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Population and Community Ecology of the Rare Plant *Amsinckia grandiflora*

Tina Marie Carlsen
PhD Thesis

November 1996

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Tina Marie Carlsen

Doctor of Philosophy
Thesis

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Population and Community Ecology
of the Rare Plant *Amsinckia grandiflora*

BY

Tina Marie Carlsen

B. A. (California State University, Stanislaus) 1984

M. S. (University of San Francisco) 1990

DISSERTATION

Submitted in partial satisfaction of the requirements for the c

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of the

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ABSTRACT

Research was conducted between the fall of 1992 and the spring of 1993 on the population and community ecology of the rare annual plant *Amsinckia grandiflora* (Gray) Kleebl. ex Greene (Boraginaceae). The research goal was to investigate the causes of the species rarity and to collect data useful to restoration efforts. The work focused on examining competitive suppression by exotic annual grasses; comparisons with a common, weedy congener; and the role of litter cover and seed predation on nutlet germination and seedling establishment.

Annual exotic grasses reduced *A. grandiflora* reproductive output to a greater extent than did the native perennial bunch grass *P. secunda* at intermediate densities when compared on a biomass per area basis. The response of *A. grandiflora* nutlet production to grass biomass was similar to the amount of cover and was linear for *P. secunda* biomass, whereas the same response to the annual exotic grass biomass was logarithmic.

Comparative studies between *A. grandiflora* and *A. tessellata* showed that nutlet germination responses to be similar between the two species. *A. grandiflora* greenhouse pollination studies revealed *A. grandiflora* to be completely self-incompatible, and floral seed cache (nutlets/flower) approached that of the self-compatible *A. tessellata*. *A. grandiflora* output could fully compensate for lower floral seed cache, resulting in similar nutlet output between *A. grandiflora* and *A. tessellata* in the greenhouse, but could not fully equal *A. tessellata* nutlet output in the field.

Manual removal of litter was shown to alter *A. grandiflora* nut microclimate. Depending on the ambient temperature at the time of germination subsequent to litter removal, nutlet germination and establishment were reduced. Nutlet predation also significantly reduced seedling establishment. The existing climatic conditions at the time of litter removal and the timing of the initiation of germination determine which factor exerted the primary controlling influence.

Population and Community Ecology
of the Rare Plant *Amsinckia grandiflora*

Abstract

Research was conducted between the fall of 1992 and the spring of 1993 on the population and community ecology of the rare annual plant *Amsinckia grandiflora* (Gray) Kleeb. ex Greene (Boraginaceae). The research goal was to investigate the causes of the species rarity and data useful to restoration efforts. The work focused on examining competitive suppression by exotic annual grasses; comparisons with a common, weedy congener; and the role of litter cover and seed predation on nutlet germination and seedling establishment.

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INTRODUCTION

AND OVERVIEW

I conducted research between the fall of 1992 and the spring of 1993 on the population and community ecology of the rare plant *Amsinckia grandiflora* (Gray) Kleeb. ex Greene (Boraginaceae). I performed greenhouse studies to investigate ecological factors which could affect fitness components of *A. grandiflora*, and thus may be contributing to the extreme rarity of this plant. The goal of my research was to obtain information which could be used in restoration efforts on this species.

A. grandiflora is a federally-listed endangered species; an annual herb that is part of the California winter annual grassland. Originally described by Gray in 1876 as a variety of *A. vernicosa*, it was recognized as a distinct species by Greene in 1894. Over one hundred years later, at the time of its listing in 1985, *A. grandiflora* was known from only one population. Its range had declined from several thousand individuals to less than one hundred. It is currently known from three natural populations containing individuals numbering from fewer than 30 to several thousand. These populations occur in grasslands which border on blue oak woodland and coastal sage scrub on steep, well-drained, north-facing slopes of intermediate (300 m) elevations. All three populations are located in the Altamont Hills of the Diablo range, about 50 miles east of San Francisco, California on or near Site 300. Site 300 is a Lawrence Livermore Laboratory high-explosive testing facility, operated by the University of California for the U.S. Department of Energy. A heterostylous species, *A. grandiflora* produces two different flower forms (also known as morphs); pin and thrum flowers. Each individual plant has only one

of flower. Pin flowers are characterized by having a stigma exserted beyond the anthers within the corolla tube. Thrum flowers have the opposite morphology, with anthers exserted and the stigma within the corolla tube.

Prior to the initiation of my research, two main hypotheses had been proposed as the cause of this species rarity. First, some evidence had been found that suggested *A. grandiflora* was competing poorly with exotic annual grasses. Second, there was evidence that the inefficient heterostylous mating system of *A. grandiflora* was resulting in competitive displacement of *A. grandiflora* by sympatric, weedy congeneric species. It should be noted that these two primary hypotheses are not necessarily mutually exclusive. And they are not the only possible causes for the rarity of this plant. For example, little previous work had focused on factors affecting seed germination. It is possible that environmental (such as temperature) and biological (such as predation) factors could be controlling the growth and establishment of this species in the field. At a minimum, genetic and environmental controls could have significant implications during restoration efforts at creating additional *A. grandiflora* populations. My research was designed to test these three hypotheses concerning the rarity of *A. grandiflora*.

The first research paper, "Competitive Suppression of a Rare *A. grandiflora* by Native Perennial and Exotic Annual Grasses", describes my research on the role of competitive suppression by exotic grasses in the decline of *A. grandiflora*. I reasoned that if exotic grasses were contributing to the decline of this plant, then its performance should be enhanced in a matrix of native perennial bunch grasses. To test this hypothesis, during the 1993-94 California wet season, I conducted a competition experiment in which *A. grandiflora* seedlings were transplanted into 0.64 m² experimental plots. The plots consisted

3-cm-dia. plants of the native perennial bunch grass *Poa secunda* in three densities, or exotic annual grasses established in four through manual removal of existing plants. All plots were arranged in a randomized, complete-block design. Both grass types significantly reduced the production of *A. grandiflora* inflorescences (which is correlated with nutlet output). The annual grasses reduced *A. grandiflora* inflorescence number to a greater extent than did *P. secunda* when compared on a biomass per area basis. The response of *A. grandiflora* nutlet production to *P. secunda* biomass was linear, whereas the same response to the exotic grass biomass was logarithmic, and appeared to be related to the grass cover. This may be due to the different morphologies exhibited by the two grass types. I concluded that *A. grandiflora* performance, as measured by inflorescence number, was greatest in a community dominated by intermediate densities of *P. secunda* when compared with a community dominated by exotic annual grasses, at least under the conditions during this single growing season experiment.

The second research paper, "The Role of Intrinsic Factors in the Reproductive Ecology of an Endangered Plant", presents a set of five greenhouse experiments I conducted to evaluate intrinsic limitations to the fitness of *A. grandiflora* as compared to *A. tessellata*, a weedy, relative. A greenhouse pollination study was conducted in which flowers on 20 pin plants and 20 thrum plants received one of four pollination treatments, 1) no manual pollination, 2) manually-assisted self-pollination, 3) manually-assisted intramorph pollination, and 4) manually-assisted intermorph pollination. Results of this study indicated that distylous *A. grandiflora* is not completely self-incompatible. The floral seed cache (seeds/flower) was observed in the intramorph

pollinations, and was comparable to that observed in field grown *A. tessellata*. Germination trials consisted of germinating nutlets on plates containing moistened filter paper, or in Steuwe tubes containing soil from one of the natural field populations. The results indicated that *A. grandiflora* and *A. tessellata* have germination responses typical of many annual species found in the California grasslands. Similar germination responses were observed for both species. Interspecific comparisons of survivorship and nutlet production were conducted on greenhouse-grown and field-grown plants. In the field, mortality was high for both species, although it was greater for *A. tessellata* at one site. In the greenhouse, *A. grandiflora* can compensate for low floral output by increasing floral output (flowers/plant) as compared to *A. tessellata* through increasing the number of flowers per inflorescence. Although not statistically significant, *A. grandiflora* floral output was also higher than *A. tessellata* in the field, but it did not fully compensate for the loss of seed cache. The presence of exotic annual grasses in the field reduced the seed cache of *A. grandiflora* to a greater extent than *A. tessellata*. I concluded that under conditions of unlimited resources, it appears unlikely that *A. grandiflora* fitness was intrinsically limited when compared to its weedy relative *A. tessellata*. However, under field conditions, the reproductive ecology of *A. grandiflora* may result in low seed output.

In the third and final research paper, "Effect of Litter and Seed Predation on the Germination of a Rare Annual Forb", I present my investigations into the role of microclimate and predation on the germination of *A. grandiflora*. I had observed very poor *A. grandiflora* germination during the fall of 1992 subsequent to a controlled burn.

suggesting microclimate or predation may control *A. grandiflora* establishment. I therefore conducted a field experiment over two seasons to investigate these possible controls on *A. grandiflora* germination. During the 1993-94 growing season, two main plots were established within a single field site. The main treatments consisted of either manually clearing all litter, or leaving the litter intact. In each main treatment, two sub-treatments were established. In the cleared main treatment plot, sub-treatments consisted of either covering the plot with aviary netting, or leaving the sub-plot open. Within the litter main treatment plot, sub-treatments consisted of clipping the standing litter and replacing it on to the sub-plot, or leaving the standing litter intact. In each treatment plots were replicated three times, the main treatment plots were not replicated. A single replicate of sub-treatment plots were instrumented with thermocouples to measure soil temperature at a depth of 1 cm. During the 1994-95 growing season, the experiment was conducted at two sites. This time, the main treatments were replicated three times and sub-treatments replicated three times within each main treatment replicate. All sub-treatment plots at one site were instrumented with thermocouples. During the 1993-94 growing season, litter cover significantly increased *A. grandiflora* germination. Soil temperature indicated litter cover moderated soil temperature. Some evidence of predation was detected, as indicated by increased germination in netted plots, but was not significant. In contrast, during the 1994-95 growing season, germination success was greatly affected by predation, as the highest amount of germination was observed in the netted sub-treatment plots. In addition, soil temperature extremes were not as great during the 1994-95 growing season. These data suggest that both microclimate alterations

predation can affect *A. grandiflora* germination, with the existing conditions at the time of litter removal and the timing of the in: germination determining which factor exerts the primary controll influence.

As a result of this research, I can conclude the following with the population and community ecology of *A. grandiflora*. First, *A. grandiflora* competes poorly with exotic annual grasses, and the competitive suppression is contributing to its rarity. A corolla *A. grandiflora* performs best in a matrix of intermediate densities perennial bunch grass *P. secunda*. Second, *A. grandiflora* has the to equal the reproductive output of its weedy relative *A. tessellata* particularly under conditions of unlimited resources. Therefore *A. grandiflora* fitness is not inherently limited. However, under conditions, *A. grandiflora*'s reproductive ecology apparently contributes nutlet output as compared to *A. tessellata*. This may also be the poor competition with annual exotic grasses. Finally, I conclude germination of *A. grandiflora* can be controlled by microclimate and predation, with the predominance of one over the other changing from year to year. The results described in this dissertation provide information which should be considered during any restoration work conducted with *A. grandiflora*.

THE RESEARCH PAPERS

COMPETITIVE SUPPRESSION OF A
RARE ANNUAL FORB BY NATIVE PERENNIAL
AND EXOTIC ANNUAL GRASSES

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Abstract. Populations of the California winter annual forb *Amorpha grandiflora* may be declining because of competitive suppression by annual grasses, and may perform better in a matrix of native perennial bunch grasses. To test this hypothesis, we conducted a field competition experiment in which *A. grandiflora* seedlings were transplanted into 0.64 m² experimental plots. The plots consisted of mature 3-cm-diameter of the native perennial bunch grass *Poa secunda* planted in three or exotic annual grasses established in four densities through removal of existing plants. All plots were arranged in a random complete-block design. Both grass types significantly reduced the production of *A. grandiflora* inflorescences. At low and intermediate densities, the annual grasses reduced *A. grandiflora* inflorescence to a greater extent than did *P. secunda* when compared on a biomass area basis. The response of *A. grandiflora* seed production to *P.* biomass was linear, whereas the same response to the annual grass biomass is logarithmic, and appeared to be related to the amount of cover. This may be due to the different morphologies exhibited by grass types.

Key words: *A. grandiflora*, *P. secunda*, rare plants, native plants, interspecific plant competition, light competition, water

INTRODUCTION

The native bunch grass communities of California have undergone major transformation since the arrival of the first Europeans (Barbour and Major 1990). Grasslands occur in several California plant communities. They are a primary component in the valley grassland community, and form the understory in oak woodland (also known as foothill woodland) and cismontane woodland communities of the California Floristic Province (Barbour and Major 1990). During exploration and intensive settlement, annual grasses native to the Mediterranean area were either introduced as cereal or forage crops, or inadvertently introduced as impurities in crop seed and in packing material (Murphy and Ehrlén 1989). By the 1850's, European plants had largely displaced native annual and perennial grassland species, particularly in the valley grassland and oak woodland communities, resulting in the California winter annual grasslands of today. These grasslands, now dominated by alien species, have more recently been fragmented due to agricultural conversion and suburban sprawl (Huenneke 1989).

In recent years, the decline of locally-restricted native plants has been observed within California winter annual grasslands. Many taxa are now of conservation concern, and some have been formally listed as threatened and endangered. In their most recent inventory of endangered vascular plants of California (Skinner and Pavlik 1994), the California Native Plant Society (CNPS) listed 454 taxa found in valley grassland, foothill woodland, and cismontane woodland communities. Annual plants comprised 48% of this total (216 taxa). Of these 216, 121 are on the CNPS list of most threatened plants, with 9 taxa federally listed as threatened or endangered. Many of the 121 highly threatened taxa

occur in valley grassland or foothill woodland communities. Con research on the role of annual exotic grasses in the decline of r species, both annual and perennial, can determine the mechanisms decline, and provide recommendations required to maintain biodive annual grasslands. Several authors have shown that competition v annual exotic grasses reduces some measure of fecundity in the n species (Gordon and Rice 1993, Guerrant 1992).

The large-flowered fiddleneck, *Amsinckia grandiflora* (Gray) Kl Greene (Boraginaceae), is a rare forb native to the California gr which there is concern that competitive suppression by its exotic may have contributed to its decline (Knudsen and Tarp 1994). Dur reintroduction efforts, Pavlik et al. (1993) found that competitiv grasses reduced fecundity of *A. grandiflora*. They also found tha management of one of the native populations using a grass-selectiv herbicide for several years resulted in the numbers in this popul rebounding from fewer than 100 individuals to its present number 1500 individuals (Pavlik 1995).

The evidence that the presence of annual exotic grasses has ne impacted the reproductive performance of *A. grandiflora* implies A. *grandiflora* would perform better in a matrix of native species assumption has not been explicitly tested. Should it be true, it reaching management implications, given that much of the Californ grasslands are now composed primarily of exotic species. Intens management to restore and maintain native grass communities woul required in the effort to sustain many rare native plants. There out to test the hypothesis that this particular California native forb, *A. grandiflora*, would perform better in a matrix of native

bunch grasses than in a matrix of annual exotic grasses. We selected native bunch grass *P. secunda* J. S. Presl (formerly *P. scabrella* Nutt.) as a test, since it is abundant at the site where *A. grandiflora* is native.

METHODS

The study species

Of the fifteen species in the genus recognized by Ray and Chisaki (1957b), *A. grandiflora* is one of four heterostylous species with restricted distributions that are probably ancestors of the weedy, widespread, and homostylous congeners (Ray and Chisaki 1957a and 1957b). As a heterostylous species, *A. grandiflora* produces pin and thrum flower forms (or morphs). Each individual plant has only one type of flower. Pin flowers are characterized by having an exerted stigma and anthers within the corolla tube. Thrum flowers have the opposite morphology, with exerted anthers and the stigma within the corolla tube. As a member of the California winter annual grassland, *A. grandiflora* germinates with the onset of fall or winter rains, grows vegetatively throughout the winter, flowers in the early spring, sets seed and dies with the summer drought (Heady 1990). It is currently known from only a few natural populations containing individuals numbering from fewer than 100 to several thousand. All natural populations occur on steep, west- or north-facing slopes in the Altamont Hills, about 30 km southeast of San Francisco, California. The populations occur at low elevations (100-200 m) and border on blue oak woodland and coastal sage scrub communities.

Two of the natural populations of *A. grandiflora* occur on Lawrence Livermore National Laboratory Site 300, a high-explosive testing facility operated by the University of California for the United States Department of Defense.

Energy. This largely undeveloped 2711 ha site has been protected from livestock grazing and many other human activities since the early 1980s. Its grasslands are managed with annual controlled burns to reduce the threat of wildfire. The burns are typically conducted in June each summer. This management practice has resulted in large expanses of native perennial bunch grass *Poa secunda* (formerly *Poa scabrella* and Davilla 1986). Although the populations of *A. grandiflora* do not occur in areas undergoing the annual controlled burns, there is a significant amount of *P. secunda* cover at the sites where the natural populations are found. However, annual exotic grasses consisting of *Bromus hordeaceus* L., *B. diandrus* Roth, *B. madritensis* ssp. *rubens* (L.) Husnot, *Avena barbata* Link and *Vulpia myuros* (L.) C. Gmelin, and annual exotic forbs such as *Erodium cicutarium* (L.) L'Hér still form the primary community matrix in which *A. grandiflora* occurs.

Site selection

The experimental site was selected in the fall of 1992. This is the same canyon on Site 300 property as the originally described natural *A. grandiflora* population. The experimental site was selected to mimic the site where *A. grandiflora* occurs naturally as closely as possible. It is in a grassland bordered by blue oak woodland and coastal sage scrub. The elevation is approximately 320 m. The aspect is northwest facing with a slope ranging from 25% to 72% (average 56%). The soil consists of well-drained, deep (about 1 m) sandy clay loam. Herbaceous cover consists primarily of the annual exotic grasses *Bromus hordeaceus* and *Vulpia myuros*, with lesser amounts of *B. diandrus*, *B. madritensis* ssp. *rubens*, *Avena barbata*, and the exotic forb *Erodium cicutarium*. Native species

include the perennial bunch grass *P. secunda* as well as several legumes, including *Clarkia* spp., miniature lupine (*Lupinus bicolor* Lindley), and purple owl's clover (*Castilleja exserta* (A. A. Heller & Heckard)).

To clear the litter from the site for the experiment, a control was conducted on October 26, 1992. Wind, slope and vegetation moisture were used in a model to predict fire intensity (Andrews 1983, Burrows & Rothermel 1983). The predicted heat per unit area was 625 Btu/ft² and fireline intensity was 48 Btu/ft/s.

Treatment assignments

The experimental method consisted of treatments on 40 plots measuring 0.64 m² each (0.8 m x 0.8 m). All 40 plots were sited and were assigned to treatments in the fall of 1992. All *P. secunda* tussocks were manually removed from the plots that received the annual grass treatments. Each plot contained 4 equally spaced target plants (*A. grandiflora*) within the selected treatment. The treatments included three densities of *P. secunda* (subsequently identified as the *P. secunda* treatments), four densities of annual grasses (identified as the annual grass treatments), and four treatments which were plots containing only *A. grandiflora*. All treatments were replicated five times, with plots arranged in a randomized complete block design (Little and Hills 1978).

P. secunda treatment establishment

Between February 5 and February 10, 1993, the *P. secunda* treatments were established. Healthy, mature *P. secunda* tussocks were excavated from land surrounding the experimental site. The tussocks were

into 3-cm-dia.. plugs and transplanted into the plots in a hexagonal arrangement at densities of 11, 22 and 45 plugs per plot (17, 34 and 45 plugs/m²). The high density treatment corresponds to densities of areas of Site 300 that undergo annual controlled burns (unpublished). Four *P. secunda* transplants that were damaged by gopher activity in the first month were replaced. The transplanted plugs were allowed to grow through the remainder of the season. All annual plants were manually removed from the *P. secunda* plots throughout the growing season.

A. grandiflora transplantation

In the fall of 1993, *A. grandiflora* nutlets were germinated in a greenhouse for subsequent transplantation into the field. Nutlets for the present experiment were obtained from a 1988 common garden study conducted at the University of California at Davis by Drs. Daniel Baskin and Ronald Kelley (Pavlik et al. 1993). These nutlets can be traced to nutlets collected from the Site 300 native population by Dr. Robert Baskin at the University of California at Berkeley in the mid-1960s. On November 1993, with the onset of winter rains, *A. grandiflora* nutlets were sown in Petri dishes containing Whatman 40 filter paper saturated with distilled water at 20 nutlets per dish. Each dish was then covered with foil and maintained at approximately 25 °C. In an ambient temperature greenhouse, Steuwe tubes measuring 2.5 cm in diameter by 15 cm deep were filled with native soil collected from the field site and were covered with distilled water. Of the *A. grandiflora* nutlets sown on the 84% germinated. *A. grandiflora* germinules were transplanted into the prepared Steuwe tubes as soon as the pericarps were shed and each

germinule possessed a noticeable hypocotyl and a root length of 1 cm. Transplantation occurred over a period of four days, with the majority of germinules transplanted on December 2, 1993. Transplant success was over 95%. Distilled water was used to irrigate the tubes.

On December 9, 1993, *A. grandiflora* seedlings were transplanted into the field plots. We selected seedlings from the December 2, 1993, greenhouse transplantation that were uniform in size, had well developed cotyledons and the first true leaves beginning to emerge. Upon transplantation into the field, the soil was packed around each seedling and well wetted with distilled water. Four *A. grandiflora* seedlings were transplanted into the center of each plot in a 30-cm-square arrangement. Two seedlings sustained rodent damage and were replaced on December 29, 1993.

Annual grass treatment establishment

During *A. grandiflora* transplantation, *Bromus hordeaceus* seeds were added to the annual grass treatments to ensure a range of exotic grass densities. Depending on the assigned treatment target density, seeds were added at 500, 1000, 2000 and 4000 seeds per 0.64 m² plot. An equal amount of grass thatch was placed on all of the plots to moderate soil temperature. Plots were manually thinned to target annual grass densities on December 13-14, 1994, and again on January 31-February 1, 1994. These target densities were 900, 1800, 2700 and 3600 grass individuals per m². Non-grass monocots were removed from the plots. After February 1, 1994, only the control and *P. secunda* treatments were routinely weeded. Due to the lack of significant natural rainfall, on January 19, 1994, all plots received supplemental watering with 0.16 cm of distilled water per

Psychrometer installation and use

A total of 64 screen-caged Peltier thermocouple psychrometers calibrated in the laboratory using the Brown and Bartos model (Brown and Bartos 1982). Two psychrometers were installed within each plot of the five blocks, which spanned the range of the slope observed in the site. Psychrometers were installed at depths of 10 and 75 cm. To avoid disturbing the plot, a 1-m-deep trench was excavated directly down the plot. The psychrometer was installed by going to the desired depth in the trench, then using a wooden dowel to create a hole reaching 1 m into the plot at the same slope as the plot. The psychrometer was lowered through this hole, the excavation was filled, and the leads to the psychrometer were directed to one of six common stations. All psychrometer cable was buried. A Campbell CR-7 datalogger was used to read all psychrometers. In early February of 1993, 30 of the psychrometers were installed, and the remaining 34 were installed in February of 1994.

Pre-dawn (4 am) soil water potential measurements were obtained at both depths in two to three blocks throughout the month of April 1994. In addition, midday soil water potential measurements were obtained at both depths from each plot on April 21, 1994, which was near the end of the flowering period.

Field observations and nutlet harvest

Phenological observations for all species, and measurements on plant height and number of flowering branches (inflorescences) on each *A. grandiflora* plant were obtained throughout the 1993/1994 growing season. Calibrated ocular estimates of grass cover were made for

on March 17 and April 1, 1994. On March 22, 1994, twenty *A. grandiflora* plants (10 pin and 10 thrum) spanning the observed size range were selected and flagged for nutlet collection. Every 2 to 3 days, nutlets were manually collected from each flagged plant.

Biomass harvest

At the end of the 1992/1993 growing season, above-ground plant biomass from all of the plots was clipped and removed. In addition, biomass was collected from each of the five blocks from a randomly selected plot that had been assigned an annual grass treatment. This biomass was separated by species and weighed to establish initial species composition within the community, exclusive of *P. secunda*.

Between May 3 and May 5, 1994, biomass from plants other than *A. grandiflora* (termed neighbor biomass) was collected from each plot. *A. grandiflora* plants were not harvested, and were thus allowed to seed. For the annual grass treatments, the center 0.6 x 0.6 m of each plot was harvested. Four randomly placed sub-samples were collected within this area (the area of each subsample was delineated by a 0.15 m x 0.15 m square), and the individual plants were counted and separated by species for subsequent dry weight determination. For the *P. secunda* treatments, *P. secunda* biomass was harvested from the entire plot. Species other than *P. secunda* remaining in the plot were collected separately. Of the 15 *P. secunda* treatment plots, one medium density and one high density plot was lost to gopher damage, and thus was not harvested. All 20 annual grass treatment plots survived the experiment intact.

All neighbor biomass was oven dried at 65 °C for 5 days and weighed. Due to the manual thinning of two plots, the manual thinning of two plots was inadequate, resulting in unacceptably high levels of neighbor biomass. These two plots were excluded from subsequent analysis.

Data analysis

Due to the rarity of *A. grandiflora*, we wanted to allow the majority of plants to senesce naturally, hopefully reseeding a new population. Therefore, it was necessary to determine the best predictor of nutlet production for use in subsequent analysis of the effects of grass competition. Therefore, shoot length in centimeters and the number of inflorescences were recorded on April 12, 1994 for each of the twenty harvested *A. grandiflora* plants. These two variables were correlated with total nutlet production per plant to determine the best predictor of nutlet output. Both single and multiple correlations were performed. The predictor or combination of predictors with the highest correlation was selected for use in subsequent analysis of the effects of grass competition on *A. grandiflora*.

The annual grass densities varied greatly within each of the plots. Each plot received an annual grass treatment, which caused the grass biomass collected from these plots to vary as well. We therefore analyzed the resulting data using both regression analyses on a per plot basis and analysis of variance (ANOVA) analyses on a per treatment basis, whichever was appropriate. Caution must be used in interpreting the results of ANOVA analysis, as the treatment classification variable is somewhat artificial, due to the large degree of variation within each treatment classification. Mortality data as percentages were transformed using an arcsin transformation prior to ANOVA (Krebs 1989). A single degree of

freedom F-test (also known as an orthogonal contrast) was used to compare the combined *P. secunda* treatments to the combined annual grass treatments. Mean separation subsequent to an ANOVA to compare levels within a grass treatment was conducted using a Tukey-Kramer pair comparison. ANOVA analyses, orthogonal contrasts and mean separations were conducted using the GLM procedure of SAS (SAS Institute 1990, Little and Hills 1978). Regression analyses were conducted using the procedure of SAS and Statview II (Feldman et al. 1988, Afifi and Soudki 1990). Linear and logarithmic simple regression models were examined. The model with the largest R^2 was considered to have the best fit. For logarithmic models, the significance of the curvature in the model was estimated by fitting a multiple regression in which the second term of the model was the squared independent variable (i.e., a quadratic model). The significance of the quadratic term was examined by fitting a model examining the significance of the quadratic term. The significance level for all statistical tests was set at $\alpha = 0.05$.

RESULTS

Species composition manipulation

To investigate the effect of exotic annual grasses and native perennial grasses on the demography of *A. grandiflora*, we manipulated the natural community to create two artificial communities; one comprised primarily of exotic annual grasses, and one comprised of native perennial grasses (Figure 1). In the annual grass treatment plots, the manipulation increased the proportion of annual grasses, with *B. hordeaceus* and *V. myuros* remaining the dominant species. Annual grass species comprised 95% of the biomass in the annual grass treatment plots. In the *P. secunda* treatment plots, *P. secunda* comprised an average of 80%

biomass collected from the field plots. The remaining 20% of the was comprised primarily of annual grasses growing within the tus although some dicots were also present in the plots. Control tre plots contained primarily *A. grandiflora*, although a small amoun neighbor biomass was present (less than 10 g/m²), consisting prin annual grasses.

Phenological Observations

The first significant rain of the season (1.8 cm) occurred on 17, 1993. This stimulated germination of the annual grasses and *grandiflora*, and broke the dormancy of *P. secunda* tussocks (as ev by greening of the tussocks) on November 19, 1993 (Table 1). Howe date of first flowering was much earlier for *A. grandiflora* and compared to the exotic annual grasses (21 and 35 days earlier, re *P. secunda* was dormant by April 19, 1994, and *A. grandiflora* had senesced by May 1, 1994, whereas the annual grasses were still gr producing seed at the time of the May 1994, harvest.

Characteristics of the grass treatment plots

The range of peak standing biomass obtained within the annual treatments (54 - 111 g/m²) and the *P. secunda* treatments (67 - 135 were very similar (Table 2). We were able to maintain the *P. sec* density classes with a high degree of precision. However, it was maintain the annual grass density classes at a given level. While significant difference between the low and high density treatment achieved, there was no real separation between the two medium den annual grass treatments.

Both grass treatments affected pre-dawn (4 am) soil water potential depth of 10 cm measured between April 13 and May 3, 1994 (Figures 2b). Soil water potential recovered for the *P. secunda* and control between April 25 and May 2. Approximately 1.2 cm of rainfall occurred April 26. At this time, *P. secunda* was fully dormant, but the annual grasses were in full flower. *A. grandiflora* had completed flowering and was still filling seed (Table 1). The rate of soil moisture depletion was greatest between April 14 and April 21, 1994. During this time the rate of soil water potential change was related in a linear fashion to biomass, and was independent of grass type (Figure 2c). Both grass treatments also reduced midday soil water potential at the 10-cm depth measured on April 21, 1994, but this reduction was not significantly different between the two grass types (Figure 2d). No significant soil water potential was observed at the 75 cm depth, therefore, data are not presented.

Maximum grass cover as estimated on April 1, 1994, exceeded 70% for both grass treatments (Figure 3). The amount of cover in the *P. secunda* treatments was linearly related to the biomass of *P. secunda*, whereas the amount of annual grass cover was logarithmically related to annual biomass. The quadratic term in the fitted quadratic model was significant for the annual grass biomass (Table 3). The quadratic model resulted in a larger R^2 term than the linear model, and was similar to the R^2 of the logarithmic model (Figure 3). This indicates that the curvature is a significant component of the fitted model.

A. grandiflora nutlet output predictor

From the twenty *A. grandiflora* plants from which nutlets were collected, correlations on several plant attributes were conducted. Inflorescence number was highly correlated with nutlet production (Figure 4), as was inflorescence number combined with the natural logarithmic transformation of shoot length ($r=0.97$). Because of interpretation, the total number of inflorescences per plot (i.e. the number of inflorescences of the four individual *A. grandiflora* plants) as well as the average number of inflorescences per plant per plot were subsequently used to analyze the effects of grass neighbor competition on *A. grandiflora*.

A. grandiflora response to grass treatments

A. grandiflora mortality was very low (Table 5), with an overall mortality of 6.9%. Mortality was highest in the *P. secunda* treatments and increased with *P. secunda* density (over 30% in the high density treatments). Mortality did not appear to be related to annual grass cover in the annual grass treatments. Mortality was zero in the control treatments.

Using *A. grandiflora* inflorescence number as an index of nutlet production, we examined the reproductive response of *A. grandiflora* to the grass treatments (Figure 5). *A. grandiflora* inflorescence number only responded to soil water potential in the two grass treatments as measured on April 21, 1994 (Figure 5a). In addition, the slopes of the two regressions were significantly different. *A. grandiflora* inflorescence number did not respond to *P. secunda* cover (Figure 5b). *A. grandiflora* responded in a linear fashion to *P. secunda* cover, but in a logarithmic fashion to the annual grass cover. The quadratic term in the fitted quadratic model was significant

annual grass cover (Table 3). The quadratic model resulted in a term compared to the linear model, but was not as large as that of the logarithmic model (Figure 5b). Thus, the curvature is a significant component of the fitted model, with the logarithmic model providing the best fit. When the overall response of *A. grandiflora* inflorescence number to neighbor biomass was examined, *A. grandiflora* again responded to *P. secunda* biomass in a linear fashion, but to annual grass biomass in a logarithmic fashion (Figures 5c). The quadratic model fitted to *A. grandiflora* inflorescence number again indicated the curvature to be a significant component of the fitted model (Table 3), with the logarithmic model having the largest term (Figure 5c). At the highest densities, competitive suppression of *A. grandiflora* inflorescence number by *P. secunda* and the exotic grasses was equally severe. However, at low and intermediate densities, annual grasses suppressed *A. grandiflora* inflorescence number to a greater extent than *P. secunda* on a per biomass basis.

An ANOVA on these treatments verifies that there are significant differences between treatments (Table 6), but also reveals a significant block effect. This effect occurred because the two blocks in the more sloping portion of the study site contained more annual grass biomass compared to those plots in the steeper portion of the study site. Planned contrasts revealed that inflorescence number in the combined *P. secunda* treatments was significantly different from the combined annual grass treatments (Table 6). Besides the increased mortality observed in the highest density *P. secunda* plots (Table 5), the grass treatments reduced *A. grandiflora* fecundity by both reducing the growth of the shoot system of individual plants (Figure 6a) and reducing the number of inflorescences per plant (Figure 6b). Again, at the highest densities, the effect of

P. secunda and the exotic grasses on *A. grandiflora* growth was s but at low and intermediate densities, the exotic annual grasses *A. grandiflora* growth to a greater extent than *P. secunda*.

DISCUSSION

The results of this experiment suggest that *A. grandiflora* per (as indicated by inflorescence number) is greatest in a community dominated by intermediate densities of *P. secunda* when compared v community dominated by exotic annual grasses, at least under the conditions found during this single growing season experiment. Surprisingly, water did not appear to be the limiting resource in competition between *A. grandiflora* and its grass neighbors. Other either suggested (Menke, 1992) or demonstrated (Gordon and Rice 19 Momen et al. 1994) that competition for water can be important bet exotic and native California grassland species. Indeed, we found grass types reduced soil water potential throughout the growing s This appeared to be linearly related to the amount of grass bioma *A. grandiflora* did respond to soil water potential, but only weak possible that the variation in our data masked any competition fo that may have occurred. There was a recovery in soil water poten during *A. grandiflora*'s seed filling period in the *P. secunda* an treatment plots as a result of rainfall during this period. The moisture in these plots could have resulted in either a greater *A. grandiflora* seed number per inflorescence or greater seed wei Unfortunately, we did not collect the data required to test this I In contrast, the annual grasses were able to utilize this soil m which kept the soil water potential low. This indicated that the

grasses were more metabolically active than *P. secunda* during the drought. Both seed number and seed weight of California annuals can be affected by drought during seedfill (Ewing and Menke 1983). However, it is not from our data that *A. grandiflora* is so affected.

A. grandiflora responded much more strongly to the amount of grass cover, which in turn was related to neighbor biomass. While *A. grandiflora* total inflorescence number was reduced by both grass cover and biomass, a per unit amount of annual grass cover or biomass more severely affected *A. grandiflora* inflorescence number compared with *P. secunda* cover or biomass, especially at the highest grass densities. In addition, the annual grasses affected *A. grandiflora* in a logarithmic fashion, versus a linear effect of *P. secunda*. The linear effect of *P. secunda* is highly unusual, typically the effect of increasing neighbor biomass or density on target plant is strongly non-linear, with a large decrease in target plant response to the presence of any neighbors, and smaller effects of adding additional neighbors (Goldberg and Fleetwood 1987, Silander and Pacala 1985). The differing effect on *A. grandiflora* by *P. secunda* and the annual grasses may be a result of the different community structure exhibited by the grass types. Spacing patterns can have a marked effect on density dependence (Silander and Pacala 1985, Fowler 1984), as well as on interspecific competition (Mack and Harper 1977). A community of annual grasses forms a more or less solid sward of plant material, which likely has an effect on the amount and quality of light reaching *A. grandiflora* seedling (Taiz and Zeiger 1991), which in turn controls seedling growth. A community in which *P. secunda* is the dominant, on the other hand, will have the majority of the biomass and leaf area concentrated in well defined tussocks, resulting in well lighted,

open spaces. Our results suggest that *A. grandiflora* can readily advantage of these open spaces.

As our results show, however, *P. secunda* can strongly compete native forbs at higher densities. In addition, data collected on Site 300 where annual controlled burns have resulted in thick stands of 100% cover of *P. secunda* reveal these communities to be species poor (Taylor and Davilla 1986). Properly timed fires have been frequently used as a means of reducing annual grass competition and promoting native perennial bunch grass communities (Menke 1992, George et al. 1992). However, aggressive land management techniques aimed at creating high density bunch grass communities that can withstand invasion of exotic grasses may not result in a habitat which is favorable for forbs. In addition, *P. secunda*'s early phenology is also very similar to *A. grandiflora*, as shown here. This suggests that a community with adequate disturbance to maintain *P. secunda* at an intermediate density with minimal amounts of exotic annual grasses would result in a community with the highest species richness, and the most favorable for *A. grandiflora*. Indeed, data from Site 300 show that stands with intermediate density of *P. secunda* are species rich (Taylor and Davilla 1986). Others have also suggested that forbs do not establish well in bunch grass communities, with good forb-bunch grass associations typically occurring where environmental factors combine to keep the grasses well spaced (Edwards 1994).

It is difficult to speculate on what comprised the pristine California grassland community. It is likely that perennial bunch grass communities with numerous native annuals filling the interspaces formed a stable climax community on well-drained upland sites (such as the location of Site 300).

Site 300 experimental site), with annual herbaceous plants dominating intermediate and early successional stages (Heady 1990). The current annual grasslands are dominated by a small number of exotic annual species with a few widespread natives present. In the pristine vernal grassland, the community was probably dominated by those same widespread natives (such as *Nassella pulchra* associated with other perennial grasses like *P. secunda*), but also contained native forbs of limited distribution, resulting in a greater regional heterogeneity (Huenneke 1989, Heady 1990). The dominance of *P. secunda* at Site 300 is likely a consequence of the late spring/early summer timing of the unusually frequent annual controlled burns, as opposed to the natural occurring lightning-caused fires of late summer/early fall timing and intermittent frequency (Heady 1990). At the time of the Site 300 burn, *P. secunda* is already dormant, whereas *N. pulchra* is still physiologically active (Laude 1953; note: Laude's *P. scabrella* is equivalent to our *P. secunda*). Our results suggest that a pristine "stable" bunchgrass community may have required some type of disturbance at an intermediate frequency to maintain the open spaces. It is also possible that a bunchgrass community with grasses of both early (*P. secunda*) and late (*Nassella pulchra*) phenology may have provided a more heterogeneous community favorable to native forbs.

While this and other studies (Pavlik et al. 1993) have demonstrated that *A. grandiflora* is negatively affected by annual grass competition, we cannot say that competition with exotic grasses is the primary mechanism in the decline of this species. And although the fact that *A. gr.* performs better in a native bunchgrass community supports this hypothesis, it is not sufficient to prove it. Ornduff (1976) has

A. grandiflora is being competitively displaced by its more weedy homostylous congeners as a result of an inefficient heterostylous system reported to be self-incompatible (Weller and Ornduff 1977, Indeed, *A. grandiflora* produces fewer seeds per flower than its relatives (Ornduff 1976, Pantone et al. 1995). While these factors for *A. grandiflora* are undoubtedly important, again, their presence does not prove they are the cause in this species decline. Pantone et al. (1995) suggest that fitness component compensation (i.e., the production of fewer seeds per flower results in more flowers per inflorescence being produced) may occur within either *A. grandiflora* or *A. menziesii* var. *intermedia*. However, this analysis was made within species, not between them. To determine if *A. grandiflora* is being displaced by *A. menziesii* var. *intermedia*, it is important to conduct a comparison between the two species. For example, if *A. grandiflora* produces fewer seeds per flower than *A. menziesii* var. *intermedia* (which it does), does *A. grandiflora* produce more flowers per plant than its relative, resulting in similar seed set per plant? Pantone et al. (1995) suggests that an increased amount of inflorescence production by *A. grandiflora* could not completely compensate for the low seed set per flower when compared to *A. menziesii* var. *intermedia*, indicating an intrinsic limit to fecundity in *A. grandiflora*. Further field and greenhouse experiments should be conducted to test this assertion. Assuming *A. grandiflora* could compensate for the low number of seeds per flower by producing more flowers, it is possible that the effects of competition on *A. grandiflora* would be more severe as compared to its weedy relatives, since the larger floral structures (i.e., inflorescences) would necessarily require more resources. Pantone and Pavlik (1996) could not demonstrate a significant difference in competition between *A. grandiflora* and *A. menziesii* var. *intermedia*.

ability between *A. grandiflora* and *A. menziesii* when the two species are grown in mixture. A field competition study comparing the effect of competition on *A. grandiflora* and *A. menziesii* var. *intermedia*, would be extremely useful in assessing the relative ability of *A. grandiflora* and its weedy relatives in competing with introduced annual grasses.

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TABLE 1. Phenology at the Site 300 experimental site, November 1993 to May 1994

Stage	Annual grasses	<i>P. secunda</i>	<i>A. grandiflora</i>
Germination	11-19-93 ^a	NA	11-19-93 ^a
Tussock greening	NA	11-17-93 ^a 12-9-93 ^b	NA
Flowering	3-31-94 ^a 5-1-94 ^c	2-24-94 ^a	3-9-94 ^a 4-20-94 ^b
Senescence	>5-1-94	NA	5-1-94 ^b
Tussock dormancy	NA	3-31-94 ^a 4-19-94 ^b	NA

a) First observed. b) Complete. c) Full flower. NA = Not applicable.

TABLE 2. Summary of neighbor biomass and estimated density by treatment at the Site 300 experimental site, May 1994.

Treatment	n	Neighbor biomass g/m ² (± 1 standard error)	Neighbor density/m ² (± 1 standard error)
Poa secunda treatments			
High (PSH)	4	135 ± 11 ^a	67 ± 1.5 ^a
Medium (PSM)	4	103 ± 3.1 ^a	33 ± 0.6 ^b
Low (PSL)	5	67 ± 6.0 ^b	17 ± 0.3 ^c
Average over all treatments (PS)	13	99 ± 9.1 Range: 49 to 155	37 ± 6.0 Range: 16 to 69
Annual grass treatments			
High (AGH)	5	111 ± 20 ^a	3160 ± 580 ^a
Medium-high (AGMH)	5	87 ± 15 ^a	3185 ± 650 ^a
Medium-low (AGML)	5	95 ± 44 ^a	2950 ± 760 ^{ab}
Low (AGL)	5	54 ± 13 ^b	1840 ± 360 ^b
Average over all treatments (AG)	20	87 ± 8.8 Range: 18 to 140	2784 ± 305 Range: 975 to 5550
Control treatments (C)	3	2.3 ± 0.85 Range: 1.0 to 3.9	ND

ND = not determined. Within each grass type, treatments with different letter; significantly different at $p = 0.05$.

TABLE 3. Significance of independent variables in simple regressions and multiple quadratic regressions.

Dependent Variable	Independent Variable (X)	model	X p	X ² p	R ²
% ag cover ^a	ag biomass	0.0001	0.0001	— ^b	0.58
% ag cover	ag biomass	0.0001	0.0001	0.0018	0.74
#inflor ^c	% ag cover	0.0001	0.0001	—	0.54
#inflor	% ag cover	0.0001	0.0001	0.0145	0.64
#inflor ^d	ag biomass	0.0004	0.0004	—	0.43
#inflor	ag biomass	0.0001	0.0007	0.0133	0.56

^a See Figure 3 for logarithmic model R² and significance level; ag = annual grass.

^b Not applicable for the simple regression.

^c See Figure 5b for logarithmic model R² and significance level. #inflor = number of *A. grandiflora* inflorescences per plot.

^d See Figure 5c for logarithmic model R² and significance level.

TABLE 4. Correlations between measures of plant size and reproductive output per individual *A. grandiflora* from the Site 300 experimental site, April 12, 1994;

X ₁ ^a	X ₂ ^a	Y	r	p
ln(shoot lgth)	#inflor	# nutlets	0.97	0.0001
#inflor	— ^c	# nutlets	0.96	0.0001
ln(shoot lgth)	#inflor	ln(# nutlets)	0.94	0.0001
#inflor	—	ln(# nutlets)	0.93	0.0001
ln(shoot lgth)	ln(#inflor)	ln(# nutlets)	0.90	0.0001
ln(#inflor)	—	ln(# nutlets)	0.90	0.0001
ln(shoot lgth)	ln(#inflor)	# nutlets	0.80	0.0001
ln(#inflor)	—	# nutlets	0.80	0.0001
ln(shoot lgth)	—	ln(# nutlets)	0.80	0.0001
shoot lgth	—	ln(# nutlets)	0.78	0.0001
ln(shoot lgth)	—	# nutlets	0.67	0.0011
shoot lgth	—	# nutlets	0.67	0.0012

^a #inflor = number of inflorescences per plant; shoot lgth = length of shoot : centimeters (i.e., total height of plant); ln = natural logarithm.

TABLE 5. *A. grandiflora* mortality at the Site 300 experimental site, November 1993 to May 1994.

Treatment ^a	n	% Mortality ($\pm$ 1 standard error)
Poa secunda treatments		
PSH	4	31.25 $\pm$ 18.8 ^b
PSM	4	12.5 $\pm$ 7.2 ^a
PSL	5	0.0 ^a
PS	13	13.5 $\pm$ 6.7
Annual grass treatments		
AGH	5	0.0 ^a
AGMH	5	10.0 $\pm$ 6.1 ^a
AGML	5	5.0 $\pm$ 5.0 ^a
AGL	5	0.0 ^a
AG	20	3.8 $\pm$ 4.1
C	3	0.0
Over entire experiment	38	6.6 + 2.6

^a See Table 2 for treatment abbreviations. Within each grass type, treatments with different letters are significantly different at $p = 0.05$.

TABLE 6. Summary of Analysis of Variance on total number of *A. grandiflora* inflorescences per plot by treatment.

Dependent variable: Total Inflorescence number per plot					
Source	df	SS	MS	F	p
Model	11	17015.62	1546.87	8.55	0.0001
Error	24	4341.27	180.89		
Corrected Total	35	21356.89			
Source	df	SS	MS	F	p
Treatment	7	13843.53	1977.65	10.93	0.0001
Block	4	2307.34	576.84	3.19	0.0310
Orthogonal Contrast					
Contrast	df	Contrast SS	MS	F	p
P. secunda vs Annual grasses	1	3647.41	3647.41	20.16	0.0002

FIG. 2. Effect of (a) final *P. secunda* biomass and (b) final dry annual grass biomass by treatment on pre-dawn (4 tial at 10 cm between April 13 and May 3, 1994 (intermediate densities omitted for clarity); (c) final dry g soil water potential change per plot at 10 cm between April 13 and April 28, 1994; and d) final dry grass bi on midday soil water potential at 10 cm as measured on April 12, 1994 (treatment abbreviations); $n = 2$; error standard error

FIG. 3. Effect of final dry grass biomass (collected on May 1, 1994) on percent grass cover measured on April 1, 1994.

FIG. 4. Nutlet output of *A. grandiflorum* plants at the Site 300 experimental site as a function of the number of inflorescences measured on April 13, 1994.

FIG. 5. Response of total grandiflorae inflorescence number per plot (as measured on 12, 1994) to (a) midday soil water potential at 10 cm (as measured on 12, 1994), (b) percent grass cover (as measured on 12, 1994), and (c) final dry grass biomass (collected May 1, 1994); see Table 2 for abbreviations.

FIG. 6. Increase in (a) average inflorescence number per plant per plot by treatment and (b) average shoot length in centimeters per plant per plot by treatment (Table 2 for treatment abbreviations); error bars are one standard error

THE ROLE OF INTRINSIC FACTORS IN THE
REPRODUCTIVE ECOLOGY OF AN ENDANGERED PLANT

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Abstract. A set of field and greenhouse experiments were conducted to compare intrinsic limitations in fitness components of the rare heterostylous forb *A. grandiflora* and its weedy homostylous congener *A. tessellata*. The highest floral seed cache (seeds/ flower) was with intermorph pollinations (although intramorph pollinations also produced significant seed set), and was comparable to that observed in grown *A. tessellata*. Pin progeny nutlets (seeds) of *A. grandiflora* up to 1.14 mg/nutlet more than thrum nutlets in both greenhouse experiments. Three greenhouse germination trials showed germination responses of both species to be similar regardless of breeding system typical of many annual species found in California grasslands. Interspecies comparisons of adult plants suggested that in the greenhouse *A. grandiflora* can compensate for low floral seed cache by increasing output (flowers/plant) with a greater number of flowers per inflorescence. In the field, *A. grandiflora* floral output was also greater than *A. tessellata* but without full compensation for lower floral seed cache. *A. tessellata* average seed weight was lower than *A. grandiflora*, which along with a lower number of flowers produced, indicated lower maternal investment per nutlet than *A. grandiflora*. Thus, competition by exotic annuals in the field could affect *A. grandiflora* reproduction to a greater extent than *A. tessellata*. Under conditions of unlimited resources, it appeared that *A. grandiflora* fitness is not intrinsically limited when compared to its weedy relative *A. tessellata*. However, under field conditions, the heterostylous breeding system of *A. grandiflora* may result in low reproductive output.

Keywords: *A. grandiflora*, *A. tessellata*, rare plants, weedy plants, intrinsic limitations, pollination biology, heterostyly

INTRODUCTION

To conservation biologists, rarity has traditionally been cause for concern. Rare species have a greater likelihood of extinction than do more common species (Rabinowitz, 1981; Gaston, 1994). Because of limited distribution, small population sizes, or unusual habitat requirements, Darwin (1859) noted that rarity is a necessary precursor to extinction, with its underlying patterns and causes (Fiedler and Ahouse, 1992). Because of the link between extinction and rarity, a great deal of effort has been directed towards developing a scientific framework within which to understand those patterns and causes (Gaston, 1994; Fiedler and Ahouse, 1992; Rabinowitz, 1986; Rabinowitz, 1981). These studies typically focused on those characteristics or factors responsible for the occurrence of naturally "intrinsically" rare species, although Cody (1986) briefly mentioned anthropogenic or "extrinsic" rarity, and Fiedler and Ahouse (1992) gave some discussion of the interaction between intrinsic and extrinsic factors. As more habitats become degraded or modified due to human influence, it is becoming increasingly important to identify those intrinsic (naturally occurring) and extrinsic (anthropogenically influenced) factors that contribute to the rarity of a given species. Indeed, it may be that with anthropogenic factors that pushes a naturally rare species closer to the edge to extinction.

Amsinckia grandiflora (Gray) Kleeb. ex Greene (Boraginaceae) is an annual plant for which both intrinsic and extrinsic factors have been invoked to explain its extreme rarity. Extrinsic limits may have been imposed as the native species of its grassland habitat were reduced or eliminated, native species, and as livestock grazing and fire suppression became widespread in Northern California. There has been particular concern

that *A. grandiflora* may be poorly competing with its exotic grass neighbors (Knudsen and Tarp, 1994). During reintroduction experiments, Pavlik et al. (1993) found that competition with non-native annuals significantly reduced the fecundity of *A. grandiflora*. In addition, management of a declining natural population of *A. grandiflora* using a grass-selective herbicide has caused a rebound from less than 100 individuals to its present number of over 1500 (Pavlik, 1995). Native perennial grasses were subsequently shown to have less detrimental effects on fecundity than annual grasses (Carlsen et al., 1996), suggesting that *A. grandiflora* would perform better within a community more closely resembling the original California grasslands in which it presumably evolved.

Possible intrinsic limitations on *A. grandiflora* could result from its breeding system, or from genetic limitations as a result of low population density. Of the fifteen species in the genus recognized by Ray and Ornduff (1957a and 1957b), *A. grandiflora* is one of four rare heterostylous species with highly restricted distributions from which the more weedy homostylous congeners are thought to have evolved (Ray and Chisaki, 1957b). Ornduff (1976) has suggested that *A. grandiflora* is likely competitively displaced by the weedy homostylous congeners because heterostyly and partial self-incompatibility greatly reduce effective pollen transfer and seed set (Weller and Ornduff, 1977, 1989). Pantone et al. (1995) found that fitness-component compensation (i.e., fewer seeds per flower compensated by more flowers per inflorescence) did not occur with *A. grandiflora* or the widespread *A. menziesii* var. *intermedia* (Fernald and C. Meyer) Ganders. They also suggested that increased floral production in *A. grandiflora* could not compensate for its low seed set per flower.

to *A. menziesii* var. *intermedia*), thus indicating an intrinsic fecundity.

In order for a weedy relative to displace *A. grandiflora*, it must have a higher degree of fitness. We conducted a set of field and greenhouse experiments to evaluate intrinsic limitations in the fitness of *A. grandiflora* as compared with *A. tessellata* A. Gray, a weedy, sympatric relative. To exhibit a fitness advantage, *A. tessellata* must have either (1) a higher fecundity (seed output), or (2) higher germination rates and/or lower seedling mortality, resulting in a greater number of reproducing plants. We conducted greenhouse germination trials to compare the germinability of the two congeners. We also compared survivorship, seed output, and field performance. This included an evaluation of the distinctive breeding systems of these two congeners. We discuss the results in the context of the intrinsic limitations on the fitness of *A. grandiflora* as well as the potential interaction between intrinsic and extrinsic factors which may affect *A. grandiflora* fitness.

MATERIALS AND METHODS

The Study Species

A. grandiflora and *A. tessellata* are both members of the California annual grassland. As such, both species germinate with the onset of winter rains, grow vegetatively throughout the winter, flower in spring, set seed, and die prior to the summer drought (Heady, 1988). Precise information concerning seed banks of both species is not available although it is likely that only a small percentage of seed carry over year to year (Evans and Young, 1989). The historic range of *A. grandiflora* apparently ranged from the hills near Antioch to the north (on the

Sacramento-San Joaquin River Delta), south through the Diablo Range (including the city of Livermore) to northern San Joaquin County. *A. grandiflora* is currently known from only three natural populations containing individuals numbering from fewer than 30 to several thousand. These populations occur in grasslands which border on blue oak woodlands and coastal sage scrub on steep, well-drained, north-facing slopes at intermediate (300 m) elevations. All three populations are located in the Altamont Hills of the Diablo range, about 50 miles east of San Francisco, California, on or near Site 300. Site 300 is a Lawrence Livermore Laboratory high-explosive testing facility, operated by the University of California for the U.S. Department of Energy. Site 300 contains the three known natural populations of *A. grandiflora*; the original population (known as the Drop Tower population) which currently contains around 1500 individuals, and a second, much smaller population (known as the Draney Canyon population) which contains around 30 individuals. A third natural population is located adjacent to Site 300 and is known as the Carnegie Canyon population. This population contains several thousand individuals.

A. grandiflora exhibits a form of heterostyly known as distyly. Pin and thrum flower forms (or "morphs") are produced (Ganders, 1979). Each individual plant has only one floral morph. Pin flowers are characterized by having a stigma exserted and the anthers within the corolla tube. Thrum flowers have the opposing morphology, with the anthers exserted and the stigma within the corolla tube. This morphology represents a type of outcrossing known as phenotypic disassortative mating (in which pins pollinate thrums, and thrums pollinate pins). Many distylous species are not self-compatible (do not exhibit assortative mating or self-po-

and may be "morph"-incompatible (pollination by a separate plant of same floral morph is unsuccessful). Distyly appears to be controlled by a pair of alleles. Pin plants have the genotype recessive *ss* and thrum plants have the genotype *Ss* or *SS*. Under conditions of complete disassortative mating (no self- or intramorph pollination), the *SS* genotype does not occur and the phenotypic pin and thrum ratio in the progeny is 1:1. In a pin X pin intramorph cross, all progeny would be pins. In a thrum X thrum phenotypic cross in which the thrum originally contained the *Ss* genotype, the expected pin: thrum ratio in the progeny would be 1:1. The occurrence of either self- or intramorph thrum pollinations would result in the presence of the *SS* genotype. This genotype would alter the expected phenotypes from the thrum X thrum cross, resulting in 1/3 thrums in the progeny. Although distyly in *A. grandiflora* appears to be controlled by the *S* allele system, it appears to be only partially disassortative (Weller and Ornduff, 1977), with significant seed production from intramorph crosses.

A. tessellata is a common weedy annual that has spread throughout much of the western United States from its phylogeographic center in the coastal ranges of California (Ray and Chisaki, 1957a). *A. tessellata* is homostylous (the anthers and stigma are opposite each other), self-compatible species exhibiting a high degree of assortative mating. *A. grandiflora* and *A. tessellata* both belong to the Tessellatae section within the *Amsinckia* genus (Ray and Chisaki, 1957b). As members of the Boraginaceae family, flowers of each species can produce a maximum of four seeds, called nutlets. Ray and Chisaki (1957b) presented evidence that *A. grandiflora* ($n = 6$, smooth nutlets, heterostylous) gave rise to *A. douglasiana* A. DC ($n = 6$, rough nutlets, heterostylous), which

gave rise to *A. tessellata* (n = 6, rough nutlets, homostylous).
occurs sympatrically with *A. grandiflora* in the field.

A. grandiflora pollination studies

During the 1993-94 and 1994-95 growing seasons, greenhouse studies were conducted on *A. grandiflora* and *A. tessellata* to examine the potential nutlet output of both species. However, given the more complex heterostylous mating system of *A. grandiflora*, it was first necessary to examine the different possible pollination regimes for this species. The greenhouse studies were conducted at the Lawrence Livermore National Laboratory in Livermore, California. *A. grandiflora* nutlets for pollination studies were obtained from a 1988 common garden study conducted at the University of California at Davis by Drs. Daniel and Ronald Kelley (Pavlik et al., 1993). These nutlets were described in the collections by Dr. Robert Ornduff of the University of California in the mid-1960s from the Drop Tower natural population at Site 300. Nutlets from this common garden source are referred to as the Davis source. Nutlets had been stored at 4 °C. On 27 November 1993, *A. grandiflora* nutlets were germinated in petri plates as described in the protocol for germination Trial A. Seedlings were established in 15.25-cm. pots with native soil from Site 300. Seedlings were maintained in an open temperature greenhouse and irrigated with tap water through a drip system. Beginning in March 1994, all plants received weekly irrigation with 50% strength Hoagland's solution.

In early March 1994, 40 uniform-size *A. grandiflora* plants were randomly selected (20 pins and 20 thrums). Plants were divided into two blocks (arranged in an east-to-west orientation), each containing

and four thrum individuals. The greenhouse was completely sealed to exclude external pollinators, and airflow and disturbance were minimized. Flowers on each plant received one of four pollination treatments: (1) manual manipulation ("passive self-pollination", abbreviated ps), (2) manually-assisted self-pollination ("active self-pollination", abbreviated as), (3) manually-assisted intramorph pollination (abbreviated intra), and (4) manually-assisted intermorph pollination (abbreviated inter). This resulted in a total of eight treatments (four treatments for each morph). Pollinations were conducted on newly opened flowers on 29, 30 and 31 March 1994. This resulted in at least three flowers per treatment per plant, with totals between 56 and 64 individual flower treatments. Nutlets were carefully collected from each labeled flower on 15 through 28 April 1994. Nutlets were air dried, weighed and then stored at 4 °C.

Nutlets from each pollination treatment were germinated during the 1994-95 winter (8 months after collection). On 29 December 1994, 60 nutlets from each treatment were grouped into three or four replicates in Petri dishes of approximately 20 nutlets. Nutlets from each treatment were germinated in a minimum of five plants. Germination under ambient greenhouse temperature was recorded for 24 days.

Germination Trials

Germination trials were conducted to compare germinability of *A. grandiflora* nutlets to that of *A. tessellata* nutlets. Trial A was conducted on 27 November 1993 using *A. grandiflora* nutlets from the DA88 source. *A. tessellata* nutlets came from volunteer plants growing with *A. grandiflora* in the 1988 common garden study, and thus are also

referred to as the DA88 source. All nutlets had been stored at 4 °C and were placed into petri plates containing Whatman 40 filter paper saturated with distilled water, with 20 nutlets per plate. Each plate was covered with foil and replicated three times for a total of 60 nutlets per species. Germination at room temperature (approx. 22 °C) was recorded over 30 days. Petri plates were remoistened with distilled water as necessary.

Trial B was conducted in August 1993. *A. grandiflora* nutlets used in this trial were collected in June 1993 from a Site 300 experimental population founded from the DA88 source. *A. tessellata* nutlets were collected in June 1993 from a natural population near the *A. grandiflora* experimental population. These nutlets are referred to as the EX Nutlets. Nutlets were collected unusually late in the spring because of poor rainfall and delayed senescence. After collection, nutlets from each species were air dried in paper bags at room temperature (about 22 °C). On 1 August 1993, 120 Steuwe tubes were filled with native soil from Site 300. Each tube had been premoistened with distilled water. Each tube was sown with a single nutlet of either species at a depth of 0.5 cm. After sowing, each tube was irrigated with 0.5 cm of distilled water, placed into racks, and organized into replicates of 10 tubes each. Replicates were arranged in four blocks (arranged in an east-to-west geometry to remove the effect of differing light intensity) and placed in a warm greenhouse (approx. 22 °C daytime). Each block contained one *A. tessellata* replicate (10 tubes) and two *A. grandiflora* replicates (20 tubes). Tubes were kept moist with distilled water, and germination was recorded through 22 September 1993. On 22 September 1993, apparently ungerminated *A. grandiflora* nutlets were excavated from 38 tubes and examined for evidence of germination.

Trial C was conducted in January 1995. The *A. grandiflora* nutlet sources were: (1) nutlets collected in the spring of 1994 from the Canyon natural population (labeled CC94), and (2) nutlets collected in the spring of 1994 from a Site 300 experimental population (founded from the Davis source) located near the Drop Tower natural population (labeled EX94). *A. tessellata* nutlets were obtained in the spring of 1994 from greenhouse-grown plants (labeled GR94) founded from the Davis source. After air drying at room temperature, the nutlets were stored at 4 °C. Petri plates were prepared on 23 January 1995 as described above for Trial A except that each plate contained 10 nutlets. Plates containing nutlets from source CC94 were replicated three times, and plates containing nutlets from sources EX94 and GR94 were replicated six times. These were incubated in a cool, ambient temperature (approx. 5 °C daytime) head house. Germination was recorded over a 10-day period. After 10 days, any ungerminated nutlets were allowed to air dry for an additional 10 days. After drying, the remaining nutlets were again placed into preprepared plates and observed for 15 days to determine the occurrence of additional germination.

Interspecies comparisons in the greenhouse

Growth and reproductive attributes were compared between greenhouse-grown *A. grandiflora* and *A. tessellata* during the 1994 and 1995 winter/spring growing seasons. In January 1994, four germinules of *A. grandiflora* and *A. tessellata* from germination Trial A were transplanted into 10.15-cm. pots containing native Site 300 soil. The plants were maintained in an ambient temperature greenhouse, drip-irrigated with water, and given weekly irrigations with 50% strength Hoagland's

during the flowering period. Once all flowers had senesced, the number of inflorescences per plant (inflorescence output) and total of flowers per plant (floral output) were determined. Any available *A. tessellata* nutlets were collected, air dried and weighed (how many nutlets were lost). Due to the lack of pollinators, *A. grandiflora* did not produce a significant number of nutlets. Above- and below-ground biomass was then separated, oven-dried at 60 °C, and weighed for each plant.

In February 1995, fifteen germinules each of *A. grandiflora* EX94 and *A. tessellata* GR94 from germination Trial C were transplanted into 10 cm. pots containing standard greenhouse potting soil. Plants were maintained in an ambient temperature greenhouse, drip-irrigated with water, and given weekly irrigations with 50% strength Hoagland's solution during the flowering period. Seven *A. grandiflora* plants were mutually inter-morph pollinated. Once all flowers had senesced, inflorescence output and floral output were determined for each plant. Above-ground biomass was oven-dried at 60 °C and weighed for each plant.

Interspecies comparisons in the field

In October 1994, thirty-six 0.25-m² plots were established in two locations. One location was near the native Drop Tower population (location 1); the second location was on an ecological reserve adjacent to Site 300 of the California Department of Fish and Game (location CDFG). Each plot was seeded with nine *A. grandiflora* nutlets from the EX94 source. The nutlets were sown in three rows of three, equidistantly spaced at 10 cm. At the onset of winter rains, the germinating *A. grandiflora* nutlets were collected along with an *A. tessellata* of the same age from the plot's ambient

bank. A total of 29 species pairs were marked at the DT location pairs at the CDFG location. Plants were periodically checked for survivorship and inflorescence output throughout the growing season. After each individual flower senesced, the sepals were marked, and fully-filled, green nutlets counted. This provided an accurate estimate of floral output and floral seed cache (number of nutlets per flower plant). As we wished to allow all *A. grandiflora* nutlets to enter the bank and potentially assist in propagating the species, final above-ground biomass was not collected. Nutlet collection was limited to collecting mature nutlets from five *A. tessellata* (one from CDFG and four from DT location) and ten *A. grandiflora* plants (four from CDFG and six from DT location).

Data analysis

Percentage data were arcsine square-root transformed prior to statistical analysis (Krebs, 1989). Analysis of variance (ANOVA) statistical tests were conducted using the GLM (General Linear Model) procedure, and all linear regressions were conducted using the REG (Regression) procedure of SAS (SAS 1990). Mean separation between treatments was conducted using Dunnett's test. Slopes of the regression lines were compared between the two species. Results were considered significant if the alpha value was less than or equal to 0.05, although nearly significant results (p less than 0.1 but greater than 0.05) are also presented.

RESULTS

Pollination Studies

Intermorph pollinations of *A. grandiflora* in the greenhouse produced the highest floral seed cache (> 2.5 nutlets/flower) regardless of the morph of the parental plant (Figure 1a). The intramorph and active self-pollinations also produced a significant floral seed cache (> 1.5 nutlets/flower). Passive self-pollinations produced essentially no seed cache. Statistically significant block and treatment effects were detected for all pollination treatments. Significant block-treatment-morph and date-treatment-morph interactions were also detected (Table 1). The block effect was a result of all three blocks located near the eastern greenhouse windows generally producing a higher floral seed cache than the two blocks located in the interior of the greenhouse. Analyzing each floral morph data separately revealed a block by treatment interaction in the pin morph data. This was a result of the intramorph pollination treatment, in which the block effect was not observed across the three window blocks. The date by treatment interaction was detected for only maternal thrum treatments. In both the intra- and active self-pollinations, there were two pollination dates which produced a floral seed cache approaching or greater than 2 nutlets/flower. On other pollination dates, floral seed cache was approximately 1.5 nutlets/flower for both treatments.

Although no difference in floral seed cache was detected between pin and thrum treatments, there was a significant difference in nutlet weight (pin nutlet weight in mg/nutlet, Figure 1b). Nutlet weight of pins was approximately 1.2 mg/nutlet greater than thrum nutlet weight. For comparison, nutlet weight of the limited number of *A. tessellata* collected from plants growing in the greenhouse at the same time

pollination study is also presented. Nutlet weight of thrum plant significantly different from that of *A. tessellata*. The nutlet weight of *A. grandiflora* pin plants, as well as the combined average nutlet weight of pin and thrum plants was significantly different from *A. tessellata*.

Germination of the 6-month-old nutlets resulting from the pollination treatments was in general very low (Figure 2a). Germination was low for nutlets produced from the active self-pollination (10 - 18%) and low for all other treatments. The floral morph of the progeny plants was consistent with that expected for the type of cross (Figure 2b). The number of progeny plants resulting from each treatment was low, and the floral morph did not differ significantly from that expected based on phenotypic disassortative mating of the S allele system ($\chi^2 = 3.0$, $P = 0.08$). Due to the extremely low sample number, however, caution should be exercised in interpreting these results. All progeny plants resulting from active self- or intramorph pollination of maternal pin plants were like their mothers, providing evidence for the lack of cross contamination from the manual pollinations.

Germination Trials

Table 3 summarizes the results of the germination trials. Germination was highest in Trial A in which *A. grandiflora* and *A. tessellata* had been stored at 4 °C for 5.5 years and germinated at 22 °C. After 3 days, *A. grandiflora* germination averaged 75.0% (S.E. = 2.9%) and *A. tessellata* germination averaged 73.3% (S.E. = 10.1%). As part of a separate study, in 26 plates each containing 20 *A. grandiflora* nutlets from the Davis nutlet source, germination averaged 77.8% with a standard deviation of 2.7% within the first 3 days (unpublished data).

In contrast, Trial B exhibited the lowest observed germination three trials. This trial examined *A. grandiflora* and *A. tessellata* that had been collected 2 months previously from field-grown plants, air dried in warm conditions, and germination attempted under warm conditions in soil. No *A. grandiflora* or *A. tessellata* germinules were observed breaking the soil surface during the course of the trial. *A. grandiflora* nutlets subsequently excavated, seven (18%) showed evidence of germination. Six of these exhibited radicle extrusion, but none had completely germinated, although the fully-developed cotyledon was able to break the soil surface and subsequently died.

Trial C examined the germination of 9-month-old seed sources which had been stored for several months at 4 °C, and germinated under warm conditions (Figure 3). Germination for all three seed sources occurred slowly and sporadically over the first 10-day period. Nutlets collected from the *A. grandiflora* experimental population (EX94) had significantly higher germination compared with nutlets from both the natural population (CC94) and greenhouse-grown *A. tessellata* (GR94). However, after allowing the nutlets to air dry for 10 days, the nutlets from the *A. grandiflora* population experienced significantly more germination during the second germination period compared with the greenhouse-produced *A. tessellata* nutlets. The natural *A. grandiflora* population experienced a substantial amount of germination during this period, while germination during this second period occurred extremely slowly and sporadically. Although the total amount of germination for all three sources was not significantly different, *A. tessellata* nutlets had the highest average percent germination, followed by *A. grandiflora* nutlets and

natural population, with nutlets from the *A. grandiflora* experimental population having the lowest average percent germination.

Greenhouse interspecies comparisons

In both 1994 and 1995, floral output of greenhouse-grown *A. grandiflora* was twice that of *A. tessellata* (Figure 4a). Floral output in plants of both species in 1994 was twice that of plants grown in 1995, although plants of similar size with respect to above-ground (aerial) biomass (Figure 4b). Plants in 1994 were grown from nutlets obtained from the DA88 source, whereas the plants in 1995 were grown from nutlets collected from the GR94 (*A. tessellata*) or EX94 (*A. grandiflora*) source. As discussed above, germination of both the GR94 and EX94 nutlet sources was less than that observed for the DA88 nutlets. These nutlet sources may have produced seedlings which were less robust compared with those produced in 1994, and thus directed the majority of their resources into vegetative structures at the expense of reproductive structures. In addition, plants were grown in 10.15-cm pots in 1994, and in 15.25-cm pots in 1995. The larger pot in 1995 may have encouraged biomass allocation to roots at the expense of aerial organs. Unfortunately, root biomass was not determined in 1995.

When grown in a greenhouse without pollinators, *A. grandiflora* seed cache was zero (Figure 1). It is possible that *A. grandiflora* increases floral output more than *A. tessellata* because resources are allocated to floral output instead of floral seed cache. However, this is not the case. Floral output in hand-pollinated *A. grandiflora* plants was twice that of *A. tessellata* (Figure 4b). Floral output of the pollinated *A. grandiflora* was significantly greater than that of *A. tessellata*.

this trial. This was not the case for the unpollinated *A. grandiflora* due to greater variability in floral output (CV of 76% vs 37% for *A. grandiflora* and 44% for *A. tessellata*). The destructive analysis of a small number of plants in 1994 (Figure 5a) suggested that under conditions of unlimited water and soil nutrients, *A. grandiflora* apparently converted more of its resources into additional shoot and root biomass, which in turn produced more inflorescences and flowers. However, in terms of total floral output and below-ground biomass, *A. grandiflora* showed much more statistical variation compared with *A. tessellata*.

For an equivalent amount of aerial biomass, *A. grandiflora* floral output was also twice that of *A. tessellata* in the greenhouse in 1995 (Figure 6a). Using the floral seed cache observed in the field in 1994 for each species, the estimated output per unit of aerial biomass was similar for *A. grandiflora* (1.6 nutlets/flower) and *A. tessellata* (0.8 for *A. grandiflora*, Figure 6b). If inflorescence output is used instead of floral biomass (Figures 6c and 6d), similar relationships are observed, but the slopes of the resulting lines are not as steep.

Field interspecies comparisons

Mortality for both species was very high at both sites (Table 4). For *A. grandiflora* and *A. tessellata* seedlings was equal at the DT site. However, *A. tessellata* mortality (75%) was higher than that of *A. grandiflora* (50%) at the CDFG site. Most of the mortality occurred within the first month of the growing season. The cause and timing of mortality did not appear to differ between the two species.

Under field conditions, floral seed cache in both *A. grandiflora* and *A. tessellata* was less than the maximum of four nutlets per flower (Figure 7a). (Because there was no statistical difference in reproductive output between the two sites, the data were combined.) *A. tessellata* produced 1.6 nutlets/flower (S.E. 0.19), which was a little more than twice that of *A. grandiflora* (0.8 nutlets/flower, S.E. 0.12). Floral output was significantly greater in *A. grandiflora*, but this difference was not statistically significant (Figure 7b). Seed output in *A. tessellata* was greater than *A. grandiflora* (Figure 7b), but again this difference was not significant. Floral output per inflorescence was greater in *A. grandiflora* (Figure 7c), resulting in similar seed output per inflorescence between the two species (Figure 7d). This was similar to the results observed in the greenhouse. Thus, *A. grandiflora* appears to be able to compensate for the low floral output by producing more flowers per inflorescence.

Nutlet weight for field grown *A. tessellata* plants was consistently less than that of the combined nutlet weight of pin and thrum *A. grandiflora* plants by 1.1 to 1.4 mg/nutlet (Table 5). Nutlets produced by *A. grandiflora* plants weighed much more than *A. tessellata* nutlets, by as much as 1.1 to 1.4 mg/nutlet. Nutlets from thrum *A. grandiflora* plants also generally weighed more than *A. tessellata* nutlets, although by only 0.5 to 0.7 mg/nutlet. The field results were similar to those observed in the greenhouse. Nutlet weight of both species was lower in 1993 when compared to 1995. *A. tessellata* nutlet weight was reduced to a greater extent than *A. grandiflora* (a reduction of 0.83 mg/nutlet as compared to 0.50 mg/nutlet).

DISCUSSION

Our pollination study results indicate that *A. grandiflora* is not self-incompatible. Although differential pollen-tube growth is a cause of *A. grandiflora*'s cryptic self-incompatibility (Weller and Ornduff 1989), our data suggest that in the absence of pollen competition nutlet production can occur from illegitimate (intramorph) pollen. This was also observed by Weller and Ornduff (1977), whose results are in Figure 1a. In their study, the highest floral seed cache was the intramorph pollinations (2.2 nutlets/flower for pins, 1.3 nutlets for thrums), with a floral seed cache in the intermorph pollinations and 1.3 nutlets/flower for pins and thrums, respectively. In our results, self-pollinations (which we assume to be active self-pollination) produced less than 1 nutlet/flower. Deviations from disassortative mating by intramorph pollination in the field could account for the deviation from the expected morph ratio of 1:1 that has been observed, particularly if population sizes are small (Ornduff, 1976; Pavlik, 1994). Our greenhouse and intermorph hand-pollinated *A. grandiflora* plants produced a floral seed cache (>2.5 nutlets per flower) that was much higher than observed by Ornduff (1976), and was comparable to that of the field *A. tessellata* observed by Ornduff (1976) in 1967 (3.02 nutlets/flower) and 1968 (2.49 nutlets/flower). It is possible our higher intermorph pollination seed cache was a result of applying all four pollination treatments to each individual plant. Our passive self- and intramorph pollinations produced lower seed caches, which could have made additional maternal reserves available for intermorph pollination seed set. However, if flowers on an individual plant received the same pollination treatment (which was presumably done in Weller and Ornduff, 1977), there would

equal competition for maternal reserves. If this is the case, the results of Weller and Ornduff (1977) imply that intramorph pollination is more effective at obtaining maternal reserves to convert into nutrients. This possibility, and its implications, should be further explored.

Our results suggest that the floral seed cache of *A. grandiflora* approaches that of *A. tessellata* under conditions of unlimited water, nutrients, and maximum pollination. Therefore, it is important for field populations to maximize the presence of pollinators. This is important, if not more, than ensuring an equal pin:thrum ratio, even intramorph pollination can result in significant seed set. In fact that we detected a significant block effect suggests that floral development is sensitive to environmental conditions, such as light. Resource limitation is known to reduce seed number, usually through seed abortion (Harper and Westoby, 1988). Competition for light could therefore negatively affect *A. grandiflora* floral seed cache.

The results of the germination trials indicate that both *A. grandiflora* and *A. tessellata* have germination responses typical of many annual species native to the California grasslands. Although many of the introduced grasses can germinate as early as August, native species generally do not respond until October (Chiariello, 1989). Temperature and water availability can interact in complex ways to determine germination timing (Young and Evans, 1989). Our results suggest that *A. grandiflora* and *A. tessellata* may have an after-ripening requirement which includes summer dormancy and delays germination until autumn. Such an after-ripening requirement has been observed in other winter annuals (Baskin et al., 1968; Baskin and Baskin, 1976). Attempts to germinate fresh *A. grandiflora* nutlets in petri plates in July 1994 were also unsuccessful.

(unpublished data), further suggesting that the response observed due to this after-ripening requirement, and not due to a light in a result of being covered with soil.

Although our germination trials were not extensive enough to determine the type of dormancy and the exact physical requirement (light, temperature and precipitation) for germination initiation (nutlet maturation, in general we observed similar germination response in *A. grandiflora* and *A. tessellata*. This was true in spite of the fact that the average nutlet weight of *A. grandiflora* was generally greater than that of *A. tessellata*. Both species appear to have the ability to respond to precipitation throughout the cool winter months, thus increasing germination. However, there did appear to be a difference in germination response from nutlets collected from an experimental population of *A. grandiflora* (EX94) and nutlets collected from the large, native population (CC94). Germination was greater for the CC94 nutlets during the first 10 days (Trial C, Figure 3). After 10 days of air-drying, germination during the second 15 days was greatest for nutlets from the EX94 population. This resulted in roughly equal germination for the two groups by the end of the trial. We cannot say whether this is due to different environmental conditions experienced by the maternal plants between the two populations or to a genetic difference between the large, native population and the small, experimental population. Nutlets used to found the small experimental population can be traced to the Drop Tower natural population. If this germination response is genetically based, it appears that the Drop Tower population may have developed the capacity to delay germination until after several rain events.

Such diversified germination may be a risk-spreading strategy some seedling establishment occurs even if early germinating cohort lost due to a return of unfavorable conditions (Haig and Westoby, Silvertown and Doust, 1993). This was apparently observed in the Tower population in the 1990-91 growing season. At this time, Ca was under severe drought conditions. Rain in the fall of 1990 and a small amount of germination of *A. grandiflora*. However, there was subsequent rain until March of 1991. During the "March Miracle" rain fell to stimulate additional germination and establish mature reproducing plants (Pavlik, 1991).

Our interspecies comparisons indicate that *A. grandiflora* can compensate for low floral seed cache by increasing floral output compared with *A. tessellata* under greenhouse conditions. This does appear to be a function of simply larger plants, but a result of fewer per inflorescence for similar sized plants. However, this is very low floral seed cache. Field floral seed cache for *A. tessellata* is about twice that of *A. grandiflora* (1.7 nutlets per flower for *A. tessellata* vs. 0.6 for *A. grandiflora*). Pantone et al. (1995) found *A. menziesii* var. *grandiflora* floral seed cache to be 2.6 nutlets per flower vs. 0.6 for *A. grandiflora* whereas Ornduff (1976) reported a field floral seed cache for *A. grandiflora* between 2.5 and 3.0 and for *A. grandiflora* of between 0.7 and 1.5. We observed a similar floral seed cache distribution in our experiment. The floral output of *A. grandiflora* could not have equaled that of *A. tessellata* although *A. grandiflora* floral output was greater than *A. tessellata* in the field, this difference was not statistically significant. As a result, the observed greater seed output for *A. tessellata* compared with *A. grandiflora* was also not statistically significant. While these results suggest

output in *A. grandiflora* can be comparable to *A. tessellata*, it is questionable as to whether or not *A. grandiflora* can consistently compensate for low floral seed cache by increasing floral output. Year variation could result in significantly greater *A. tessellata* output compared with *A. grandiflora*.

The average weight of nutlets collected from both field- and greenhouse-grown plants of the common *A. tessellata* species was consistently less than that of the rare *A. grandiflora*. This might initially seem surprising because seed size is generally thought to be advantageous in terms of emergence and growth, at least within a species (Haig and Westoby, 1988; Fenner, 1985). In a study of rare and common prairie grasses, the rare species had lighter caryopses, whereas the common grasses had heavier caryopses (Rabinowitz, 1978). However, a study on mariposa lilies of the genus *Calochortus* showed two of the three rare species examined to have smaller seeds compared to the common species (Fiedler, 1987). In the case of graminoids, it was suggested that the rare species were colonizers and thus had light caryopses for dispersal to suitable but rare sites. In the case of *Calochortus* and *Amsinckia*, both the common and rare species have highly overlapping habitats. Also, in at least the *Amsinckia* genus, the common species is a highly aggressive colonizer. It would appear that in both *Calochortus* and *Amsinckia*, the common species has successfully made the trade-off between seed size and seed number (Haig and Westoby, 1988; Fenner, 1985). Our data show that germination was not negatively affected by *A. tessellata*'s lower nutlet weight. However, field seed mortality did appear to be greater in *A. tessellata* compared to *A. grandiflora*, which might be related to the lower seed reserves of *A. tessellata*. *A. tessellata* nutlet weight showed a greater change between 1993 and

than did *A. grandiflora*, although germination of the 1993 nutlets similar. This again suggests *A. tessellata* can "get away with" less maternal reserves into each nutlet, responding to environmental limitations through reduction in nutlet weight while still producing nutlets. *A. grandiflora* nutlet weight, on the other hand, is relatively plastic, and *A. grandiflora* appears to respond to environmental limitations through a reduction in nutlet number.

What was more surprising than the difference between *A. grandiflora* and *A. tessellata*'s nutlet weight was the greater *A. grandiflora* progeny nutlet weight when compared to thrum progeny nutlet weight. Progeny nutlets weighed from 0.76 up to 1.14 mg/nutlet more than pin progeny nutlets (Table 5, Fig. 1b). Although the number of maternal progeny nutlets germinating was somewhat less than that of maternal pin progeny nutlets (Fig. 2b), the overall number of nutlets germinating was small to reach any meaningful conclusion. The evolutionary and restoration implications of such a difference in pin and thrum progeny nutlet weight remain to be explored.

Our field results may have been confounded by the fact that the grasses were growing in a community dominated by exotic annual grasses. Although we carefully paired the two species in an attempt to minimize the confounding impact of the community, it is possible that *A. grandiflora* was affected to a greater extent by the presence of exotic annual grasses than *A. tessellata*. It has been shown that *A. grandiflora* competes poorly with exotic grasses (Pavlik et al., 1993) and performs better in a matrix of perennial bunch grasses (Carlsen et al., 1996). The greater root system of *A. grandiflora* may provide a competitive edge in the relatively undisturbed perennial bunch grass community, where substantial root

competition may occur (Wilson and Tilman, 1993). In the case of grasses, it appeared that competition for light was the primary factor which reduces the number of inflorescences, and thus number of flowers (Carlsen et al., 1996). Our data suggests that *A. grandiflora* must invest more resources into additional floral structures to produce an equal number of nutlets compared with *A. tessellata*, even under conditions of unlimited resources. That *A. grandiflora* does not simply increase floral number when presented with unlimited resources is probably a consequence of its reliance on pollinators. By increasing the number of flowers produced prior to pollination, overall pollination probability may be increased. Thus, selfing *A. tessellata* could reduce the number of flowers produced, thus relying on pollinators. Fewer number of flowers produced, along with lower nutlet weight, means maternal investment for an equal number of progeny nutlets is lower for *A. tessellata* than *A. grandiflora*. This suggests that competition for resources with exotic grasses may be more intense for *A. grandiflora* to a greater extent compared to *A. tessellata*. In a controlled environment in the absence of other competitors, it was found that *A. grandiflora* competed effectively with *A. menziesii* with respect to biomass accumulation, but was not effective in converting those resources into seeds (Pantone and Pavlik, 1996). This was likely due to the lack of natural pollinators. Extrinsic factors such as parasitic nematodes have been shown to be important in the competitive ability of *A. menziesii* and *A. intermedia* (Pantone et al., 1989). A controlled field competition experiment comparing the impact of exotic annual grasses on both *A. grandiflora* and *A. tessellata* is required.

We conclude that under conditions of unlimited resources, it is that *A. grandiflora* fitness is intrinsically limited when compared to its weedy relative, *A. tessellata*. Under field conditions, it appears that the reproductive ecology of *A. grandiflora* may result in lower seed counts than *A. tessellata*. However, this may be a result of an interaction with an extrinsic factor of competition with exotic plants.

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Table 1. Summary of analysis of variance on floral seed cache of *A. grandif* different greenhouse pollination treatments.

Dependent variable: Floral seed cache					
Source	df	SS	MS	F	p
Model	79	580.73	7.35	9.41	0.0001
Error	402	314.14	0.78		
Corrected Total	481	894.87			
Source	df	SS	MS	F	p
Block	4	11.37	2.84	3.64	0.0063
Pollination date	5	6.60	1.32	1.69	0.1361
Pollination treatment	3	356.13	118.71	151.91	0.0001
Floral morph	1	0.24	0.24	0.31	0.5804
Morph (treatment)	3	3.92	1.31	1.67	0.1724
Block x morph (treatment)	28	60.82	2.17	2.78	0.0001
Date x morph (treatment)	35	42.05	1.20	1.54	0.0288
By Floral morph					
Pins					
Source	df	SS	MS	F	p
Block	4	23.21	5.80	8.20	0.0001
Pollination date	5	6.52	1.31	1.84	0.1059
Pollination treatment	3	184.77	61.59	87.06	0.0001
Block x treatment	12	27.98	2.33	3.30	0.0002
Date x treatment	15	14.46	0.96	1.36	0.1691
Thruns					
Source	df	SS	MS	F	p
Block	4	9.77	2.44	2.87	0.0243
Pollination date	5	2.92	0.58	0.69	0.6345
Pollination treatment	3	175.90	58.64	68.83	0.0001
Block x treatment	12	13.73	1.14	1.34	0.1962
Date x treatment	15	25.54	1.70	2.00	0.0168

Table 2. Representation of floral morphs in progeny grown from nutlets obtained from artificial pollinations in the greenhouse during spring 1994.

Pollination ^a	No. pin progeny	No. thrum progeny	Expected segregation	χ^2	df
P x P as	12	0	1:0	0	1
P x P intra	4	0	1:0	0	1
P x T inter	3	0	1:1	3.00	1
T x T as	3	4	1:3	1.19	1
T x T intra	0	2	1:3	0.67	1
T x P inter	3	3	1:1	0	1

^a Seed parent x pollen parent: as = active self; intra = intramorph pollination; intermorph pollination

^b Significant at $p = 0.05$

Table 3. Summary of germination trial results.

Trial	Species	Nutlet Source ^a	Nutlet storage	Germination Conditions	#replicates/ container type	#nutlets/ replicate	Germination (% ± 1 se)
A							
(27 Nov 93 to 7 Dec 93)	A. grandiflora	DA88	4°C, 5.5 years	22°C, dark	3 / petri plates	20	75.0 ± 2.9
	A. tessellata	DA88	4°C, 5.5 years	22°C, dark	3 / petri plates	20	73.3 ± 10.1
B							
(23 Aug 93 to 22 Sept 93)	A. grandiflora	EX93	22°C, 2 months	27°C day dark	80/Steuwe tubes	1 in 4 blocks	0
	A. tessellata	EX93	22°C, 2 months	27°C day dark	40/Steuwe tubes	1 in 4 blocks	0
C-1							
1st 10 days (23 Jan 95 to 2 Feb 95)	A. grandiflora	CC94	4°C, 9 months	5°C day dark	3 / petri plates	10	43.3 ± 8.8
	A. grandiflora	EX94	4°C, 9 months	5°C day dark	6 / petri plates	10	13.3 ± 8.0
	A. tessellata	GR94	4°C, 9 months	5°C day dark	6 / petri plates	10	55.0 ± 5.6
C-2							
2nd 15 days (13 Feb 95 to 28 Feb 95)	A. grandiflora	CC94	Air dried 10d	22°C 5°C day dark	3 / petri plates	4 - 7	13.3 ± 3.3
	A. grandiflora	EX94	Air dried 10d	22°C 5°C day dark	6 / petri plates	5 - 10	32.8 ± 8.1
	A. tessellata	GR94	Air dried 10d	22°C 5°C day dark	6 / petri plates	2 - 6	6.6 ± 3.3
C-Total							
(23 Jan 95 to 28 Feb 95)	A. grandiflora	CC94	See C-1 and C-2	5°C day dark	3 / petri plates	10	56.6 ± 6.6
	A. grandiflora	EX94	See C-1 and C-2	5°C day dark	6 / petri plates	10	46.1 ± 10.4
	A. tessellata	GR94	See C-1 and C-2	5°C day dark	6 / petri plates	10	61.6 ± 4.8

a DA88 = nutlets collected from a common garden study conducted at the University of California, Davis in 1988. EX93 = from an experimental population at Site 300 in 1993. EX94 = nutlets collected from an experimental population at Site CC94 = nutlets collected from the Carnegie Canyon natural population in 1994. GR94 = nutlets collected from greenhouse in 1994.

Table 4. Survivorship of pairs of *A. grandiflora* and *A. tessellata* marked in the field during the 1994-95 growing season.

Site ^a	No. marked pairs	Mortality (%)	
		<i>A. tessellata</i>	<i>A. grandiflora</i>
CDFG	24	75	50
DT	29	55	55

^a CDFG = California Department of Fish and Game Reserve; DT = Drop Tower Site on Site 300

Table 5. Average nutlet weight (mg/nutlet) of field grown *A. grandiflora* and *A. tessellata* plants with greenhouse plant results provided for comparison.

Year ^b	<i>A. grandiflora</i> ^a			<i>A. tessellata</i> ^{d,e}
	Pins ^{c,e}	Thruns ^{c,e}	Combined ^d	
1993 (1)	4.15 ± 0.140 (21) ^{a‡}	3.39 ± 0.297 (11) ^{b‡}	4.00 ± 0.829 (48) ^a	2.58 ± 0.072 (7) ^{b‡}
1994 (2)	4.79 ± 0.345 (10) ^a	3.82 ± 0.109 (10) ^b	4.31 ± 0.209 (20) ^a	ND
1995 (3)	4.73 ± 0.217 (6) ^{a‡}	3.89 ± 0.325 (3) ^b	4.50 ± 0.203 (10) ^a	3.41 ± 0.347 (5) ^{b‡}
Δ1995-1993	0.58	0.50	0.50	0.83
1994 (4)	4.99 ± 0.076 (129) ^{a‡}	3.85 ± 0.079 (130) ^{b‡}	4.42 ± 0.065 (259) ^a	3.13 ± 0.038 (14) ^{b‡}

^a Results are presented ± one standard error. Number in parentheses indicate number of plants, except in the case of 1993 *A. tessellata* and 1994 greenhouse where it indicates number of nutlet sub-samples. Combined *A. grandiflora* p do not equal the sum of pin and thrum plants due to inclusion of plants that senesced prior to floral morph determination.

^b Nutlets collected from (1) EX93 *A. grandiflora* experimental population with naturally occurring *A. tessellata*, (2) EX94 *A. grandiflora* experimental population with naturally occurring *A. tessellata*, (3) Combined *A. grandiflora* experimental plots at DT and CDFG locations with naturally occurring *A. tessellata*, and *A. grandiflora* plants in greenhouse pollination study and *A. tessellata* greenhouse plants.

^c Different letters indicate significant difference between pins and thrums at

^d Different letters indicate significant difference between combined *A. grandiflora* and *A. tessellata* at $p = 0.05$

^e ‡ indicates significant difference between *A. tessellata* and different floral morphs of *A. grandiflora* at $p = 0.05$.

Figure 1. Average floral seed cache (1a) and average nutlet weight (1b) from artificial pollinations in the greenhouse. Average nutlet weight of *tessellata* presented for comparison. ps = passive self-pollination; as = active self-pollination; intra = intramorph pollination; inter = intermorph pollination; all trt = treatments for individual morphs; species = *A. grandiflora* = *A. tessellata*. Treatments with the same letter are not significantly different. A solid bar represents results of W and Ornduff (1977) assuming their self-pollination treatment is equivalent to our active pollination treatment. Error bars are one standard error.

Figure 2. January 1995 percent germination (a) and floral morph (b) of nutlets from spring 1994 pollination studies. as = progeny from active self-pollination; intra = from intramorph pollination; inter = progeny from intermorph pollination with T different letters are significantly different. Error bars are one standard error.

Figure 3. Trial C germination of three nutlet sources collected in the spring of 1994. A. tessellata nutlets from 1994 greenhouse plants; EX94 and CC94 nutlets from 1994 experimental population; CC94 grandiflora plants from 1994 Carnegie Canyon natural population. For each separate germination period, nutlet sources with different letters are significantly different at $p < 0.05$. Error bars are one standard error.

Figure 4. Floral output by greenhouse (*A. tessellata* and *A. grandiflora*) Results of 1994 and 1995 comparisons. b) 1995 results in which *A. grandiflora* plants were hand intermorph pollinated. * Significantly different at $p = 0.05$. * Significantly different at $p = 0.005$. Error bars are one standard error

Figure 5. Distribution of dry biomass of greenhouse-grown *A. tessellata* and *A. grandiflora* plants. a) Root and above-ground (aerial) biomass grown in 1994, R/A = root:aerial biomass ratio; b) Above-ground (aerial) biomass in 1994 and 1995. * Significantly different at $p < 0.05$. Error bars are one standard error

Figure 6. Reproductive attributes of 1995 greenhouse-grown *A. tessellata* and *A. grandiflora* plants. a) Floral output per aerial biomass. b) Estimated seed output per aerial biomass. c) Floral output per inflorescence output. d) Estimated seed output per inflorescence output. *** Slope of line significantly different from zero. Slopes with different letters are significantly different from each other at $p = 0.005$. Slopes with # are significantly different from each other at $p = 0.05$.

Figure 7. Reproductive attributes of 1995 *A. tessellata* and *A. grandiflora* plants. a) Floral seed cache. b) Floral output and seed output. c) Floral output per inflorescence output. d) Seed output per inflorescence output. Error bars are one standard error. * * Significant difference $p < 0.05$. * * * Slope of line significantly different from zero. Slopes with different letters are significantly different from each other at $p < 0.05$.

EFFECT OF LITTER AND SEED PREDATION
ON THE GERMINATION OF A
RARE ANNUAL FORB

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Abstract. The poor germination of *A. grandiflora* after a cont suggested that changes in microclimate or predation may control establishment of this endangered plant. A field experiment was t conducted over two growing seasons to investigate the role of lit and post-dispersal seed predation on *A. grandiflora*. The main t consisted of either manually clearing all litter, or leaving the Two sub-treatments were established within each main treatment pl the cleared main plot, sub-treatments consisted of either coverin with aviary netting, or leaving the plot open. Within the litter i sub-treatments consisted of clipping the standing litter and repl the subplot, or leaving the standing litter intact. A subset of i instrumented to a depth of 1 cm with thermocouples to measure soi temperatures. During the 1993-94 growing season, litter cover (c standing) increased *A. grandiflora* germination. Soil temperatur moderated by litter cover. Some predation was detected, but its l not significant. In contrast, germination success during 1994-9! greatly affected by predation, and high germination was confined netted sub-treatment. Soil temperature extremes were not as grea this year, with little influence by litter cover. Thus, both mic alteration and predation can affect *A. grandiflora* germination, k relative importance could vary depending on the timing of litter and germination, and the environmental conditions peculiar to a particular growing season.

Keywords: *A. grandiflora*, microclimate, soil temperature, germ: litter, predation.

INTRODUCTION

The use of fire to manage grasslands and rangelands is becoming increasingly common (Humphrey 1949, Howe 1994, Glitzenstein et al. 1994). In California, fire has been used to control exotic annual grasses introduced from the Mediterranean region and to re-establish native perennial bunch grasses (Menke 1992, George et al. 1992). However, the use of controlled burns in the late spring or early fall essentially removes all litter cover that may be present. This has the potential to alter the microclimate during the critical initial rains of the California rainy season which typically begins in late fall or early winter. The first significant rainfall (approx. 1.3 cm, Heady 1956) stimulates annual seeds to germinate and establish seedlings that grow vegetatively throughout the winter months (Heady 1990). The lack of an insulating layer of litter usually results in extremes in soil temperatures and may result in reduced seed germination or post-germination seedling establishment (Heady 1956, Evans and Young 1970, Evans and Young 1989).

If fire was a natural component of the original California perennial grasslands (Heady 1990), then native annual species probably evolved mechanisms to tolerate such microclimate variation. One such mechanism may be ample seed production or prolonged seed longevity which ensures a sufficient number of seeds are present in the seed bank in the event of a year of poor germination. There is evidence that germination and post-germination seedling establishment of exotic grass species in the California grasslands is reduced as a result of unfavorable microclimate due to lack of litter cover, but sufficient seeds remain in the seed bank to maintain the population (Evans et al. 1975). Alternatively, the lack of a litter layer may not result in lower seed germination. Many alien

unaffected by the lack of a litter layer (Evans and Young 1970). annual species actually have higher seed germination without litter. We use the greater temperature extremes as an indication of the lack of competitors in order to germinate (Evans et al. 1974, Rice 1985).

Invasive exotic species, agricultural conversion and suburban development have placed many native annual species in peril. Many such taxa are of conservation concern, and some have been formally listed as threatened and endangered. In their most recent inventory of rare and endangered vascular plants of California (Skinner and Pavlik 1994), the California Native Plant Society (CNPS) listed 216 annual plant taxa found in valley grassland, foothill woodland, and cismontane woodland communities in California. Of these, 121 are on the CNPS list of most threatened taxa, with 9 taxa federally listed as threatened or endangered. Ninety-one highly threatened annual taxa occur in valley grassland or foothill woodland communities. Consequently, restoration efforts are currently underway for several of these rare annual species (Skinner and Pavlik 1994).

There is evidence that competition by invasive exotic annual grasses reduces some measure of fecundity in both annual and perennial native species (Gordon and Rice 1993, Guerrant 1992, Pavlik et al. 1993). Therefore, the use of fire to control exotic annual grasses would be appropriate. But it is during early restoration efforts, when populations of rare annual plants may be limited, that poor germination as a result of an unfavorable microclimate may be particularly important. During this period, there would likely be a lack of a significant seed bank to allow species to span a year of low germination. Failure of the seeds to germinate could result in the loss of a significant percentage of the extant

A loss of propagules occurred during restoration efforts on *A. grandiflora* (Gray) Kleeb. ex Greene (Boraginaceae), a rare forb in the California grasslands. As part of a study on the competitive native perennial and exotic annual grasses on *A. grandiflora*, in 1992, a site selected for reintroduction of *A. grandiflora* underwent a controlled burn of 625 Btu/ft² (heat per unit area), with a fire intensity of 48 Btu/ft/s (Carlsen et al. 1996). After the burn, 3800 *A. grandiflora* (seeds) were sown into the site. Significant rainfall did not occur in December, at which point daytime temperatures were at or below freezing. Germination at both sites was below 5%. This was very unexpected because nutlets from the same source had laboratory germination of 80%, and reintroduction efforts using this nutlet source had field germination exceeding 60% (Pavlik et al. 1993). The reintroduction site was selected based on similar criteria, and was near the site where *A. grandiflora* occurs naturally (Carlsen et al. 1996).

The low germination of *A. grandiflora* nutlets observed in the field suggested that the fire created an unfavorable microclimate as a result of the total removal of the overlying litter. However, it could also be the result of predation, some unknown effect of the burn, or the site may have been unfavorable for *A. grandiflora* germination for other unknown reasons. Therefore, a set of field experiments were conducted to determine the role of litter cover in the germination of *A. grandiflora*.

METHODS

Plot selection and design

In the fall of 1993, a 64-m² site was selected just outside of the burned area. This site (elevation ≈320 m) is in the same area as the site where *A. grandiflora* occurs naturally (Carlsen et al. 1996).

canyon where the originally described natural population of *A. g.* exists, located in the Altamont Hills of the inner Coastal Range, miles southeast of San Francisco, California. The property is part of Lawrence Livermore National Laboratory's Site 300, a high-explosive testing facility operated by the University of California for the Department of Energy. The site was selected to mimic the location where *A. grandiflora* occurs naturally, in annual grassland bordered by woodland and coastal sage scrub. The aspect is north-west facing slope ranging from 25% to 72% (average 56%). The soil consists of well-drained, deep (about 1 m) sandy clay loam. Herbaceous cover consists primarily of the annual exotic grasses *Bromus hordeaceus* L. and *Myuros* (L.) C. Gmelin, with lesser amounts of *B. diandrus* Roth, *B. madritensis* ssp. *rubens* (L.) Husnot, *Avena barbata* Link, and the forb *Erodium cicutarium* (L.) L'Hér. Native species include the bunch grass *Poa secunda* J. S. Presl, and several forbs and legumes including *Clarkia* spp., miniature lupine (*Lupinus bicolor* Lindl.) and purple owl's clover (*Castilleja exserta* (A. A. Heller) Chuang &

On 9 November 1993 the site was split into two 4 x 8 m main plots. The main plot cover treatments consisted of clearing the litter (designated "cleared" main plot treatment), or retaining the litter (designated "litter" main plot treatment). Litter is defined as the previous year's leaves, and inflorescences of plants (primarily herbaceous) sensu Young (1970). The treatments were assigned to the main plots randomly. The "cleared" treatment was applied by manually removing the litter down to the bare mineral soil. Six 0.25-m² sub-plots were established in each main plot. Within each main plot, two sub-treatments were assigned to the six sub-plots, replicated three times. The

treatments within the litter main plot consisted of 1) the litter standing litter (the "standing" sub-treatment), and 2) the litter then placed back onto the plot (the "clipped" sub-treatment). Within the cleared main plot treatment, the two sub-plot treatments consisted of plots covered with aviary netting (the "netted" sub-plot treatment) and plots left open (the "open" sub-plot treatment). Because of a limited *A. grandiflora* nutlet supply, the main plot treatments were not replicated.

The experiment was repeated in the fall of 1994. This time, two sites were selected. The first site was located at Site 300 within the cleared main plot treatment, which was burned in the fall of 1992 (the "S300" site). The second site was located on an ecological reserve adjacent to Site 300 belonging to the California Department of Fish and Game (the "CDFG" site). Efforts to establish *A. grandiflora* at this site had been underway for several years (since 1994). The experimental design for each site was identical to that used for the previous year's experiment except that each main treatment was replicated three times in a randomized complete block design. A rake was used to remove litter from the cleared main plot treatments.

Nutlet source and sowing

The nutlet source used in 1993-94 was the same as used in the fall of 1992. These nutlets were originally collected from the nearby native *A. grandiflora* population, and further propagated at the University of California, Davis in 1988 (known as the DA88 source). Each plot was sown with nine *A. grandiflora* nutlets at a depth of 1 cm equidistantly spaced at 10 cm intervals in 3 rows. There had been no fall rain up to the time of sowing and establishment. The following day (10 November 1993), there was 1.5 cm of rainfall.

The *A. grandiflora* nutlets used in 1994-95 were obtained in the fall of 1994 from plants established as part of a competition experiment at the site that had been burned in the fall of 1992 (the "EX94" nutlet site). Nutlets were air dried after collection and maintained at room temperature throughout the summer. Nutlets were sown on 5 October 1994 at S300 and on 6 October 1994 at CDFG. The first significant rain (1.52 cm) occurred on 7 November 1994.

Microclimate instrumentation and data collection

In 1993, all plots, with the exception of the netted plots, were instrumented to a depth of 1 cm with thermocouples on 15 November 1993. A Campbell Scientific CR-7 data logger was used to obtain soil temperature data. Temperature data were collected once per hour over a 24-hour period for one set of standing, clipped and open sub-plots on 29 November 1993 and again on 29 December 1993. *A. grandiflora* germination data were collected from 7 November 1993 through 30 November 1993. At this time the aviary netting was removed. The number of established reproductive plants was recorded on 15 November 1994. On 5 May 1994, neighbor biomass was collected from 0.1 m² in the center of each plot, dried at 65 °C and weighed.

In 1994, all subplots at the S300 site were instrumented with thermocouples at a depth of 1 cm. Germination was carefully recorded from 7 November 1994 through 13 December 1994. A Campbell Scientific CR-7 data logger was used to continuously read the soil temperature at a rate of once per hour. The aviary netting was removed at the end of the period. The number of established reproductive plants was recorded on 13 March 1995. Neighbor biomass was collected from the 0.1 m² in the

of each plot on 30 May 1995 at the CDFG site, and on 1 June 1995 at site. All biomass was dried at 65 °C and weighed.

Daily minimum and maximum temperatures were also obtained for both 1993 and 1994 from the nearby Castle Rock Radiation Lab meteorological station (Station number B80 1583-00). This station maintained at Site 300 for the State of California Department of W Resources. Although the station is not collocated at the experim temperature data from this station were used to evaluate relative differences in air temperature between the two years.

Data analysis

Because each main treatment did not contain identical sub-trea the experiment could not be analyzed as a traditional split-plot. Therefore, the main treatments (litter and cleared) were first as two separate experiments, each containing two treatments (that is and standing treatments within the litter experiment, and open an treatments within the cleared experiment). While this makes com across "experiments" difficult (such as comparing the open treatr clipped treatment), some general observations are possible. Next investigate the effect of cover (the main treatment, that of litter if no significant differences were observed between sub-treatment each main treatment "experiment", then all sub-plots within a mai were pooled and considered sub-samples of the main plot. The exp was then analyzed as a two treatment (litter vs. cleared) randomi complete block with each block containing 6 sub-samples per treat However, if significant differences were observed between the sub treatments within the main treatment, then only the open sub-plot

the cleared main plot, and the standing sub-plots within the main main plot were used in the analysis. This resulted in a two treatment randomized complete block experiment, with each block containing three samples per treatment. Because the main plots were not replicated in the 1993-94 season, treatment in this case is confounded with location. It is possible that any observed treatment effect is actually a location effect. The small study site in 1993-94 was uniform with respect to aspect, slope, vegetation cover and soil type. Therefore, we believe any "treatment" effect to be meaningful. Statistical analyses were conducted using the General Linear Model procedure of SAS (SAS 1990). All percentages were arcsine square-root transformed prior to statistical analysis (Sokal and Rohlf 1989). Results were considered significant if the alpha value was equal to or less than 0.05.

RESULTS

On 30 Nov 1993 the germination of *A. grandiflora* nutlets was not significantly different between sub-treatments within each main treatment (Fig 1a). The open sub-treatment resulted in the lowest amount of germination (4%), whereas the clipped sub-treatment resulted in the highest amount of germination (52%). The litter main treatment had significantly greater *A. grandiflora* germination (48%) compared to the cleared main treatment (9%, Fig. 1b) using the pooled sub-treatments as sub-samples of each main treatment (Table 1).

There was no significant difference in *A. grandiflora* nutlet germination between the two sites in 1994, and therefore the data were pooled to increase the power of the analysis. This resulted in three samples per treatment. At the end of the germination monitoring period

A. grandiflora nutlet germination in the netted sub-treatment with cleared main treatment was significantly greater than that observed in the open sub-treatment (33% vs. 24%, Fig. 1c). Sub-treatments within the main treatment were not significantly different at the end of the germination monitoring period (Table 1), although they were significantly different earlier in the germination period (Fig. 1c). The netted treatment resulted in the greatest amount of *A. grandiflora* nutlet germination (33%), whereas the standing sub-treatment resulted in the lowest amount of germination (16%). Because significant differences were detected between sub-treatments, only the open sub-plots within the main plots and the standing sub-plots within the litter main plot were used in the statistical analysis on the effect of cover on *A. grandiflora* nutlet germination. Although the cleared main treatment had greater *A. grandiflora* nutlet germination compared to the litter main treatment (24% vs. 16%, Fig. 1d), this difference was not significant at the end of the germination monitoring period (Table 1). However, significant differences between these two treatments were detected earlier in the germination period (Fig. 1d). The difference in nutlet germination between the two treatments observed in 1994 was not as great as that observed in 1993. The maximum amount of *A. grandiflora* nutlet germination observed in 1994 was lower than that observed in 1993 (33% vs. 52%), whereas the minimum amount of germination was greater (16% vs. 4%). A significant block by sub-treatment interaction was observed in 1994, which was the result of the netted sub-plots in one block at each site exhibiting lower *A. grandiflora* nutlet germination than observed in the open sub-treatments in the other block.

Differences in soil temperature between the sub-treatments were detected (Fig. 2). In 1993, 19 days after initial rainfall, the clipped sub-treatments resulted in cooler temperatures during the warm part of the day (by approx. -1.5°C), and warmer temperatures during the coldest part of the day (by approx. $+1^{\circ}\text{C}$) as compared to the open treatment (Fig. 2a). This difference could still be detected 49 days after initiating rainfall, although the temperature differential during the warm part of the day had reduced to 0.5°C (Fig. 2b). In general, this was observed in 1994 although the temperature differential was not as large. One day after the germination initiating rainfall, the clipped sub-treatment was approximately 1°C cooler than the netted sub-treatment during the warmest part of the day, with the open and standing sub-treatment intermediate between (Fig. 2c). This differential was maintained 9 days later. In both years, the clipped sub-treatment produced more uniform soil temperatures than the standing sub-treatment, as indicated by the smaller temperature variation. In general, minimum air temperatures in 1994 were not as low as in 1993 during the first 25 days after the germination initiating rainfall (Fig. 3). During this period, the average minimum air temperature in 1993 was 1.3°C (SE 3.9°C), while in 1994 it was 2.8°C (3.8°C).

The percentage of reproductive *A. grandiflora* plants established the following spring was not significantly affected by the main treatment in either year (Fig. 4, Table 2). The percentage of established plants was generally lower in the spring of 1995 as compared to the spring of 1994. Again, there were no site differences in 1995. In 1994, the clipped sub-treatment resulted in the greatest establishment (63%), with the open treatment exhibiting the lowest (22%), although these differences were not statistically significant (Fig. 4a). However, in 1995, there were

differences in adult establishment between sub-treatments within treatments (Fig. 4b). The netted sub-treatment resulted in the greatest amount of adult establishment (25%), with the standing sub-treatment exhibiting the lowest (12%). The significant block X sub-treatment interaction was again the result of the same two blocks within the sub-treatments which exhibited low germination, this time exhibiting establishment compared to the open sub-treatments within the same blocks.

The main cover treatments did not significantly affect the amount of final dry neighbor biomass collected from the plots in 1994 (Fig. 3). However, in 1995, the litter main treatment had significantly greater biomass than the cleared main treatment (33 vs. 20 g/0.1 m², Fig. 3). Sub-treatments within the main treatments were not significantly different. In general, a greater amount of final dry biomass was collected in 1995 as compared to 1994 (an average maximum of 37 vs. 10 g/0.1 m²).

DISCUSSION

Litter cover has been shown to be an important factor in the germination and establishment of annual plants in grassland communities. It reduces high temperatures during the day which could result in the loss of critical soil moisture. During the night, litter helps prevent temperatures from dropping below levels which could inhibit cellular metabolism and promote dormancy. Evans and Young (1970) found litter cover to reduce surface soil temperatures 9 °C lower during the warmest part of the day and 5 °C higher at the coldest part of the day. At a depth of 1.5 cm, temperature differentials were 2 °C and 1 °C, respectively. They found that the germination of alien grasses to be generally enhanced by litter cover.

the germination of alien forbs was unaffected. In contrast, litter apparently can increase the germination of the native forb, *A. gr* as was observed in the fall of 1993. Litter cover, either clipped or increased germination during the first few weeks after the initiation. Soil temperatures at 1 cm was in the range of that observed by Evans and Young (1970) and is consistent with the hypothesis that litter cover moderates extremes in soil temperature. Clipped litter apparently moderates soil temperatures more effectively than standing litter due to more even coverage of the soil surface. In 1993, the soil temperature data were not collected until 19 days after the initial rainfall, and minimum air temperatures were fairly high (Fig. 3). Had soil temperature data been collected earlier (when air temperatures were colder), it is possible the soil temperature differential between the sub-treatment plots during the coldest part of the day would have been even greater. Soil temperatures are a major limiting factor in annual grass germination (Evans and Young 1970, Evans and Young 1989). Although many annual species (particularly exotics) will germinate over a wide range of temperatures, temperatures below 2 °C are generally inhibitory (Evans and Young 1989, Young et al. 1973).

The apparent reversal of the litter cover effect on germination in the fall of 1994 (in which greater germination was observed in the main plots compared to the litter plots) could have been the result of several factors. First, temperatures were slightly warmer during the 1994 germination period as compared to 1993 (Fig. 3). Second, the small amount of litter left after using a hoe to clear the main plots may have been sufficient to prevent temperature-induced reductions in germination. Third, if any of these factors could have contributed to the smaller temperature effect

observed between the sub-treatments during 1994 as compared to 1993. Finally, the initial rainfall did not occur for a full month after the seeds were sown, whereas in 1993 it occurred the following day. This provided a longer opportunity for predation of *A. grandiflora* nutlets by ground birds and small rodents.

Nutlet predation was more important than soil temperature during 1994-95 as compared to 1993-94. Herbivory on seedlings was also evident in the non-netted sub-plots in 1994-95. Although germination was lower in the netted sub-plots during the initial germination period, the lower apparent germination observed in the open sub-plots as compared to the netted sub-plots may have been due to a combination of nutlet predation and herbivory. Predation of seeds and grazing on shoots and roots by rodents is common in western grasslands (Borchert and Jain 1978, Detling and Jain 1983, Hoffmann et al. 1995). There was also a suggestion of lower germination in 1993-94, as the netted sub-plots resulted in a larger amount of germination compared to the open sub-plots, however, this difference was not statistically significant. In 1994-95, the standing sub-plots exhibited both the lowest amount of *A. grandiflora* germination and the lowest number of reproductive plants. Seed-eating birds such as sparrows (*Passerculus sandwichensis*) and towhees (*Pipilo* spp.) were observed foraging in the study area. Granivorous mammals such as mice (*Peromyscus maniculatus*), Heermann's kangaroo rats (*Dipodomys heermanni*) and valley pocket-gophers (*Thomomys bottae*) have been trapped out of this and other *A. grandiflora* reintroduction sites. Rodents often preferentially forage in areas that provide some protection from predators (Bartholomew 1970), and may have preferred the standing cover sub-treatment plots over the other sub-treatment plots. In

the relatively large *A. grandiflora* nutlets (approx. 4 mg), would be attractive to granivores (Hoffmann et al. 1995).

During the 1993-94 growing season, germination of *A. grandiflora* nutlets continued after the initial germination period, as evidenced by an increase in established adult reproductive plants relative to the seedling cohort. In contrast, there were fewer established adult plants during 1994-95 compared with the initial seedling cohort. The decline in the adult population was due in part to ongoing seedling herbivory and also due to greater mortality because of the more intense neighbor competition (there was significantly greater neighbor biomass in 1994-95 than in 1993-94, composed mostly of exotic annual grasses). *A. grandiflora* has been shown to compete poorly with exotic annual grasses (Pavlik et al. 1996; Carlsen et al. 1996). Although in both years there was a lack of statistically significant main treatment effect on adult plant establishment, there were large differences in the mean values. Removal of the enclosures after the initial germination period subjected all *A. grandiflora* plants to potential herbivory. This may have increased the variability observed in the final plant establishment.

The generally lower germination observed in 1994-95 as compared to 1993-94 may have also been an effect of nutlet source. The nutlets from the 1993-94 source had been stored for several years, and had laboratory germination exceeding 70% (Carlsen and Pavlik 1996). This germination was highly asynchronous, with most of the germination occurring during the first 30 days. The 1994-95 nutlet source had been collected in the spring of 1994. These nutlets were kept at room temperature, and did not experience the extremely warm summer temperatures typical of the in situ seed bank. Such warm temperatures are often necessary to complete afterripening.

winter annuals (Baskin and Baskin 1976). Laboratory germination nutlet source, conducted in January 1995, was approximately 45% and occurred over a period of 28 days (Carlsen and Pavlik 1996). Because of a large amount of replication conducted in the 1994-95 experiment, it is unlikely the nutlet source impacted the cover effects, although it undoubtedly added to the variation observed in the resulting data.

These data suggest that both microclimate alteration, nutlet predation, and seedling herbivory can affect germination and establishment of reproductive plants after litter cover removal. The existing climatic conditions at the time of litter removal, the timing of germination, and condition of the predator population can determine the levels of mortality that affects *A. grandiflora* nutlets and seedlings. It is likely that both factors operated during the fall and winter of 1992-93 when *A. grandiflora* germination and plant establishment was less than in previous years. Nutlets were exposed to predation for well over a month, and when germination finally occurred, air temperatures were extremely low. Thus, caution should be exercised when using fire as a tool for the restoration of rare forbs. In the context of California annual grasslands, fire is commonly used to control exotic annual grasses. Consideration should be given to the use of a surrogate for the litter cover after a controlled burn, particularly in the case of a rare plant whose seeds occur subsequent to the burn. The use of other comparable mulching material could both moderate temperatures and possibly reduce the risk of predation, particularly from birds. Unfortunately, straw may provide cover for rodent populations, thus potentially increasing the risk of rodent predation. However, fresh litter lying on the ground surface appears to provide less protection to rodents than standing litter. In very cold years, the risk from

microclimate conditions may outweigh the risk from rodent predation, a meaningful way of predicting such a climate does not readily come to mind.

If the restoration goal is focused solely on the establishment of native forbs, an alternative to the use of fire, at least on a fairly small scale, is the application of a dilute solution of grass-selective herbicide. Work done in mid-winter, this has been shown to reduce annual grass competition without completely removing the protective litter layer (Pavlik et al. 1998). However, unless applied manually in a very careful, precise manner, the use of grass-selective herbicides can reduce native perennial grass populations. This limits the utility of such herbicides on a large scale, and their use is clearly inappropriate if the restoration goal is to maintain native grasses along with native forbs. However, the combined use of grass-selective herbicides, fire and appropriate mulch may prove a powerful restoration tool. Herbicides could be used initially on a small-scale to establish native forb populations. Once the rare forb population has developed a large seed bank to buffer against the occasional poor germination year, herbicides could be periodically used on a larger scale to maintain native grass populations.

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TABLE 1. ANOVA on *A. grandiflora* percent germination at end of germination monitoring period. All data were arcsine transformed prior to analysis.

Source	DF	SS	MS	F	p
Germination on 30 November 1993 ^a					
Cover	1	2923.44	2923.44	14.34	0.0036
Error	10	2037.75	203.78		
Germination on 13 December 1994 ^b					
Cover	1	243.64	243.64	1.84	0.1880
Block	5	848.04	169.61	1.28	0.3056
Block X Cover	5	596.83	119.37	0.90	0.4965
Error	23	3043.31			
Cleared Experiment					
Subtrt	1	572.17	572.17	7.12	0.0135
Block	5	831.38	166.28	2.07	0.1049
Block X subtrt	5	1663.40	332.68	4.14	0.0075
Error	24	1929.87	80.41		
Litter Experiment					
Subtrt	1	353.54	353.54	3.25	0.0866
Block	5	330.93	66.19	0.61	0.6948
Block X subtrt	5	481.05	96.21	0.88	0.5101
Error	20	2177.54	108.88		

^a All sub-plots within each main plot were combined.

^b Only open and clipped sub-plots were considered.

TABLE 2. ANOVA on *A. grandiflora* percent establishment of reproductive plants
All data were arcsine transformed prior to analysis.

Source	DF	SS	MS	F	p
Establishment on 15 March 1994 ^a					
Cover	1	461.28	461.28	1.87	0.2016
Error	10	2469.26	246.93		
Establishment on 25 March 1995 ^b					
Cover	1	388.15	388.15	2.07	0.1645
Block	5	429.65	85.93	0.46	0.8032
Block X Cover	5	1023.62	204.72	1.09	0.3933
Error	22	4130.06	187.73		
By Cover: Cleared					
Subtrt	1	403.01	403.01	3.78	0.0637
Block	5	339.49	67.90	0.64	0.6738
Block X subtrt	5	2244.82	448.96	4.21	0.0069
Error	24	2559.32	106.64		
By Cover: Litter					
Subtrt	1	1000.20	1000.20	6.47	0.0198
Block	5	601.74	120.35	0.78	0.5772
Block X subtrt	5	445.48	89.10	0.58	0.7174
Error	19	2936.19	154.54		

^a All sub-plots within each main plot were combined.

^b Only open and clipped sub-plots were considered.

TABLE 3. ANOVA on final dry neighbor biomass.

Source	DF	SS	MS	F	p
Final dry neighbor biomass on 5 May 1994 ^a					
Cover	1	4.54	4.54	0.32	0.5844
Error	10	142.10	14.21		
Final dry neighbor biomass on 30 May 1995/1 June 1995 ^a					
Cover	1	2461.21	2461.21	23.03	<0.0001
Block	5	1830.58	366.12	3.43	0.0097
Block X Cover	5	880.85	176.17	1.65	0.1645
Error	50	5343.05	106.86		

^a All sub-plots within each main plot were combined.

FIG. 1A. *grandiflora* germination in (a) the fall of 1993 by sub-treatments (n=3), (b) the fall of 1993 by main treatments (n=2), (c) the fall of 1994 by sub-treatments (n=18), and (d) the fall of 1994 by main treatment using only the open and standing sub-treatments (n=2). Error bars are one standard error. Sub-treatments within main treatments with different lower case letters are significantly different at $p=0.05$. Main treatments with different capital letters are significantly different at $p=0.05$.

FIG. 2. Effect of sub-treatments on soil temperature at 1.5 cm. (a) 19 days after initial rainfall (29 November 1993), rainfall (29 December 1993), (c) 1 day after initial rainfall (8 November 1994) (n=9) and (d) 9 days after initial rainfall (n=9). Error bars are one standard error

FIG. 3. Minimum daily temperature during *grandiflora* germination period in the fall of 1993 and 1994.

FIG. 4. Effect of main treatments and sub-treatments on establishment of reproductive plants of *grandiflora* (a) the spring of 1994 (n=3) and (b) the spring of 1995 (n=18). Error bars are one standard error. Main treatments with different capital letters are significantly different at $p=0.05$. Sub-treatments within main treatments with different lower case letters are significantly different at $p=0.05$.

FIG. 5. Effect of main treatments and sub-treatments on final dry neighbor biomass (a) the spring of 1994 (n=3) and (b) the spring of 1995 (n=18). Error bars are one standard error. Main treatments with different capital letters are significantly different at $p < 0.05$. Sub-treatments within main treatments with different lower case letters are significantly different at $p < 0.05$.