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*Amsinckia grandiflora***

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The Recovery of an Endangered Plant.

I. Creating a New Population of *Amsinckia grandiflora*

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Abstract: *The recovery of endangered plants often requires the creation of new populations in order to decrease the risk of extinction. Despite numerous attempts, no plant species have been fully recovered by creating new populations. Here we report on initial efforts to recover *Amsinckia grandiflora* Kleeb. ex Gray (Boraginaceae) by re-establishing the species in appropriate habitat within its historic range, with consideration given to genetic and demographic characteristics of the founding population. An experimental framework with demographic monitoring was used to follow the fates of nutlets (propagules) from two sources (one wild, one cultivated) and to evaluate the effects of habitat manipulations (fire, herbicide application, clipping) for reducing competition from introduced grasses. Founding nutlets from two sources had different germination and genetic characteristics. Nutlets directly descended from a wild population had half the germination potential of nutlets from a cultivated population because of their greater age (about 25 years in cold storage). Levels of genetic variability, as measured by allozyme electrophoresis at 18 loci, were low overall, but much more so in the cultivated population. These data were used to maximize genetic variability among founding nutlets and to predict the frequency of alternative allele carriers in the new population. After sowing and plot treatment at*

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La recuperación de una planta en peligro de extinción. I. Creando una nueva población de *Amsinckia grandiflora*

Resumen: *La recuperación de plantas en peligro de extinción requiere usualmente de la creación de una nueva población para disminuir el riesgo de extinción. A pesar de numerosos intentos, ninguna especie vegetal ha sido totalmente recuperada creando nuevas poblaciones. Nosotros reportamos sobre los esfuerzos iniciales para recuperar *Amsinckia grandiflora* Kleeb. ex Gray (Boraginaceae), reestableciendo especies en hábitats apropiados dentro de su rango histórico, dándole consideración a las características genéticas y demográficas de la población fundadora. Un marco experimental con monitoreo demográfico fue utilizado para seguir el destino de los propágulos desde dos fuentes (una silvestre y otra cultivada) y para evaluar los efectos de las manipulaciones del hábitat (fuego, aplicación de herbicida, desbroce) para reducir la competencia de pastos introducidos. Propágulos fundadores obtenidos de dos fuentes distintas tuvieron germinación y características genéticas diferentes. Los propágulos que descendieron directamente de la población silvestre tuvieron la mitad del potencial de germinación que los propágulos de la población cultivada debido a su mayor edad (~25 años en almacenaje en frío). Niveles de variabilidad genética, medido con electroforesis de aloenzimas en 18 loci, fueron en general bajos, pero mucho más en las poblaciones cultivadas. Estos datos*

the Lougher Ridge re-establishment site, the 3460 founding nutlets produced a large number of germinules (1774) during the 1989–1990 growing season, and many (1101) survived to reproduce. From these plants, and estimated 35,800 nutlets were produced, indicating that the population had a high potential for growth during subsequent years. Introduced grasses had significant negative effects on survivorship to reproduction, plant size, and nutlet production in the new *Amsinckia* population. Grass cover was effectively reduced by using fire or a grass-specific herbicide. Burning significantly decreased *Amsinckia* mortality rates early in the growing season and significantly increased survivorship to reproduction and maximum plant size. Herbicide treatment had no effect on mortality rates or survivorship to reproduction, but it significantly increased plant size and, therefore, nutlet output per plant and per plot. The new population should be able to maintain itself within the existing grassland community, but growth and short-term viability would be assured by management practices that increased the availability of low-competition habitat patches for *Amsinckia* occupation.

Introduction

Recovery plans for endangered plants often call for the creation of new, self-sustaining populations within historic range and characteristic habitat (Knudsen 1987; Whitten 1990). Self-sustaining populations are those in which plants are able to complete all life-history phases in the wild and, therefore, have demonstrated a potential for growth and long-term persistence. Unfortunately, creating new populations that possess the genetic and ecological characteristics of natural populations remains a great challenge. Despite numerous attempts (see Brookes 1981; Morgan & Wilson 1981; Griggs & Jain 1983; Havlik 1987; Falk 1988; Olwell et al. 1987, 1990; Parenti & Guerrant 1990; Reichenbacher 1990; Simonich & Morgan 1990), few created populations appear to be self-sustaining (see DeMauro 1993 for an exception), and no endangered plant taxa have, in fact, been fully recovered. In the United States, recovery leads to “down-listing,” a change from endangered to threatened status, or “de-listing,” removal of protected status, under the Endangered Spe-

han sido utilizados para maximizar la variabilidad genética entre los propágulos establecidos y para predecir la frecuencia de los portadores de alelos alternativos en la nueva población. Después de sembrar y de tratar los plots en el sitio de re-establecimiento en “Lougher Ridge”, los 3460 propágulos establecidos produjeron un gran número de germinules (1774) durante la estación de crecimiento de 1989–1990 y muchos sobrevivieron (1101) para reproducirse. De estas plantas, se estima que 35,800 propágulos fueron producidos, indicando que la población tuvo un gran potencial de crecimiento durante los años subsiguientes. Los pastos introducidos tuvieron un efecto significativamente negativo sobre la supervivencia hasta la edad reproductiva, en el tamaño de la planta y en la producción de propágulos en la nueva población de *Amsinckia*. La cobertura de pastos fue efectivamente reducida con el uso de fuego o con un herbicida específico. La quema decreció significativamente la tasa de mortalidad temprana en la estación de crecimiento en la población de *Amsinckia* e incrementó significativamente la supervivencia hasta la edad reproductiva y el tamaño máximo de las plantas. El tratamiento de herbicida no tuvo efecto en las tasas de mortalidad o supervivencia hasta la edad reproductiva pero incrementó significativamente el tamaño de la planta y por consiguiente la cantidad de propágulos por planta y por plot. La nueva población tiene que ser capaz de mantenerse por ella misma dentro de la comunidad de pastizal existente, pero el crecimiento y la viabilidad a corto plazo debe ser asegurada con prácticas de manejo que incrementen la disponibilidad de una baja competencia en los patches que vayan a ser ocupados por *Amsinckia*.

cies Act if the minimum number of viable populations are achieved (Bartel 1987; Rohlf 1991).

Recovery projects that attempt to create populations of endangered plants have fallen short for a variety of reasons. Some do not address issues of genetic variation with respect to the new population or the taxon as a whole (Clegg & Brown 1983; Hamrick 1983; Guerrant 1992). Others do not use a detailed demographic approach to monitoring that would predict short-term trends and analyze factors that limit establishment and growth of the new population (Menges 1986; Pavlik 1987, 1993). Many simply lack the design and statistical strength of a good experiment, even though these ventures are, by their very nature, experimental (Travis & Sutter 1986). Without rigorous hypothesis testing, no evaluation of demographic characteristics, habitat conditions, or management techniques is possible, and so no conclusions regarding the “success” of the effort will be forthcoming.

In this paper we report on an effort to initiate recovery of a highly endangered plant, *Amsinckia grandiflora* Kleeb. ex Gray (Boraginaceae), by creating new

populations within its historic range. This project was specifically designed to (1) re-establish a self-sustaining population within the northernmost portion of the species' historic range, (2) determine the genetic characteristics of two propagule sources and their demographic expression within the founding population, (3) provide detailed demographic data that would allow evaluation of the new population, and (4) conduct experiments to determine the effects of introduced annual grasses on *A. grandiflora* and to test some potential techniques for minimizing competition.

The Study Species

*Amsinckia grandiflora*¹ is an annual plant of the winter-wet, summer-dry grasslands of northern California. These grasslands were once a rich mosaic of native perennial bunchgrasses and spring forbs that bordered oak savanna prior to the arrival of European settlers. The introduction of livestock grazing, fire suppression, and non-native plants led rapidly to domination of the grasslands by annual species of *Avena*, *Bromus*, *Lolium*, and *Hordeum* (Heady 1977). Native forbs such as *Amsinckia* are still present, but they are faced with deteriorating habitat quality and catastrophic disturbance from changes in land-use patterns and intensity.

Between 1869 and 1917, *Amsinckia* was collected from the hills near Antioch (on the Sacramento–San Joaquin River Delta) and in scattered locations south through the Diablo Range to northern San Joaquin County. It is now known from only three natural populations that lie approximately 24 km east of Livermore, in Alameda and San Joaquin counties. One population is large (about 3200 individuals in 1991) and apparently stable. It is on private rangeland, portions of which have been intensively grazed. The remote, steep, dry, and often rocky topography of the canyon site has excluded most cattle, and so there are only a few signs of light grazing. The second population is much smaller (23 to 355 individuals in recent years), having once been comprised of “thousands” in the mid 1960s (R. Ornduff, University of California at Berkeley, 1989, personal communication), while the third has only 16 to 29 individuals. The second and third populations are found in adjacent canyons on Site 300 of Lawrence Livermore National Laboratory, protected from grazing for nearly 40 years and fire for the last decade or so. With so few, small populations, a deteriorating habitat, and a high potential for disturbance, *Amsinckia grandiflora* has been listed as endangered under both federal and state law. The recovery plan, drafted by the U.S. Fish and Wildlife Service, called for establishing four new *Amsinckia* populations within historic range.

¹*Amsinckia grandiflora* will often be referred to by its generic epithet. Nomenclature follows Munz and Keck (1964).

In addition to deteriorating habitat quality and high potential for anthropogenic disturbance, the species is threatened by several intrinsic characteristics. These include a moderate degree of habitat specificity (deep, loamy soils of sedimentary origin; mesic, north-facing slopes) that may have given the species a restricted and patchy geographic distribution prior to European settlement. Juvenile *Amsinckia* plants are caulescent and perhaps more sensitive to livestock impact than weedy, rosette-forming relatives (for example, *A. intermedia*). Finally, the *in situ* reproductive output of the species is apparently low. Average-sized plants at Site 300 usually produce less than 20 nutlets each, compared to 200–300 by common congeneric species (Pavlik 1988). This has been attributed to the possession of a primitive form of heterostyly (pin-thrum floral morphs) with cryptic self-incompatibility and inbreeding depression (Ornduff 1976; Weller & Ornduff 1977, 1991). Populations of *A. grandiflora* are composed of pin individuals (having flowers with long styles and short stamens) and thrum individuals (having flowers with short styles and long stamens), and seed set is promoted by intermorph pollen transfer. Intramorph pollen transfer and self-pollination greatly reduce seed set (Ray & Chisaki 1957; Barrett 1990), but competition from introduced grasses may also be important.

The possession of heterostyly and cryptic self-incompatibility indicates that *A. grandiflora* has an outbreeding mating system with a potential for high levels of heterozygosity and genetic recombination. This could be maintained only if both pin and thrum individuals were available as mates in the founding population (Clegg & Brown 1983). Pin/thrum ratios in different generations at Site 300 vary between 0.75 and 2.0, but are commonly close to 1.0 (Ornduff 1976). Therefore, it is likely that both pin and thrum individuals would be present in a large founding population and that outcrossing would be maintained.

Methods and Materials

Site Selection and Microsite Evaluation

The site selection process for the new *Amsinckia* population was described in detail by Pavlik and Heisler (1988). Many factors were taken into consideration, some ecological (macroclimate, soil, slope, exposure, community associates, habitat size, and degree of disturbance), and others logistic (land use history, road access, property ownership, size). Ranking of the ecological factors was done by consensus: five members of the recovery team, all of whom had field experience with the species, were asked to evaluate the relative importance of the factors with respect to population vigor. This provided a subjective ecological profile against which site characteristics could be compared. A total of 55 “candidate” sites within historic range were first

identified on topographic and soil survey maps using soil type and slope criteria from the profile. These were further evaluated with respect to logistic factors: a high scoring site would be on public land, with dirt road access, no nearby residential or agricultural development, and at least 1.6 km across its longest dimension. This left 27 "nominee" sites that were examined for community characteristics using recent aerial photographs and then surveys in the field. Final selection of the Stewartville site was based on favorable habitat characteristics (mesic grassland on north-facing slopes, loamy soils of the Altamont-Fontana complex) and public ownership (East Bay Regional Park District), and because two collections of the species had been made in the general vicinity between 1869 and 1917.

The exact location of the re-establishment site (the microsite) was determined from field and laboratory studies conducted during the spring of 1989. A field survey of five potential microsites near Stewartville was conducted. Each met several major criteria for the re-establishment experiment (suitable habitat, large size, relatively homogeneous in character, remote but accessible). Furthermore, data on soils, flora, and standing crop were obtained and compared to similar data from natural populations at Site 300. These comparisons showed that Lougher Ridge was the only microsite that was similar to existing *Amsinckia* habitat in terms of soil moisture, the presence of co-occurring species, and total standing crop of dominant grasses. In addition, *Amsinckia* nutlets grown in Lougher Ridge soil under greenhouse conditions had the best performance in terms of germination, survivorship, and growth.

Acquisition and Characteristics of the Founding Propagules

HISTORY OF THE NUTLETS

All of the propagules (nutlets) of *Amsinckia* used in this recovery effort were ultimately derived from collections made by Dr. Robert Ornduff (University of California at Berkeley) at Site 300 in the mid-1960s. At this time the population was very large, and nutlets were taken from many different individuals. As a result, the collection probably contained a representative sample of the existing genetic variation (Guerrant 1992). Nutlets were stored in paper pouches at 4° C and periodically removed for propagation and study. Dr. Ornduff donated about 2000 nutlets (subsequently called the Site 300 or wild source) to the recovery project in 1989.

In the mid-1980s, Dr. Ornduff had also given nutlets to Drs. Daniel Pantone and Ronald Kelley at the University of California at Davis, who were studying several species in the genus. Pantone and Kelley were able to grow *Amsinckia* in an experimental garden and to ob-

tain large, reproductive plants during 1987, 1988, and 1989. Unlike plants observed under natural conditions at Site 300, the Davis plants grew to exceed 1 m in height, and each produced hundreds of nutlets. About 3000 nutlets from the 1988 crop (referred to as the Davis or cultivated source) were donated to this project in 1989.

GERMINATION TRIALS

The Site 300 and Davis sources constituted a pool of nutlets to serve as founders of the new population. Laboratory germination trials were conducted in July 1989 (four months prior to sowing) to estimate the maximum number of germinules that could be produced in the field. An average of 59% of the Davis and 31% of the Site 300 nutlets germinated on moist filter paper after 10 days in constant darkness and at room temperature. Greenhouse germination in soil from the microsite was generally lower, with 55% of the Davis and 20% of the Site 300 nutlets producing germinules. Seedlings from laboratory tests were then used to determine the genetic characteristics of the nutlets by means of starch gel electrophoresis.

ALLOZYME VARIATION

Information on genetic variation within nutlet sources was needed in order to maximize the frequency of alternative alleles in the founding population (Clegg & Brown 1983; Hopper & Coates 1990). A beginning hypothesis was that the nutlets from the Site 300 source, taken from a once large natural population, would possess higher allelic diversity and heterozygosity than the population currently in existence. In addition, preliminary studies of plants grown from the 1987 Davis source revealed a pin/thrum ratio of 4.0, indicating that sampling or selection under cultivation had skewed the genetic structure of that source population (Lacy 1987; Pavlik 1988). Any such differences would have to be taken into account when formulating the mixture of nutlets for re-establishment and for making predictions about levels of genetic variability in subsequent generations of the new population.

Plant material and extraction. Seedlings from the July 1989 germination trials were transferred to tissue culture boxes (Magenta®) containing sterile perlite. These were given 0.5 × Hoagland solution and kept under a fluorescent light (14 hr/10 hr photoperiod). Seedlings were allowed to grow for 20 days after germination, during which time they attained the 4–6 leaf stage (excluding cotyledons).

The fresh weight of individual seedlings ranged from 25 to 100 mg wet weight. Seedlings (including primary leaves, roots, and stems) were cut into pieces, placed in the wells of a cold acrylic plate, and homogenized with

a stainless steel pestle in extraction buffer (see Nickrent 1986). Depending on the size of the individual, 80 to 100 μ l of a pH 7.5 extraction-buffer (Nickrent & Wiens 1989) containing 5% (w/v) PVP-40 and 0.1% mercaptoethanol was used. The homogenate was absorbed onto filter paper wicks and loaded immediately into the gel.

Starch gel electrophoresis. Eleven enzyme systems encoded by 18 presumptive gene loci were resolved for this study. Two buffer systems were used: the pH 8.1 lithium hydroxide system reported in Ridgeway et al. (1970) and the pH 6.0 histidine-citrate system described in Nickrent and Wiens (1989). The following enzyme system loci were resolved on the lithium hydroxide buffer: AAT-1, -2, -3 (aspartate amino transferase, E.C. 2.6.1.1), LAP (leucine amino peptidase, E.C. 3.4.11.1), PGI-1, -2 (phosphoglucoisomerase, E.C. 5.3.1.9), and PGM (phosphoglucomutase, E.C. 2.7.5.1). The following enzyme system loci were resolved on the histidine-citrate buffer: ACO (aconitase, E.C. 4.2.1.3), ALD (aldolase, E.C. 4.1.2.13), G-3-PDH (glyceraldehyde 3-phosphate dehydrogenase E.C. 1.2.1.12), IDH (isocitrate dehydrogenase, E.C. 1.1.1.44), MDH-1, -2, -3 (malate dehydrogenase, E.C. 1.1.1.37), 6-PGD-1, -2, -3 (6-phosphogluconate dehydrogenase, E.C. 2.7.5.1), and SKDH (shikimate dehydrogenase, E.C. 1.1.1.25).

Electrophoresis methods were essentially as reported in Shaw and Prasad (1970). Enzyme staining followed Soltis et al. (1983). Genotypes were inferred directly from enzyme banding patterns based upon knowledge of the overall conservation of isozyme subcellular location, subunit composition, and isozyme number in plants (Gottlieb 1982; Weeden & Wendel 1989). *A. grandiflora* is diploid ($2n = 12$; Munz & Keck 1964), so that interpretation of banding patterns was straightforward and in conformity with typical diploid patterns seen in other plants. Allozyme variation was described by the mean number of alleles per locus (A), proportion of loci polymorphic (P), and heterozygosity expected given Hardy-Weinberg equilibrium (H_{exp}). A locus was considered polymorphic if any variation was encountered. Genetic diversity statistics were assessed using BIOSYS-I (Swofford & Selander 1981). An estimate of the proportion of individuals in a nutlet source that could potentially carry alternate alleles into the founding population (the alternate allele carriers) was obtained by summing the number of individuals that had at least one locus that was homozygous for alternate alleles with the number of individuals that were heterozygous at one or more loci and dividing by the total number of individuals in the sample.

Combining Nutlet Sources for Sowing

Since genetic variability in small populations of endemic species is often low (Hamrick 1983; Waller et al. 1987)

and can be ecologically restrictive (Clegg & Brown 1983; Lesica et al. 1988), it is important to maximize allelic diversity in new populations to insure long-term persistence and evolutionary viability (Frankel 1983; Hopper & Coates 1990; Soltis & Soltis 1991). In the case of *Amsinckia*, both wild and cultivated nutlet sources had a common origin at Site 300, so panmixis per se was not an issue. The goal adopted here was to maximize genetic variability using the limited number of available propagules and their relative germination abilities. Allozyme electrophoresis data indicated that the Site 300 source had more genetic variability than the Davis source (see results). Laboratory and greenhouse germination tests, however, showed that Site 300 nutlets were also less likely to germinate due to their greater age (about 25 years in cold storage). Germination of Site 300 nutlets was one half to one third that of the Davis nutlets (discussed above). Consequently, a maximum population of 100 seedlings in each experimental plot would require a minimum of 100 Davis nutlets or 200–300 Site 300 nutlets to produce. The latter, which represents the maximum infusion of genetic variability into the new population, was not possible because there were too few Site 300 nutlets available to sow 20 experimental plots in such a manner (see below). Consequently, only 30 of the 100 sowing positions in each plot were allocated to Site 300 nutlets, but these were triple-sown (three nutlets per position) to compensate for low germination. The remaining 70 positions were single-sown with Davis nutlets.

Plot Design and Treatments

After selection of the Lougher Ridge microsite, a large area (14 \times 17 m) was fenced with barbed wire to exclude large mammals and prepared to receive plots, treatments, and data acquisition equipment (Fig. 1). A stratified-randomized design was used to assign treatments to 20 plots, including three treatments and control, with five replicates each. Each treatment patch was 2 \times 2 m, centered around a 1 \times 1 m experimental plot. Buffer zones and designated paths between patches ensured that no human impact occurred where nutlets were to be sown. The five control plots contained high cover by introduced annual grasses (*Avena barbata*, *A. fatua*, *Bromus mollis*, *Hordeum leporinum*, and *Lolium multiflorum*), and a few native and introduced forbs (*Claytonia perfoliata*, *Dichelostemma pulchellum*, *Brassica geniculata*, *Erodium cicutarium*). After sowing, five patches were burned, five were clipped by hand to remove all shoots 2 cm above the soil surface, and five were sprayed with a dilute solution of a grass-specific herbicide (fluazifop-p-butyl or "Fusilade[®]", ICI Corp.).

Five patches were burned on October 20, 1989, immediately after they were sown and just one day before

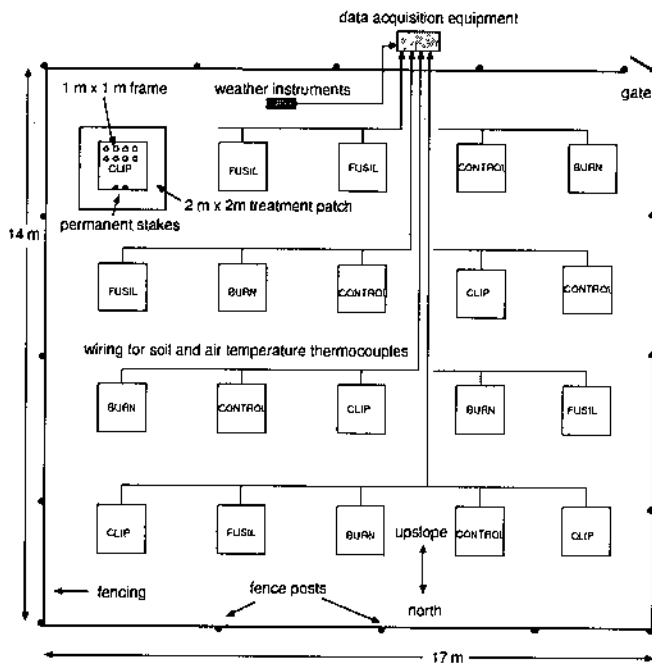


Figure 1. Layout of plots at the microsite. Treatments were randomly assigned to plots within a row. Data acquisition equipment measured soil and air temperatures within each plot as well as precipitation and air temperatures for the microsite as a whole.

the first fall rains. A 2×2 m galvanized metal box, which stood 70 cm tall, was used to block wind and contain flames. A fire was started along one interior side of this "burn box" and allowed to cross the patch. As small flames escaped beneath the sides or at the corners of the box, they were doused with a liquid CO_2 fire extinguisher to insure that moisture or solid chemicals were not added.

Another five patches were hand-clipped prior to sowing and twice again during the growing season (November 16 and January 4) so that grass blades did not exceed 15 cm in height above the soil (compared to 30–50 cm in the control patches). Plant material from the first clipping of 2×2 m was removed to prevent thatch buildup but was subsequently allowed to fall in place.

Herbicide was not applied to the five "fusil" patches until November 16 because Fusilade® works after tiller emergence. This allowed grasses to grow vigorously for 26 days after the first rains. Each fusil patch was treated only once with a 1/10th-strength solution, based on the manufacturer's full-strength specifications. Exactly 5.0 ml of Fusilade® concentrate was mixed in 5.0 l of distilled water with 12.5 ml of a nonionic surfactant (Monterey Herbicide Helper, Lawn and Garden Products Inc., Fresno, California). A hand sprayer was used to apply 1.0 l of this solution to the 2×2 m patch in a fine mist that wetted blades and culms. Care was taken to prevent overspray, and very little if any of the liquid dripped

onto the soil surface. Dieback of the grasses was obvious by December 12 and complete by January 4. Native forbs, including the *Amsinckia* seedlings, were not noticeably affected by this single application.

A microcomputer-based data acquisition system (CR 21x, Campbell Scientific, Logan, Utah) was used to monitor soil surface and +10 cm air temperatures within each plot. The remote probes were copper-constantan thermocouples, shielded from direct solar radiation and mounted on a stainless rod. They were automatically scanned every half hour, and data were summarized (daily means, maxima, minima) and stored. These data were used to detect microclimatic variability between the treatment plots that might be linked to germination, survival, and seed production of the plants. In addition, the system recorded standard meteorological parameters for the microsite as a whole, including precipitation (with a tipping bucket rain gauge) and bulk air temperature.

Sowing the Nutlets

A 1×1 m experimental plot was centered within each 2×2 treatment patch, allowing a 0.50-m border to minimize edge effects. The 1-m^2 plots were permanently marked with two stainless steel rods driven into the soil so that 8 cm protruded above the surface. The rods positioned a removable wooden frame, 100 cm \times 105 cm, into which a grid of 100 holes (10 holes \times 10 holes, each 2.5 cm in diameter) had been drilled. The holes allowed exact placement of nutlets within the plot and subsequent monitoring of seedlings and juvenile plants (see Pavlik 1993).

A total of 3460 nutlets of *Amsinckia*, 1800 from the Site 300 source and 1660 from the Davis source were sown on October 19 and 20. (A counting error led to an extra 260 nutlets being sown into one of the clip plots. There should have been only 1400 Davis nutlets sown.) All of the nutlets were of high quality, fully formed with a 1.5–3.0 mg range of weight per nutlet (Pavlik 1988).

Using the wooden frames, each plot was sown with 160 nutlets, 70 Davis (1 per hole) and 90 Site 300 (3 per hole, see above). The Site 300 holes were in a pattern that distributed these germinules among those from Davis to better insure crossing of the two sources. Nutlets were pressed into shallow depressions in the mineral soil made with a blunt nail, covered with about 20 cc of loose, native soil, and tamped down uniformly. Each plot took one person 45–60 minutes to sow.

No supplements of water or nutrients were applied during the experiment, even though the site received less than 60% of normal precipitation for the growing season.

Demographic Monitoring of the New Population

Demographic monitoring of all plots was conducted to identify those factors that could limit the establishment

or growth of the new population (Pavlik & Barbour 1988; Pavlik 1993). The monitored parameters included *in situ* 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 were followed separately so that the effects of electrophoretically-detectable genetic differences could also be assessed.

Monitoring was facilitated by the repeated use of planting frames to locate and identify individuals. After all nutlets were sown on October 20, 1989 (= day 0), plots were censused with the frames on October 24 and 29 (days 4 and 9), November 6 and 16 (days 17 and 27), December 1 (day 42), and January 4, 1990 (day 76). After the last date, each plant was marked with a slender wooden stake (20-cm potsticker) on which was coded the source and whether multiple germination had occurred at that frame position (from the triple-planted Site 300 wells). After December 1, seedlings of *Amsinckia intermedia* were removed in order to simplify monitoring. Plots continued to be censused on January 25 (day 97), February 18 (day 120), March 19 (day 150), and April 9 (day 171).

Nutlet output per plant and per plot were estimated from measurements of shoot length or from the sum of the inflorescence lengths (Pavlik 1988; Pavlik & Barbour 1988). In order to develop the necessary correlations, a total of 18 plants were selected on April 9, 1990, after growth and nutlet production had ceased at Lougher Ridge. A complete range of shoot lengths (15.5–49.0 cm), treatments, and nutlet sources were included in the sample. Maximum shoot length was measured and plants were clipped at soil level, sealed in separate polyethylene bags, and refrigerated. Total inflorescence length and the number of branches, inflorescences, flowers, and nutlets were determined at the lab. Each flower was examined for the presence of filled 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 nonlinear regression analyses were based on total shoot length or 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 relationship from *in situ* plants with the highest regression coefficient was used to convert maximum shoot length of each plant in every plot to nutlet output at the peak of fruit set (March 19 and 20, 1990). Plot analyses were made by summing the nutlet output of all plants in a single treatment plot.

Evaluation of the treatments was made by comparing germination, mortality rates, survivorship to reproduction, and nutlet output per plot between experimental plots and the appropriate control plots. Statistical analysis of differences was made using analysis of variance (ANOVA) with arcsine transformation when appropriate.

Results

Genetic Characteristics of the Nutlet Sources

As a whole, the 26 *Amsinckia grandiflora* seedlings analyzed by electrophoresis had very low levels of allozyme variability at the 18 loci examined (Table 1). The mean number of alleles per locus for the two seed sources was 1.13, with only 17% of all loci being polymorphic. Nutlets from the Site 300 sources had higher levels of polymorphism than did Davis nutlets that had been cultivated twice over a two-year period. Site 300 nutlets were polymorphic at the IDH-1, PGI-2, PGM, and SKDH loci, while Davis showed variation only at LAP and PGM. The proportion of potential alternate allele carriers in the Site 300 nutlet source was 41.7%, compared to 28.6% in the Davis source.

Effects of the Plot Treatments on the Lougher Ridge Grassland

On December 4, approximately one month after the first rains, live grasses constituted 44% of the total cover in the control plots and ranged between 17% and 23% in the treatment plots. Burn plots were very open because fire had removed much of the thatch, leaving only a thin layer of ash. Clip and fusil plots had less open ground and more grass thatch, either because clipping left the culm bases in place or because herbicide killed grass shoots that were left standing. The low height of grasses and thatch in the burn and clip plots allowed light to reach the soil surface during much of the day. The standing live and dead grasses in the fusil plots, however, produced a shady environment similar to the

Table 1. Genetic variability at 18 loci in *A. grandiflora* seedlings derived from two nutlet source populations along with means for the combined source populations.

Nutlet source	Mean number of alleles per locus (<i>A</i>)	Mean % polymorphic loci (<i>P</i>)	Mean heterozygosity per locus (H_{exp})	Polymorphic loci
Site 300 (<i>n</i> = 12)	1.22	22.2	0.044	PGM-1, PGI-2, IDH-1, SKDH
Davis (<i>n</i> = 14)	1.08	11.1	0.028	PGM-1, LAP-1
Combined (<i>n</i> = 26)	1.13	16.6	0.034	

controls at this time. *Amsinckia* and other forbs constituted a very low percentage of live cover (<5%) in the plots at this time.

During the peak of reproduction, however, grass cover in the control and some of the treatment plots was high and similar (Table 2). Grasses in the burn plots were as tall or taller than the flowering *Amsinckia*, although the total cover by *Amsinckia* was still twice that of the controls. Fusil plots were strikingly different from burn and control plots because their live cover was completely dominated by large, profusely-flowering individuals of *Amsinckia grandiflora*, with a surprisingly lush "understory" of mostly native forbs (such as *Amsinckia lycopsoides*, *Claytonia perfoliata*, *Galium aparine*, *Lithophragma affine*, and *Triteleia laxa*). Live cover by introduced annual grasses was effectively eliminated by the herbicide.

There was no significant effect of the treatments on air and soil surface temperatures within the plots (unpublished data). Mean daily temperatures in the vicinity of *Amsinckia* plants in different plots were the same throughout the growing season when comparisons were made between all treatment plots and controls (the slope of the correlation was 0.95–0.98 with no consistent deviation). Soil moisture levels were also equivalent during the fall and early winter (unpublished data), suggesting that treatments did not alter the physical interception or storage of precipitation. Treatment effects on *Amsinckia* were, therefore, due to alterations of biological rather than physical factors in the plots, and most likely due to differences in levels of competition with annual grasses.

Demography of the New Population

IN SITU GERMINATION

The first significant rains fell during October 22–24, with 38.1 mm received at Lougher Ridge. The nutlets, sown only four days before, began to germinate immediately. On October 29 more than 30% of the sown nutlets had already germinated. Eight days later more than 50% of the sown nutlets had germinated, constituting 90% of all germination that was to occur during

the 1989–1990 season. There were no significant differences in germination rate among the treatment and control plots, although there was a slight delay in burn plots during the first nine days. Nutlets continued to germinate sporadically throughout the growing season, with the last germinules recorded on February 18, 1990, 120 days after sowing.

When compared to lab germination trials, total *in situ* germination (% of nutlets sown) during the October 29 to February 18 period was higher than expected, with 43% of the Site 300 and 70% of the Davis nutlets finally emerging (Table 3). Differences between the two sources were consistent among plots, reflecting age-specific rather than environmental effects on germination. Even the passage of fire across nutlets in the burn plots had no significant effect, although slightly higher Site 300 and slightly lower Davis germination were observed. As a result, total germination of both nutlet subpopulations averaged between 54% and 55%, and initial seedling density was equivalent in all plots.

POPULATION GROWTH AND MORTALITY

Regardless of treatment the Lougher Ridge population grew rapidly, attaining a maximum of 1774 live plants in seedling and juvenile stages. Totals of 443, 443, 456, and 432 individuals were found within the control, burn, clip, and fusil plots, respectively, during the growing season. Each plot had 70 to 80 plants initially, with an average of about 40% from the Site 300 source. There were no statistically significant treatment effects on the proportion of live plants derived from the two nutlet sources, but burn plots consistently had more Site 300 plants (about 50% of the total) from mid-November until peak flowering in mid-March.

Although the first seedling deaths were detected 17 days after sowing, they were not frequent until 27 days (mid-November) and later (Fig. 2). At that time there were significant differences in mortality among plots. The average mortality rate in the control plots (about 9% per week) was more than twice that in the burn and clip plots, but statistically equivalent to that in the fusil plots. A significant difference between control and burn plots was also found in early December but not after-

Table 2. Composition of live plant cover within the Lougher Ridge plots, March 30, 1990.

	Total relative live cover (%)	Live cover			Cover dominants
		Grasses (%)	<i>Amsinckia grandiflora</i> (%)	Other forbs (%)	
Control	84.1 ± 5.8	69.2 ± 6.0	8.6 ± 2.8	6.4 ± 2.1	<i>Avena, Bromus, Hordeum</i>
Burn	82.0 ± 7.0	54.2 ± 3.8	22.0 ± 4.7	5.8 ± 3.5	<i>Avena, Ag, Marah</i>
Clip	79.7 ± 6.1	60.6 ± 10.3	10.2 ± 4.2	9.0 ± 4.4	<i>Avena, Hordeum, Erodium</i>
Fusil	80.7 ± 10.0	1.1 ± 2.2	60.8 ± 12.9	18.8 ± 8.0	<i>Ag, Claytonia, Galium</i>

Cover was estimated in replicate 0.25-m² circular quadrats in each plot. Ag = *A. grandiflora*.

Table 3. Total *in situ* germination (% , October 29, 1989, to February 18, 1990) of *A. grandiflora* nutlets as a function of plot treatment and source.

	Germination (%)		
	Davis	Site 300	Both sources
Control	72.9 ± 9.9	41.8 ± 10.0	55.4 ± 5.2
Burn	62.9 ± 12.2	49.5 ± 11.9	55.4 ± 9.9
Clip	70.1 ± 4.6	40.9 ± 6.4	54.1 ± 4.8
Fusil	73.7 ± 8.0	38.7 ± 10.3	54.0 ± 8.1

Values (mean ± SD) in a column were not statistically different at $p < 0.05$ (ANOVA, arcsine transformed data).

wards. Thus, the treatments (burn, clip) that minimized *Amsinckia*—grass interactions during this time effectively reduced mortality of seedlings and young, established plants. The herbicide treatment, since it was not administered until after the grasses had emerged in mid-November, had no effect on seedling survival. After wards, mortality rates in all plots declined to 1–3% and then tended to rise slowly towards spring. There was no difference in mortality between plants derived from Site 300 and Davis sources, indicating that neither genetic differences nor triple sowing (producing intraspecific competition) were important during the early, nonreproductive stages of the population.

The causes of mortality were not always clear. In some cases, seedlings vanished between census dates, and their deaths could not be assigned to a particular stress category. However, symptoms of water stress (wilting of leaves), grazing by microherbivores (chewed leaves, cotyledons, and stems), and light or nutrient deficiency (chlorosis) were often observed. Despite the fact that 1989–1990 was another in a series of drought years, only a small percentage of live plants wilted during the growing season, ranging between 2% and 7% among all plots (Table 4). Herbivory was much more prevalent, with at least 30% of all live plants losing stem, leaf, and cotyledon tissue. There were no significant treatment or source effects on stress due to wilting or grazing. Chlorosis, however, was much more common in the control and fusil plots and was lowest in the burn plot, especially when the plants were young and small (the first 42 days). This suggested that the grass canopy in both the control and fusil plots affected seedling mortality through competition for light and/or mineral nutrients at this time.

FLOWERING AND NUTLET OUTPUT

Inflorescences of *Amsinckia* were first observed on January 4, 76 days after sowing. They were tightly coiled, with closed flower buds, and borne on stems 4–5 cm above the soil surface. Most were found in the burn plots and most were from Site 300 nutlets. By January 25 (day 97), 6% of all live plants had inflorescences, and a few of these had open flowers. All of the plants with

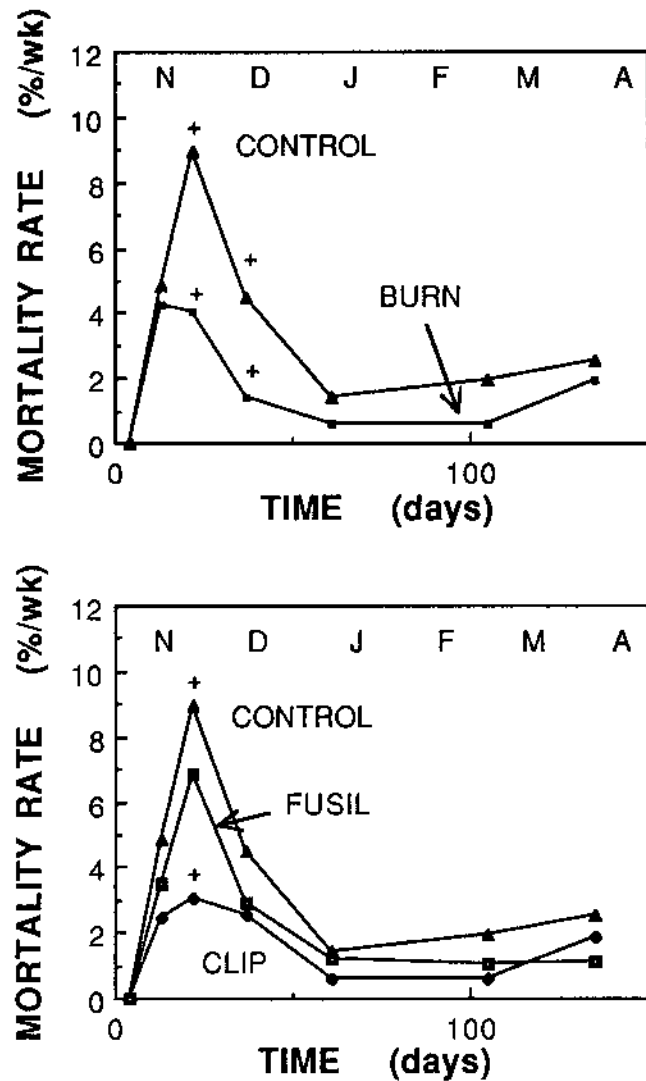


Figure 2. Seasonal course of mean mortality rates of *Amsinckia* plants in treatment and control plots. "+" indicates a mortality rate in treatment plots that was significantly different from the control plots at that time (ANOVA, $p < 0.05$, arcsine transformed).

open flowers were thrams from Site 300 nutlets. This pattern was accentuated by February 18 (day 120), when 70% of all live plants had inflorescences. Of all live plants with open flowers at that time, 80% were from Site 300 nutlets, 84% were thrams, and 90% were found in the burn plots. Although no plants with open flowers were in the fusil plots, 78% had inflorescences compared with 82% in the burn plots, 60% in the control plots, and 56% in the clip plots.

The peak of flowering was reached in mid-March (day 150), when 1101 out of 1310 living plants (84%) had open flowers and/or inflorescences with flowers undergoing anthesis (orange petals expanding beyond the calyx lobes). These were regarded as the reproductive plants, those likely to set nutlets before the end of the

Table 4. Assessment of stress in the treatment plots.

	% of Plants		
	Wilted	Grazed	Chlorotic
Control	6.5	35.4	31.6
Burn	1.8	31.8	9.3
Clip	3.3	44.5	16.0
Fusil	6.2	29.6	23.4

Each value is the percent of the total live individuals during the growing season (November to April) that exhibited wilting, tissue loss due to micrograzers, or chlorosis.

growing season. The remaining 198 plants were unlikely to reproduce because they were small (generally less than 16 cm tall and unbranched) and either had no inflorescences (25 of the 1310, or 2%) or had new, tightly coiled inflorescences with no sign of impending anthesis (173 or 13%). Reproductive plants in the burn plots (339) outnumbered those in the control, clip, and fusil plots (191, 289, and 282, respectively). In mid-March, however, plants from Site 300 nutlets were no more likely to be reproductive than plants from Davis nutlets. Nearly all of the early-flowering Site 300 plants (January–February) observed in the burn plots survived and continued to produce flowers during the peak period in March.

The burn plots contained significantly more reproductive plants and, therefore, had higher survivorship to reproduction (expressed as a percentage of germinated nutlets) when compared to controls (Table 5). Clipping and herbicide did not significantly enhance survivorship, indicating that only the low seedling mortality rates observed in burn plots (Fig. 2) were of any consequence at this time. There was no treatment effect on pin/thrum ratio, which averaged 1.36 in all plots, and no differential reproductive survivorship of plants derived from Site 300 nutlets. Control, clip, and fusil plots had slightly fewer Site 300 plants while burn plots had slightly more, but the differences were not significant.

The output of nutlets by individual plants at Lougher Ridge was linearly related to shoot length and the sum of the inflorescence lengths (Table 6; Fig. 3). The largest

plants (with shoot lengths ranging from 35 to 50 cm) produced between 150 and 182 nutlets each. Ovule production 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 (the slope of the sum of inflorescence lengths versus reproductive efficiency = 0). Typically, medium to large plants had low reproductive efficiencies (nutlet/ovule ratios), with a mean of 0.20 and a maximum value of 0.34.

An estimated 35,768 nutlets were produced by the 1101 reproductive individuals at Lougher Ridge by the end of March 1990. Because 3460 founder nutlets were put into the site, the new seed bank population of *Amsinckia* was amplified by about a factor of 10. Approximately 40% of the new nutlets were derived from the Site 300 source (the proportion of Site 300 plants in the reproductive population), since differential survivorship (Table 5) and differential nutlet output (Table 8, below) were not detected.

The burn and herbicide treatments significantly enhanced one or more measures of plant size and, consequently, nutlet production (Table 7). Mean maximum shoot length was greater in both the burned and fusil plots, while mean shoot length was greater only in the fusil plots. Burning alone did not release all individuals from competition because there were many small, presumably suppressed individuals in the burn plots along with the larger, dominant ones. *Amsinckia* plants in burn plots were twice as fecund as plants in control plots, but the difference was not statistically significant. Plants in fusil plots, however, produced more than three times the number of nutlets as did control plants, and the difference was significant. Consequently, the mean nutlet output per plot was decidedly greater in the fusil plots (more than four times that of the controls, $p < 0.025$) but only enhanced ($p < 0.07$) in the burn plots.

Clipping the grasses had the unexpected result of reducing shoot length of *Amsinckia* plants (Table 7) and the degree of branching as well (unpublished data). Although statistically equivalent to the controls, plants in

Table 5. Treatment effects on population size, survivorship to reproduction, and pin/thrum ratio of *A. grandiflora* during the period of maximum flowering (mid-March 1990).

	Mean number of reproductive plants per plot			Survivorship to reproduction (% of germ)			Pin/thrum ratio		
	Davis	Site 300	Σ	Davis	Site 300	\bar{X}	Davis	Site 300	\bar{X}
Control	22.6 ^a	15.6 ^a	38.6 ± 15.8 ^a	45.4 ^a	38.9 ^a	42.7 ± 16.5 ^a	1.17 ^a	2.11 ^a	1.38 ± 0.33 ^a
Burn	33.6 ^a	35.0 ^a	67.2 ± 19.8 ^b	75.1 ^b	76.1 ^b	75.3 ± 11.6 ^{b*}	1.44 ^a	1.40 ^a	1.40 ± 0.36 ^a
Clip	37.8 ^a	20.0 ^a	57.8 ± 16.5 ^a	68.3 ^a	55.5 ^a	63.1 ± 12.0 ^a	1.26 ^a	1.47 ^a	1.27 ± 0.82 ^a
Fusil	35.6 ^a	20.8 ^a	56.4 ± 15.6 ^a	70.5 ^a	59.5 ^a	64.4 ± 10.8 ^a	1.22 ^a	1.40 ^a	1.40 ± 0.49 ^a

* Different at $p < 0.01$.

Data for Davis and Site 300 nutlet sources are presented separately, then summed (Σ) or averaged (\bar{X}). Values (means, $n = 5$) in a column followed by the same letter are not statistically different ($p < 0.05$, ANOVA, arcsine transformed % and ratios).

Table 6. Linear correlations between measures of plant size and nutlet output, ovule output, or reproductive efficiency per individual *A. grandiflora* from Lougher Ridge, March 19, 1990.

X	Y	Sample	n	Slope	Intercept	r	P
Σ inflor lgth (cm)	# nutlets	all plants	18	2.51	-5.9	0.95	<0.01
Σ inflor lgth (cm)	# nutlets	Davis	12	2.43	-6.2	0.95	<0.01
Σ inflor lgth (cm)	# nutlets	Site 300	6	3.29	-14.1	0.98	<0.01
Shoot length (cm)	# nutlets	all plants	18	4.60	-79.2	0.77	<0.01
Σ inflor lgth (cm)	# ovules	all plants	18	10.95	7.2	0.99	<0.01
Shoot length (cm)	# ovules	all plants	18	20.58	-328.4	0.81	<0.01
Σ inflor lgth (cm)	repro eff	all plants	18	0.001	0.14	0.43	ns

ns = not significant; Σ inflor lgth = sum of the lengths of all inflorescences; repro eff = reproductive efficiency (nutlet/ovule ratio).

clipped plots were weaker, less branched, and lacked the showy inflorescences typical of plants in other treatment plots. This was despite the fact that the grass canopy in each clipped plot was low and open throughout the growing season.

Comparisons between Davis and Site 300 plants of mean maximum shoot length, mean shoot length, and nutlet output per plant did not yield any statistically significant differences (Table 8). Both sources exhibited similar responses to burning (increased plant size), clipping (no effect or slight diminution of plant size and nutlet output), and herbicide (increased plant size and nutlet output).

Discussion

This effort to create a new population of *Amsinckia grandiflora* at Lougher Ridge was successful, at least in its first year. More than half the nutlets sown had germinated ($n = 1774$), and nearly two thirds of those survived to reproduce (1101). Many plants grew vigorously without supplements of water or nutrients, especially if the habitat was manipulated to reduce cover (and, therefore, competition) by introduced, annual grasses. The overall ratio of pin to thrum individuals (1.36) was well within the range of 0.75 to 2.0 found in the natural population at Site 300 (Ornduff 1976), suggesting that the relative abundance of floral morphs would not be an impediment to outcrossing. Floral displays in the burned and herbicide-treated plots were showy, bright orange patches that attracted and detained more native pollinators than did the less showy displays of control plots. The ratio of total nutlet output to input (35,800/3460) indicated that the population has the potential of growing by an order of magnitude, depending on weather, microhabitat factors, and site management during the years to come.

An experimental design, coupled with a detailed program of demographic monitoring, provides a basis for managing this population and establishing others within the historic range of the species. Comparisons of sub-

population demography between control and treatment plots revealed that non-native annual grasses had a profound and immediate impact on the new population of *Amsinckia*. Although there was no effect on *in situ* germination, grass competition significantly increased mortality rates and significantly decreased reproductive survivorship, plant size, and reproductive output of *Amsinckia*. Therefore, annual grass cover must be managed to improve habitat quality and to promote population growth of this highly endangered plant.

Not all of the grass management techniques tested in this study, however, were equally effective. Hand clipping the grass canopy to keep it low and open significantly decreased seedling mortality rates early in the year. But it was surprising that mature *Amsinckia* plants in clipped plots were the same size as those in grassy control plots (or smaller) and had the same low nutlet output. Nutlet production in clipped plots (mean = 473, Table 6) was somewhat above nutlet input (160, the number sown in each plot), but in one replicate it was much less. This small margin for growth would be further reduced by embryo abortion (Weller & Ornduff 1991), post-dispersal mortality (predation or dispersal into low-quality habitat), and dormancy in the soil. Population growth of *Amsinckia*, therefore, would not be promoted by the use of clipping as a technique for reducing grass competition. In fact, clipping appears to intensify competition by some unknown mechanism. Perhaps initial removal of clipped grass shoots from the plots accentuated a soil nutrient deficiency. Although clipping generally does not increase biomass allocation to roots in grasses (Richards 1984), it may result in higher photosynthesis and resource use by the remaining canopy (Coughenour et al. 1985), thus increasing below ground competition. Although the treatment used in this study does not mimic the patterns or intensity of grazing by livestock or other large mammals, it suggests that removal of the grass canopy by grazers during the growing season reduces the growth and fecundity of plants like *Amsinckia* and could lead to population decline. Further studies are needed to evaluate all the impact (trampling, soil compaction, herbivory) as well as the benefit (increased habitat patchiness) of

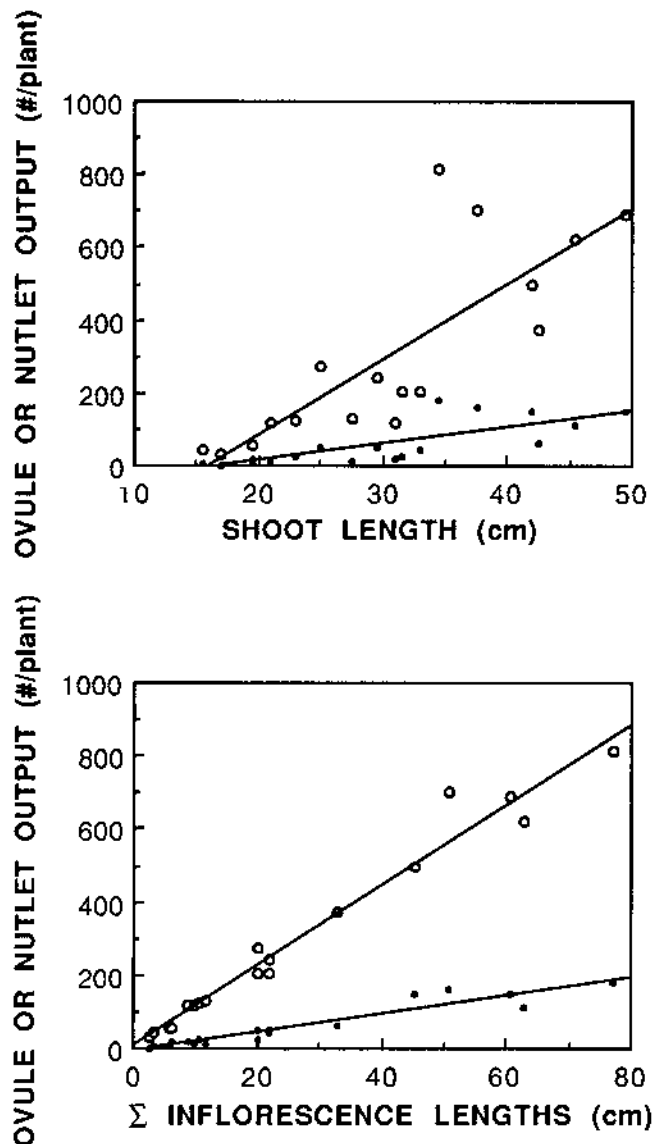


Figure 3. Ovule (open symbol) or nutlet (closed symbol) output of *Amsinckia* plants at Lougher Ridge, March 1990, as functions of shoot length and the sum of the inflorescence lengths. See Table 6 for line equations and coefficients.

using livestock to favor native herbs over introduced annual grasses in parks and preserves.

The use of fire to manage grass cover decidedly improved habitat quality for *Amsinckia*. Fire significantly reduced mortality rates early in the growing season and significantly increased survivorship to reproduction and maximum plant size at maturity. Nutlet output per plant was not significantly enhanced because annual grasses re-established themselves after the burn and grew vigorously late in the season. Reproductive output was probably diminished by interspecific competition with annual grasses rather than intraspecific competition among *Amsinckia* plants. This is because grass cover in

the burn plots during late March was the same as in control plots (54% and 69%, respectively), and more than three times greater than the cover contributed by *Amsinckia* (Table 2). The lack of intraspecific competition is further supported by the observation that high *Amsinckia* densities were accompanied by large plant size and high nutlet output in herbicide-treated plots. Development of competition between grasses and *Amsinckia* in the burn plots was to some extent an artifact of small plot size. Caryopses of *Avena fatua* and *Bromus mollis* in the immediate vicinity could readily disperse into burn plots after treatment and take advantage of the open conditions. In these plots they were able to achieve large sizes and become effective competitors by late winter and early spring. Thus, small-scale fires are unable to eliminate the competitive effects of annual grasses on *Amsinckia* later in the growing season. More extensive burns, however, would increase the distance between caryopsis sources and the *Amsinckia* population. Large burns would also create a mosaic of variations in grass cover where topography and soil conditions (such as depth and surface roughness) change over the hillside, and they increase the number and size of patches with reduced levels of interspecific competition.

Controlling annual grasses by spraying with the grass-specific herbicide Fusilade® had no effect on mortality rates or survivorship to reproduction, but it significantly increased plant size later in the growing season. As a result, it significantly increased the number of nutlets produced per plant and nutlet production per plot. Such relationships between competition, plant size, and reproductive output are important in the demography of all plants (Harper 1977; Winn & Werner 1987), but especially so in annuals (Watkinson 1981; Lee & Bazazz 1982). The persistence of rare annuals such as *Amsinckia* and our ability to create new populations for purposes of recovery may often depend on effective management of introduced competitors. Therefore, the judicious use of selective herbicides on a small scale during the critical, initial phases of re-establishment could be justified, especially if other management tools were less likely to improve the chances of species persistence. Additional trials are needed to determine the range of species, habitats, and land-use situations in which herbicides could be used safely, responsibly, and effectively to meet conservation objectives.

This study has demonstrated that *Amsinckia* can complete all of its life history phases at the Lougher Ridge re-establishment site. Even in control plots with intense competition from annual grasses, nutlet output exceeded nutlet input by a ratio of 5 to 1. High reproductive output combined with appropriate site conditions have allowed the new population to grow at a rate of 18–26% per year following the 1989–1990 reintroduction (unpublished data). Reproductive individuals

Table 7. Treatment effects on plant size (length of main shoot) and nutlet production (per plant and per plot) of *A. grandiflora* (both sources) during the period following maximum flowering (mid-March to April 1990).

	Plant size		Nutlet production	
	Mean maximum (cm)	Mean (cm)	Mean (#/plant)	Mean (#/plot)
Control	26.0 ± 3.1 ^a	18.6 ± 2.8 ^a	15.1 ± 10.1 ^a	835 ± 606 ^a
Burn	33.7 ± 5.3 ^b	22.9 ± 3.2 ^a	29.1 ± 14.4 ^a	2324 ± 1379 ^{a†}
Clip	23.1 ± 3.7 ^a	14.9 ± 2.9 ^a	6.6 ± 5.6 ^a	473 ± 364 ^a
Fusil	40.5 ± 4.1 ^{b*}	28.6 ± 3.3 ^{b*}	53.5 ± 16.5 ^{b**}	3522 ± 1274 ^{b*}

† p < 0.07.

* p < 0.01.

** p < 0.005.

Mean maximum plant size calculated from the 10 largest individuals in each plot. Values (mean ± SD) in a column followed by the same letter are not statistically different from the control (p < 0.05, ANOVA unless otherwise indicated).

numbered 1301 in the spring of 1991 and 1640 in the spring of 1992, indicating that natural patterns of nutlet dispersal, habitat availability, and plant establishment were apparently favorable. The natural establishment of new plants and growth to reproductive maturity were also used as criteria for evaluating the success of re-established populations of *Hymenoxys acaulis* var. *glabra* in Illinois (DeMauro 1993). These results support the conclusion that the potential for self-maintenance is high in the Lougher Ridge population of *Amsinckia grandiflora* and that the methods of site selection, microsite evaluation, plot design, habitat treatment, and monitoring will be effective for finishing the recovery effort.

Near-term population viability may also depend on achieving a critical minimum population size, however, one large enough to withstand stochastic disruption and catastrophic disturbance (Shaffer 1987). Although the minimum viable population size is unknown for this species (and for other annual plants as well), a general range of 500 to 2500 individuals has been suggested (see Mace & Lande 1991). If an animal-based model can be extrapolated to plants, then smaller bodied, shorter-lived species require larger minimum populations than larger, longer-lived species (Soulé 1987; Belovsky 1987). This is to say that the new *Amsinckia* population

will have to be managed for growth during its first few years in order to achieve a mean size of at least 1500–2000 reproductive individuals. Growth of the population at Lougher Ridge appears to be limited by the availability of high quality (low competition) habitat, rather than by intrinsic factors such as inbreeding depression or heterostyly. It may be essential, therefore, to promote expansion of the population beyond the study plots (by artificial dispersal, for example) in order to take full advantage of naturally-occurring habitat variation that provide additional low-competition patches for *Amsinckia* occupation. Bare soil surfaces created by fossorial mammals (Hobbs & Mooney 1985) and sparse grass neighborhoods created by variations in rainfall and soil moisture storage (Pitt & Heady 1978; Rice & Menke 1985) may be particularly important to native forb populations in California annual grassland. Extensive burning has already been suggested as a means of increasing the heterogeneity of grass cover, but spot-spraying of dilute, grass-specific herbicide would also be effective in producing patches that support young *Amsinckia* plants. Ultimately, restoration of the original bunchgrass physiognomy may be the most effective way of increasing habitat patchiness and promoting *Amsinckia* population growth.

Long-term viability of the population will depend on

Table 8. Treatment effects on plant size (length of main shoot) of *A. grandiflora* from Davis and Site 300 sources during the period following maximum flowering (mid-March to April 1990).

	Davis source			Site 300 source		
	Mean maximum shoot length (cm)	Mean shoot length (cm)	Mean nutlet output (#/plant)	Mean maximum shoot length (cm)	Mean shoot length (cm)	Mean nutlet output (#/plant)
Control	24.9 ± 2.9 ^a	18.4 ± 3.6 ^a	15.5 ± 9.8 ^a	21.1 ± 5.3 ^a	18.0 ± 3.8 ^a	13.8 ± 11.3 ^a
Burn	31.2 ± 5.3 ^{a†}	23.0 ± 3.9 ^a	30.6 ± 15.2 ^a	30.0 ± 5.3 ^{b*}	22.3 ± 3.6 ^a	27.3 ± 14.1 ^a
Clip	22.0 ± 4.2 ^a	14.8 ± 3.8 ^a	7.2 ± 6.7 ^a	18.2 ± 2.8 ^a	13.9 ± 2.7 ^a	4.9 ± 4.1 ^a
Fusil	38.7 ± 2.8 ^{b**}	28.5 ± 3.2 ^{b**}	54.4 ± 14.4 ^{b**}	35.4 ± 2.8 ^{b**}	27.8 ± 5.4 ^{b*}	52.4 ± 21.5 ^{b*}

† p < 0.07.

* p < 0.025.

** p < 0.001.

Mean maximum plant size calculated from the 10 largest individuals in each plot. Values (mean ± SD) in a column followed by the same letter are not statistically different (p < 0.05, ANOVA unless otherwise indicated).

genetic composition as well as size (Lande & Barrowclough 1987). It is likely that this species has passed through a recent bottleneck that effectively removed much of the variability from its gene pools. The bottleneck probably preceded recent declines in the Site 300 population, because the other large, natural population is genetically depauperate as well (unpublished data). Compared to other dicot, annual, endemic, and outcrossing taxa, *Amsinckia grandiflora* has fewer alleles per locus, a much lower percentage of polymorphic alleles, and very low heterozygosity overall (Hamrick et al. 1979; Soltis & Soltis 1991). Similar observations have been made for endangered populations of *Pedicularis furbishiae* (Waller et al. 1987) and *Howellia aquatilis* (Lesica et al. 1988), both of which show little or no allozyme variation. Although electrophoretic data are unlikely to reveal the presence of adaptive variation (Powell 1983), natural populations of *Amsinckia* appear to offer very little in terms of an allelic "cocktail" for creating new populations with evolutionary potential. As a result, selection in atypical habitats is likely to be severe, and microsites for new populations must be carefully chosen to prevent unnecessary erosion of a species' diminishing pool of genes and propagules. Perhaps this is why the population cultivated in Davis showed such a marked decrease in allozyme variation after only two generations.

In this study, genetic management has been limited to maximizing the allozyme variability in the mixture of founding nutlets (Clegg & Brown 1983). That mixture was dominated by homozygous embryos, but some of the nutlets contained embryos that were either heterozygotes or homozygotes for alternate alleles at one or more loci. For these variant embryos to contribute to a newly organized gene pool, they must germinate and survive to reproduce. These plants are the realized alternate allele carriers (AACs) in the founding population. The number of AACs in the Lougher Ridge population would, therefore, depend on: (1) the combined frequency of heterozygotes and homozygous alternates in the nutlet source populations (Davis = 0.286, Site 300 = 0.417, or the potential AACs); (2) the number of nutlets sown from each source (Davis = 1660, Site 300 = 1800); (3) in situ germination of the two sources (Davis = 0.699, Site 300 = 0.427); and (4) in situ survivorship of the two sources (Davis = 0.648, Site 300 = 0.575). Given these values, a total of 215 AACs from the Davis source and 184 from the Site 300 source would be expected, or approximately 36% of the 1990 population (1101 reproductive individuals). Whether or not this proportion was realized in the Lougher Ridge population remains to be seen. Preliminary electrophoretic data indicate that the actual proportion of AACs in 1990 was closer to 25%, but sampling was complicated by differential survivorship in some of the treatment plots. Further studies will be required to document ge-

netic variability in the new population and to compare it with model predictions and levels in the existing natural populations.

There were no immediate demographic effects that could be attributed to genetic differences between nutlet source populations. Plants from the Site 300 source did not demonstrate better performance than those from the Davis source. Germination, mortality rates, survivorship to reproduction, and nutlet output per plant were the same for both subpopulations. Therefore, the observed differences in the number of alleles per locus, the percentage of polymorphic loci, and mean heterozygosity per locus for the enzyme systems examined had no demographic expression in the first generation of *Amsinckia* at Lougher Ridge (but see Schmidt & Levin 1985). Consistent differences between the two nutlet sources in response to burning and reproductive phenology could, however, produce significant effects at the population level after many generations. But until fitness components for individuals with known genotypes are measured, much of the ecological, evolutionary, and conservation significance of the genes contained in the new population will remain obscure.

Conclusions

New, self-sustaining populations of plants as unusual and endangered as *Amsinckia grandiflora* must be created within their historic ranges for purposes of recovery. In order to maintain the existing pools of genes and propagules of diminishing species during such recovery efforts, several considerations are of utmost importance. First of all, microsites for new populations should be carefully chosen because natural selection in atypical habitats (including those in the greenhouse or garden) is likely to be severe. The loss of a few variant alleles under such circumstances in a genetically depauperate species (and many rare plants appear to be so) may jeopardize the long-term fate of the new population and is contrary to the purposes of biological conservation. Secondly, electrophoretic screening of source populations can be used to genetically compose the founding population, with the goal of maximizing allelic variability in the propagule mixture. Finally, any efforts to create new populations of endangered plants must have a sound, empirical framework using demographic monitoring as the centerpiece for evaluating the reestablishment experiment and the performance of the population itself (Pavlik 1993). In the case of *Amsinckia grandiflora*, demographic monitoring identified the effects of competition from annual grasses on critical life-history attributes (survivorship to reproduction, reproductive output) and evaluated the prospects for population growth, self-maintenance, and recovery. It also provided information on alternative management

techniques for minimizing grass competition and maximizing performance of the target species. These same techniques are now being used to recover the serpentine endemic *Acanthomintha duttonii* (known from only one natural population) and could be modified to accommodate a wider variety of plant life forms.

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