing by the Los Vaqueros Program Manager.

Dr. Pavlik and DFG agree to meet with CCWD staff periodically, if requested, to discuss the project and to provide CCWD with copies of all final reports produced as a part of this study.

The CCWD agrees to allow Dr. Pavlik to conduct an experimental establishment of large-flowered fiddleneck at the designated site in the Kellogg Creek watershed and to monitor the results of the initial experiment for five years. The experimental establishment will be carried out according to the specifications in the DFG Contract #FG-9517 and in the "Proposal for Continuing Reintroduction and Recovery Efforts Related to Amsinckia grandiflora." For the purposes of completing this study, CCWD will grant Dr. Pavlik, a small number of students under his direct supervision, and DFG staff, access to the study site.

If the results of the five-year monitoring program indicate that the large-flowered fiddleneck is likely to survive over the long-term at the Los Vaqueros site, then CCWD and DFG will explore options for providing protection in perpetuity for the area occupied by the fiddleneck population.

When this agreement has been signed by all parties, please return a copy to Ms. Susan A. Cochrane, Chief, Natural Heritage Division, at the letterhead address.



Mr. Ed Seegmiller, General Manager  
Contra Costa Water District  
Concord, California

Signed,

11-21-90

Date



Mr. Pete Bontadelli, Director  
Department of Fish and Game  
Sacramento, California

11-15-90

Date



Dr. Bruce Pavlik, Professor of Biology  
Mills College  
Oakland, California

11-30-90

Date

cc: Ms. Susan A. Cochrane, Chief  
Natural Heritage Division



**CONTRA COSTA  
WATER DISTRICT**

1331 Concord Avenue  
P.O. Box H20  
Concord, CA 94524  
(415) 674-8000 FAX (415) 674-8122

(415) 439-9169 Toll Free from  
Eastern Contra Costa County

December 10, 1990

Directors  
Bette Boatman  
*President*

Ronald E. Buller  
*Vice President*

Donald P. Freitas  
Daniel L. Pellegrini  
Paul F. Hughey  
Ed Seegmiller  
*General Manager*

**Mr. Pete Bontadelli, Director  
Department of Fish and Game  
P. O. Box 944209  
Sacramento, CA 94244-2090**

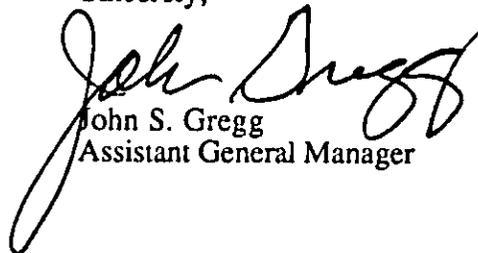
Attention: Ms. Susan A. Cochrane  
Natural Heritage Division

Dear Mr. Bontadelli:

I am enclosing a copy of the Letter of Agreement concerning the Large-flowered Fiddleneck introduction on lands of the Contra Costa Water District. All three parties have signed the Agreement and a copy has been transmitted to Dr. Bruce Pavlik.

The Water District is proud to play a part in the recovery of this plant and we sincerely hope that our contribution results in the continued preservation of our natural heritage.

Sincerely,



John S. Gregg  
Assistant General Manager

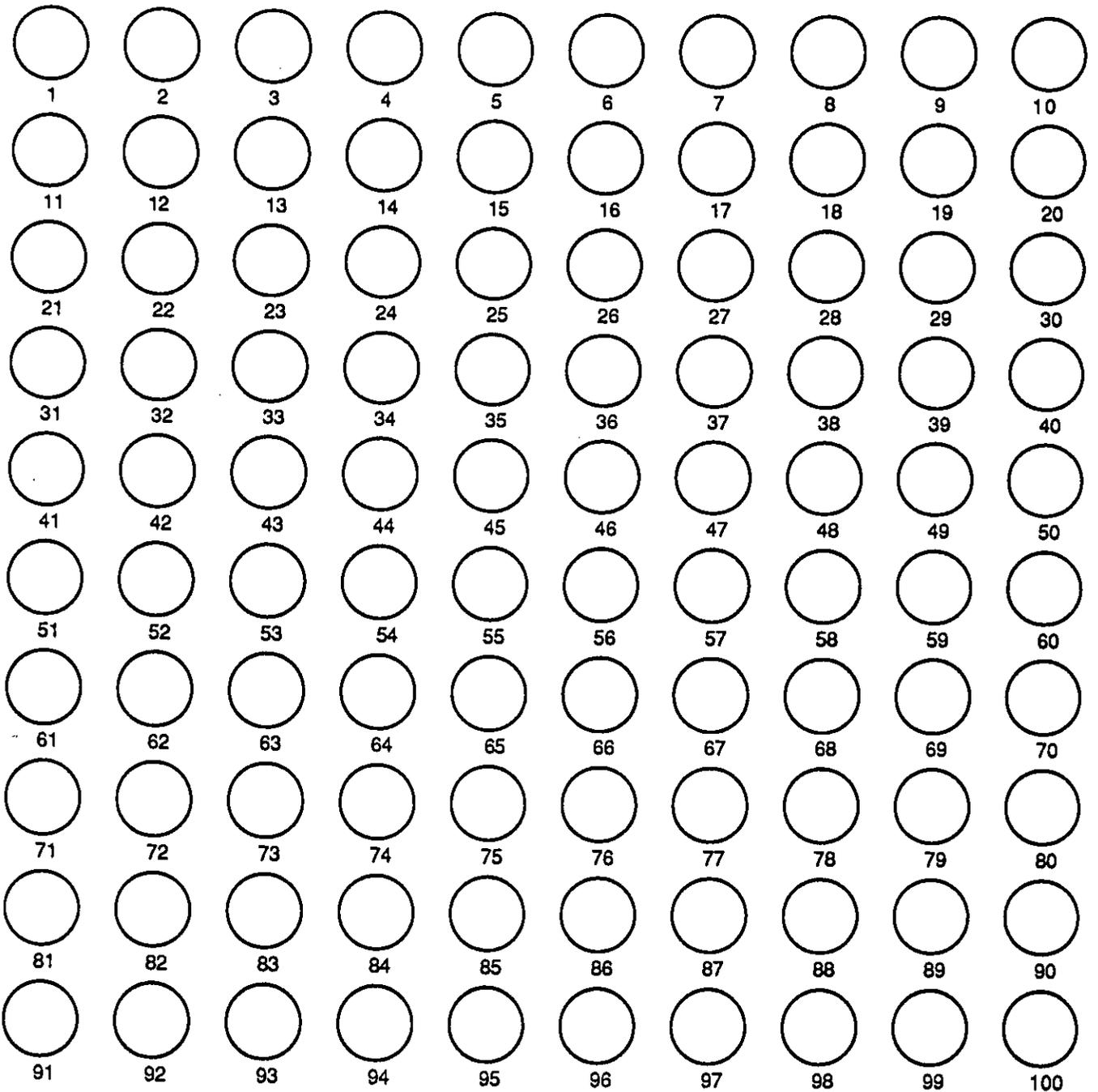
JSG:llc  
Enclosure

cc w/enc.: Dr. Bruce Pavlik  
Ed Seegmiller  
Jan Harski (original agreement)

CCN: 12464  
CO 3.56.1

Appendix C

Examples of data sheets used during the *Amsinckia grandiflora* reintroduction project.

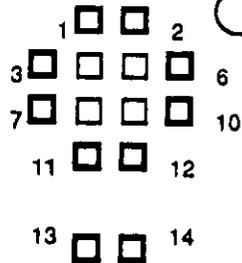


species \_\_\_\_\_ plot \_\_\_\_\_ date \_\_\_\_\_

notes \_\_\_\_\_

recorded by \_\_\_\_\_

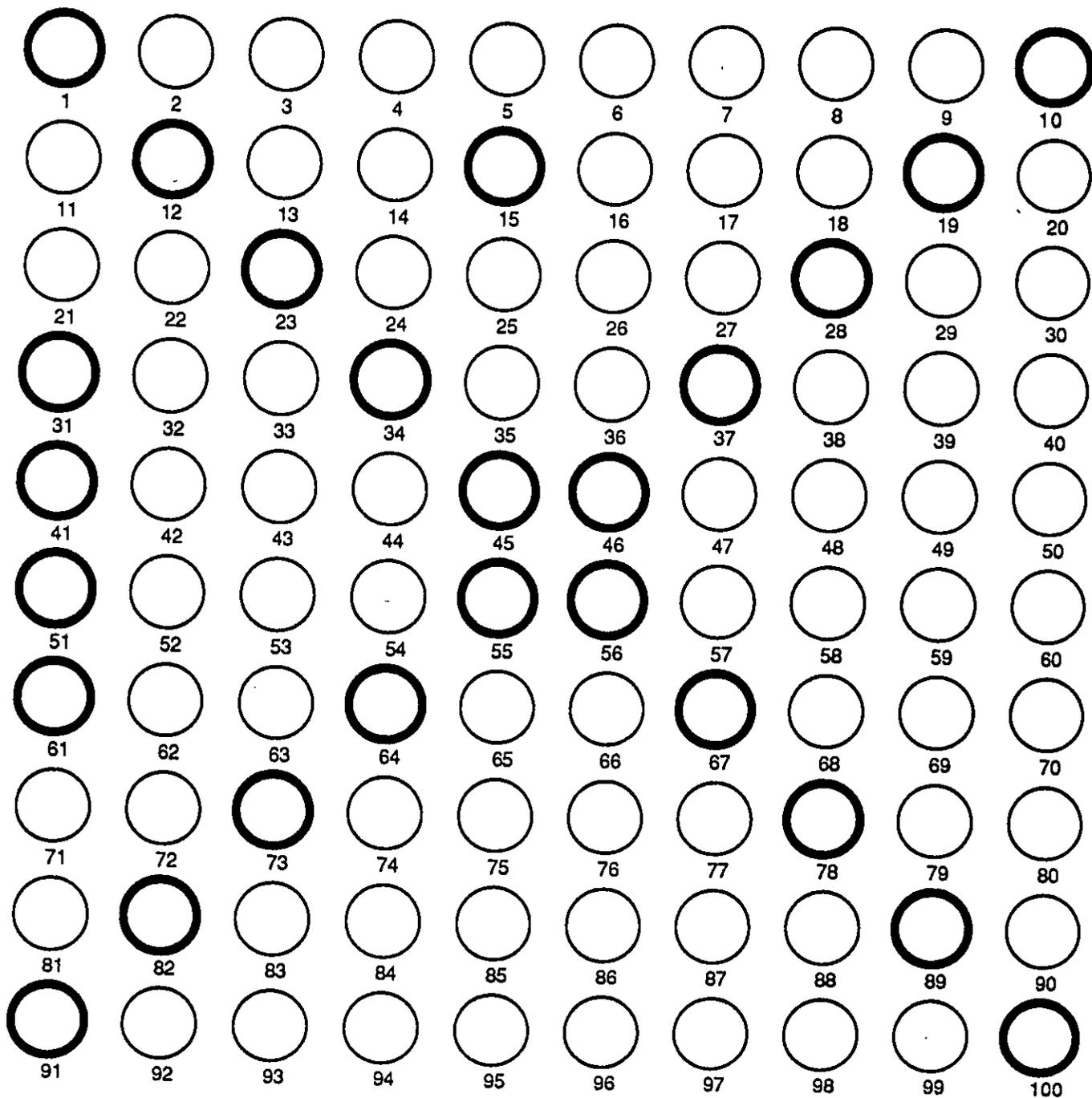
- = live      P = pin
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- X = missing



**Davis 88**

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- T =

- total**
- live =
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  - T =



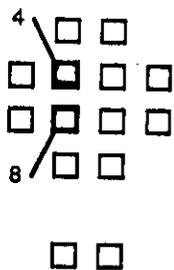
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plot \_\_\_\_\_ date 1/91

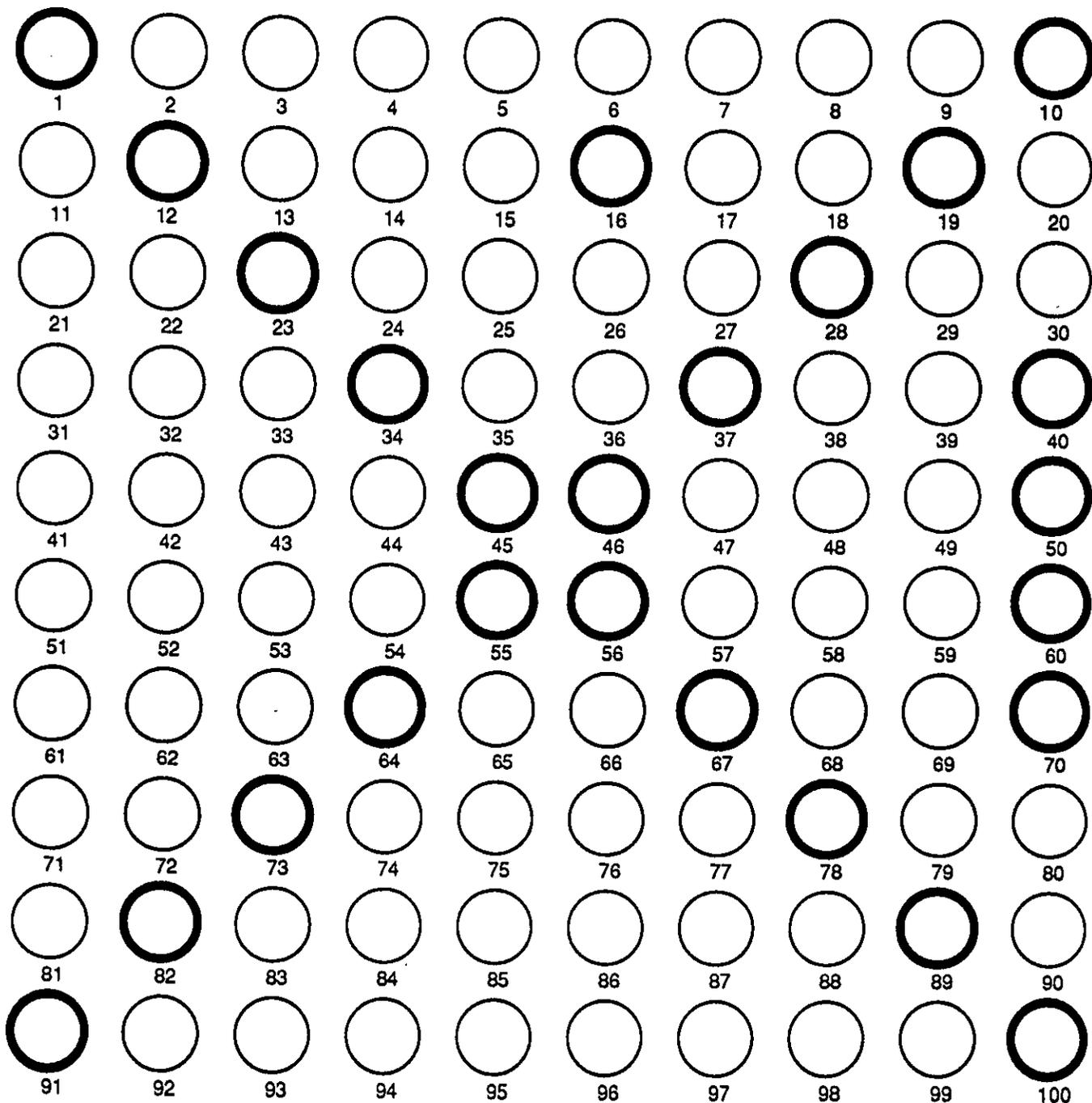
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species Amsinckia grandiflora

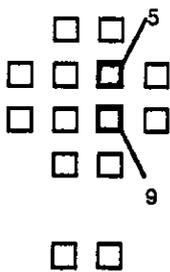
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**Davis 88**

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Appendix D

Photographs of the *Amsinckia grandiflora* reintroduction project, 1990-91.

- a) A portion of the Los Vaqueros reintroduction site immediately after sowing and burning, November 1990. Red flags mark the position of native perennial grasses. An unburned control plot is in the right foreground (bottom of photo).
- b) A portion of the Black Diamond II reintroduction site, May 1991. Note the treatment patches of *Amsinckia grandiflora*, surrounded by dense annual grass cover. An untreated control plot is behind the meter stick held by Vicki Bates with no visible flowers.
- c) Connolly Ranch reintroduction site, April 1991. Note the showy patches of *Amsinckia grandiflora* and the ground cover of *Microsteris gracilis*.
- d) *Amsinckia grandiflora* at Los Vaqueros reintroduction site, May 1991. This was a typical individual, short (15-18 cm) with few branches. Compare with photo e.
- e) *Amsinckia grandiflora* at Connolly Ranch reintroduction site, April 1991. Plant in foreground was more than 40 cm high, leafy and branched.
- f) Grazed plot #13 at Connolly Ranch, April 1991. Note large hoofprints in center of photo and the lack of flowering individuals of *Amsinckia grandiflora*. Compare with photo g.
- g) Fenced plot #10 at Connolly Ranch, April 1991. Note the large number of flowering *Amsinckia grandiflora*.