2008

Competition effects of mycorrhizae on two California grasses and B. hordeaceus

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COMPETITION EFFECTS OF MYCORRHIZAE ON TWO CALIFORNIA GRASSES AND *B. HORDEACEUS*

A Thesis

Presented to

The Faculty of the Department of Biological Sciences

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

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December 2008

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ABSTRACT

COMPETITION EFFECTS OF MYCORRHIZAE ON TWO CALIFORNIA GRASSES AND *B. HORDEACEUS*

By Noëlle Marie Antolin

Restoring native grasses is key to reestablishing healthy ecosystems, and land managers need species-specific information to determine whether or not to incorporate mycorrhizae into restoration plans. This thesis provides specific information regarding the effects of mycorrhizae collected from a California coastal prairie on two native perennial grasses, California brome (*Bromus carinatus)* and purple needle grass (*Nassella pulchra)*, and one non-native annual grass, soft chess (*Bromus hordeaceus*).

Competition experiments were set up between seedlings growing in the presence or absence of mycorrhizal inoculum in native soils under relatively controlled conditions within a greenhouse. Mycorrhizal inoculation caused greater and faster seedling emergence in all three grasses. Only *Nassella pulchra* demonstrated a significant positive growth response to inoculation, which persisted when in competition with and at the expense of *Bromus hordeaceus.* Inoculated *Bromus hordeaceus* plants, however, produced significantly more seed and more viable seed when grown alone and in competition.

ACKNOWLEDGEMENTS

My utmost gratitude goes to my major adviser, Dr. David K. Bruck, for inviting me to join his lab, his expertise, patience, and enthusiasm even when mine dwindled. Many thanks to my committee members, Dr. Rachel O'Malley, who spent hours helping me with my experimental design, and Dr. Rod Myatt, who, through his passion for botany, reminded me why this research is important. I thank Andrea Woolfolk for her time and for granting me access to the Elkhorn Slough National Estuarine Research Reserve. Thanks also to Jennifer Cross for her generosity, as well as her greenhouse assistants who kept my grasses alive. I would have been lost without the help of the Michael Allen Lab at the University of California, Riverside, who helped me to key out my mycorrhizal species, and Dr. Joe Morton, who provided me with priceless phone counseling, not to mention inoculum. Thanks also to Andrea Woolfolk for her time, Greg Schlick and Jason Hoeksema for their tricks of the trade, Troy Allman and Steve Alvarez for making field soil collection seem easy, and other friends who supported me. Above all, I thank my husband, Stuyvie, who stood beside me and believed in me all the way to the end and my parents and sister for their encouragement and love.

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INTRODUCTION

Mycorrhizal – Plant Interactions

Restoration ecologists often face the challenge of speeding up succession and reestablishing populations of native plants on soils that are highly disturbed and dominated by non-native plant species. These disturbed soils often lack mycorrhizal fungi, which develop positive symbiotic relationships with plant roots and are essential to the establishment of many plant species (Boerner et al. 1996).

Mycorrhizal fungi are widespread (Fitter et al. 2004), and approximately 70% of plant species examined have exhibited associations with them (St. John 1997). They form a network of hyphae in and/or around plant roots that extends far into the surrounding substrate. They play a role in shaping plant community structure by increasing the mineral supply to plants, reducing the uptake of heavy metals, and improving water uptake and retention and thus drought tolerance (Lapointe and Molard 1997). Mycorrhizal fungi can produce phytohormones and increase plant tolerance to pathogens through the production of secondary metabolites, such as antibiotics (Allen 2003), some of which protect plants from herbivory (Wilson and Hartnett 1998). Mycorrhizae can influence shoot and root architecture, leading to heightened vegetative reproduction (Jones and Smith 2004), and have also been found to boost stolon branching and length (Wilson and Hartnett 1998).

There are two general types of mycorrhizae: vesicular arbuscular mycorrhizae (AM), which penetrate internal root cells to develop an elaborate, extensive, and highly ramified network, and ectomycorrhizae (EcM), which extend only between root cells and to a limited degree. Usually the ectomycorrhizal hyphae form a sheath over the surface of the root.

Mutualism and Symbiosis

Scientists have debated the existence of mutualisms and their function in nature for over a century. More recently, the topic has surfaced in the literature of restoration ecology as scientists face the challenge of restoring disturbed habitats. Although the relationship between mycorrhizal fungi and plants is generally understood, the role of mycorrhizae in the management and restoration of damaged ecosystems is unclear.

In the late 1800s, de Bary (1879, cited in Sapp 2004) introduced the concept of symbiosis (Greek for "living together") after discovering that lichens are double organisms consisting of both algae and fungi (Sapp 2004). Around the same time, Frank (1877, cited in Sapp 2004) encountered a symbiosis between the roots of forest trees and fungi, which he called *mykorrhizen*, meaning fungus root. His work with mycorrhizae is recognized by many as the most significant in mycorrhizal science. He was the first to differentiate between ectotrophic mycorrhizae, which form a mantle around the root, and endotrophic mycorrhizae, which penetrate the root tissue (Jones and Smith 2004).

Many have erroneously used the terms symbiosis and mutualism interchangeably. Whereas a mutualism refers to a positive association between two organisms where both individuals are benefiting, a symbiotic association does not necessarily benefit both organisms. Some have questioned the existence of mutualisms altogether. Pound (1893), for example, believed that mycorrhizal associations only appear to be mutualistic, but, in reality, one organism always dominates over the other and in some way harms the other. While showing that fungi improved seed germination in orchids, Bernard (1902, cited in Sapp 2004) suggested that the two organisms were not experiencing a mutualism, but rather were in an ongoing conflict or competition. He believed that a mutualism could rarely exist and that these relationships were various stages of infection. Johnson et al. (1997) argued that this notion was too simplistic and that a generalization concerning all mycorrhizae and plant species could not be made because the associations are species specific. A fungus beneficial to a woody plant, for example, may be harmful to an orchid.

By the end of the 19th century, it became widely accepted that microbial symbioses were basic components of life. Supporting evidence came in the form of the dual nature of lichens, of nitrogen-fixing bacteria in the roots of legumes, and of the association between mycorrhizal fungi and forest tree roots. Mycorrhizae were also presumed to play an essential role in the colonization of land by prehistoric plants over 450 million years ago (Sapp 2004, Gifford and

Foster, 1989). Scientists of the early 1930s who studied mycorrhizae concurred in that in nutrient-limited soils seedlings grew faster in the company of mycorrhizae (Jones and Smith 2004). Because mycorrhizae are species specific, their interactions vary greatly with environmental conditions. Mycorrhizal associations are now recognized to range from parasitic to mutualistic and vary depending on the environmental setting (Sanders 2002). Mutualism is considered a key characteristic of mycorrhizae (Allen 1991).

Functions of Mycorrhizae

Mycorrhizae may perform beneficial functions that their host would be unable to complete alone (Sapp 2004). Relationships between mycorrhizae and plants occur when there is a deficiency in soil minerals, especially phosphorus and nitrogen. When an association occurs, plants may allocate more carbon to their roots (sometimes up to 20% additional carbon), making it available to the mycorrhizae (Sapp 2004). Sophisticated fungal networks then develop and acquire phosphorus and nitrogen a great distance from the roots, transport it to the plant, and absorb the plant's excess carbon (Allen et al. 2003). It is not uncommon for mycorrhizae to obtain other minerals (*e.g.*, magnesium, zinc, copper, and iron) for plants as well (Jones and Smith 2004).

Conflicting findings have characterized mycorrhizal research with respect to the effect on plant growth, due at least in part to inconsistent or inappropriate experimental factors, such as the use of inappropriate fungi, varying nutrient

content of the soil, and unsuitable inocula (Pattinson et al. 2004). However, mycorrhizal fungi have been found by many to improve plant growth in varied substrates and growing conditions but especially in soils low in phosphorus and nitrogen. As long as there is a deficiency in phosphorus or nitrogen in the soil, the symbiotic exchange will most likely continue (Allen et al. 2003). Under high phosphorus conditions, mycorrhizae often depress plant growth (Koide 1985). In these cases, phosphorus obtained by the mycorrhizae augments that by the plant, resulting in phosphorus toxicity (Koide 1985).

Plant growth depressions can also be caused by competition for carbon between the plant and fungus (Koide 1985). The effect is most commonly observed in seedlings where the young plant allocates more of its limited supply of stored photosynthate to the fungus than it can spare while not yet fully benefiting from the mycorrhizal mineral uptake (Richter and Stutz 2002). Phosphorus is not limiting in these circumstances; rather, light is deficient, impeding photosynthesis. Should the seedling survive the carbon competition, the subsequent effect is minimal or advantageous to the plant (Koide 1985).

Early successional plants are generally assumed to be non-mycorrhizal; however, a unique study by Gange et al. (1990) showed the effects of mycorrhizal fungi on the early succession of plants when he applied the fungicide, iprodione, to early seral plant communities in degraded soil. As a result, there was a noticeable reduction in mycorrhizal infection in annual forbs

and one perennial grass, and there was a significant depression in plant growth. These results suggest that mycorrhizal fungi play a role in post-seedling plant development during habitat establishment through increased nutrient acquisition.

Mycorrhizae and Habitat Restoration

Although harsh growing conditions are typical of a disturbed site, the subterranean component of restoration sites is often overlooked (Salyards et al. 2003). Soil is usually degraded in disturbed habitats, and mycorrhizae are lacking (Salyards et al. 2003). Mycorrhizae play a crucial role in many ecosystem functions, such as conferring overall sustainability, one of the main goals of restoration (St. John et al.1997). If introduced, mycorrhizae may have a significant impact on the restoration of habitats containing both mycorrhizal plants and non-mycorrhizal plants. Added moisture and nutrient uptake may allow the mycorrhizal species to out-compete the non-mycorrhizal species (Wilson and Hartnett 1998; Smith et al. 1998) and accelerate succession by recolonized plants (Allen and Allen 1988).

The benefits of mycorrhizal inoculation to habitat restoration have been examined to some degree (Pattinson et al. 2004), but most of the studies employing inoculation have been conducted in environments of extreme degradation, such as abandoned mines (Walker et al. 2004) or sites of volcanic eruption (Smith et al. 1998). Mycorrhizae have been found to be beneficial under these circumstances (Richter and Stutz 2002). They have been shown to

colonize disturbed sites at an impressive rate; for example, in one trial, mycorrhizal volume in disturbed soil increased from 1% to 90% in a single year following inoculation (Salyards et al. 2003). Few studies, however, have examined mycorrhizal inoculation in less degraded habitats, such as roadsides, and of those, only some have shown inoculation to be valuable to plants during restoration (White 2008).

Still, mycorrhizal fungi are generally regarded as beneficial to restoration, but this is a broad generalization. Positive effects of AM inoculation have been documented for native plant seeds and seedlings. St. John and Evans (1990), for example, determined that the inoculation of mycorrhizae into disturbed soil aided the establishment of native grasses and subsequently gave the plants a competitive advantage against invasive species; but Richter and Stutz (2002) discovered AM colonization on the perennial grass, big sacaton (*Sporobolus wrightii*), to have no significant effect on plant growth after 8 weeks in a greenhouse study. Wilson and Hartnett (1998) found perennial warm-season, C_4 prairie grasses responded positively to AM colonization, whereas cool-season, C_3 grasses did not. In another study in the Rocky Mountains, three late-successional plants benefited from AM colonization, whereas three early-successional plants did not (Rowe 2007).

The use of mycorrhizal application in restoration projects is desirable, if it is effective, because, (a) it is relatively inexpensive (St. John and Evans 1990),

(b) can improve soil quality while avoiding the shortcomings of fertilizer and herbicide application, and (c) has the potential to produce a fully self-sustainable ecosystem within a short period of time. But to better evaluate inoculation as a viable management tool, the interactions between specific plant species and mycorrhizal species must be better understood. More information of this kind would be valuable to land managers because it allows them to assess the importance or relevance of these types of symbiotic relationships during habitat rehabilitation.

Restoring California Coastal Prairies

Before Europeans settled into the Monterey Bay area of California, the "low-lying" or "low-elevation" uplands and terraces of Elkhorn Slough were largely composed of coastal prairie (A. Woolfolk, personal communication). Due to grazing, agriculture, and urbanization, the soils of this and many other historic grasslands have been degraded and are now dominated by non-native annual grasses (ESNERR Final Management Plan 2006). In the Elkhorn Slough region it appears that hardy, invasive grasses out-compete native grasses such as *Nassella pulchra* and *Bromus carinatus* and inhibit their re-colonization because non-natives are fast-growing, shade out native seedlings, and consume a high proportion of the available minerals and moisture in the soil.

This project had three main goals. The first was to identify the mycorrhizal fungi that occupy healthy coastal prairie ecosystems in the Monterey

Bay area in California. The second was to test their effects on two native perennial grasses, California brome (*Bromus carinatus)* and purple needle grass (*Nassella pulchra).* The tests were designed to determine whether inoculating seeds of these grass plants with AM would enable the seeds or seedlings to (1) emerge faster, (2) grow at a greater rate and to a larger ultimate size, and (3) achieve a higher state of vigor than plants whose seeds were not inoculated. The third goal was to determine how competition with a non-native annual grass, soft chess (*Bromus hordeaceus*), would affect those qualities. Experiments were set up with seedlings growing in the presence or absence of mycorrhizal inoculum in native soils under relatively controlled conditions within a greenhouse.

METHODS

Reference Site

A 0.5-acre portion of a coastal prairie at the Elkhorn Slough National Estuarine Research Reserve (ESNERR) (36° 49' 10"N, -121° 44' 17"E) served as the indirect study site for this investigation. In October of 2006, field soil from the site was collected to create a growth medium, and mycorrhizal fungal species were isolated and identified in soil samples from this location. Seeds were collected: 500 *B. carinatus* and 500 *N. pulchra*. It is considered harmless to plant survival to harvest no more than 10 percent of the seeds found on each plant (Guerrant et al. 2004), and care was taken to ensure that this percentage was not exceeded. Non-native soft chess seeds were purchased online from B and T World Seeds (Aigues-Vives, France).

Identification of AM Species on Study Site

In October of 2006, four root samples (from *B. carinatus*, *N. pulchra* and *Danthonia californica*) and soil samples were collected from the ESNERR site and examined for the presence of AM fungi. The roots were carefully rinsed, cleared in lactic acid, and stained in trypan blue, chlorazol black E, or lactophenol blue (Brundrett 1994). All stains produced AM visibility under brightfield optics with a Zeiss compound research microscope, but resolution was highest with 0.03% chlorazol black E in 1 part lactic acid, 1 part glycerol, and 1 part water.

Fungal spores were extracted from the soil by the method of Allen (1979).

Soil samples were dry sieved and centrifuged in distilled water at 2,500 rpm for 10 min. The organic matter-containing water was then poured off, a 2 M sucrose-calgon solution was added, and the samples were centrifuged again at 2,500 rpm for 20 min. The solution was filtered through Whatman no. 1 filter paper, leaving the AM spores on the filter paper, and then identified to genus via the Manual for the Identification of VA Mycorrhizal Fungi (Schenck and Pérez 1988).

Experimental Design

Five experimental blocks were set up in a greenhouse where each block contained twenty 410 ml Deepots (Stuewe & Sons, Inc., Corvallis, OR). Of the 20 pots, 10 were inoculated with mycorrhizal fungi and 10 served as uninoculated controls. One block contained two *N. pulchra* (native) plants, the second contained two *B. carinatus* (native) plants, the third contained two *B. hordeaceus* (non-native) plants, the fourth contained a combination of one *B. hordeaceus* plant and one *N. pulchra* plant, and the last contained a combination of one *B. hordeaceus* plant and one *B. carinatus* plant. A greenhouse study was chosen over a field study to avoid contamination of the controls with mycorrhizal spores (Salyards et al. 2003) and to minimize variability.

The greenhouse experiment was begun on January 7, 2008, and the duration was 18 weeks. Temperatures in the greenhouse ranged from 11-27°C, and plants received constant light of 190 μmoles/m^2/s. The potting mixture was

composed of equal parts sterilized sand and sterilized field soil from ESNERR plus 4 g "Terra-Sorb" to maintain even moisture. Mycorrhizal inoculum was added as a 40 ml layer over the potting mix, and seeds were placed at this level. Every pot (inoculated and uninoculated) was then covered with a final 1 cm layer of potting mix.

Inoculum

Initially, inoculum was prepared with field soil from ESNERR, according to the method of Miyasaka et al. (2003). This process involved layering a small amount of field soil between two layers of 1 part sterilized peat moss and 3 parts perlite in 87 six-inch pots. Two known live hosts (*Triticum aestivum* and *Trifolium incarnatum*) were added followed by a low P fertilizer (Apex 19-5-12). After three months, water was withheld and the vegetative portions of the hosts were removed, triggering AM sporulation. The resulting inoculum was composed of roots, spores, and hyphae. The final product was unsuitable for use, because sterilization of the peat moss during soil preparation resulted in the release of humic acid (J. Morton, personal communication), which in turn killed all AM fungi present. A more appropriate method substitutes sand for peat moss (Brundrett 1994; Richter and Stutz 2002). The inoculum used in this study was a mixture of *Glomus deserticola, G. etunicatum, G. intraradices, G. clarum,* and *Acaulospora delicata*, purchased from the University of West Virginia International Culture

Collection of (Vesicular) Arbuscular Mycorrhizal Fungi, chosen on the basis of my earlier identification of the composition of fungi in ESNERR soil.

Data Collection

After seedling emergence, various parameters of plant size and vigor were measured weekly for 13 weeks. Growth was determined by the length of the longest leaf and the number of tillers (Richter and Stutz 2002). At week 8, percent AM root tip colonization was measured by means of the Gridline Intersect Method (McGonigle et al. 1990) under a compound microscope at 200X. Root segments 1.0 cm in length were placed on microscope slides and, using a hairline graticule as a line of intersection, the presence or absence of mycorrhizae was recorded at 150 intersection points where p = no fungal structures, q = arbuscules, r = vesicles, s = arbuscules and vesicles, u = mycorrhizal hyphae (observed at arbuscules or vesicles), and v = hyphae not seen to be connected to arbuscules or vesicles. Percent root infection was then calculated by: 100[(q+r+s+t+u+v2)/G] where G= p+q+r+s+u+v.

Total moisture content was determined by comparison to dry weight after complete oven drying. The Agricultural Analytical Services Laboratory at Pennsylvania State University (University Park, PA) analyzed phosphorus, nitrogen, and other mineral levels.

Statistics

Unpaired t-tests were used to identify differences between treatments. GLM Repeated Measures ANOVA was applied to measure the effects of treatments over time, and a two-way ANOVA was used to compare effects between plants grown independently and plants grown in competition (Zar 1999).

RESULTS

Mycorrhizal Identification

Mycorrhizae were identified in the field samples of all of the grasses by the method of Schenck and Pérez (1988). *Glomus* and *Acaulospora* were observed in the samples (Figure 1) with *Glomus* being the more abundant. Colonization in the field appeared as in (Figure 2), indicating that the grasses found in ESNERR did have a mycorrhizal association and with the morphological features expected of a healthy, fully functional mycorrhizal population.

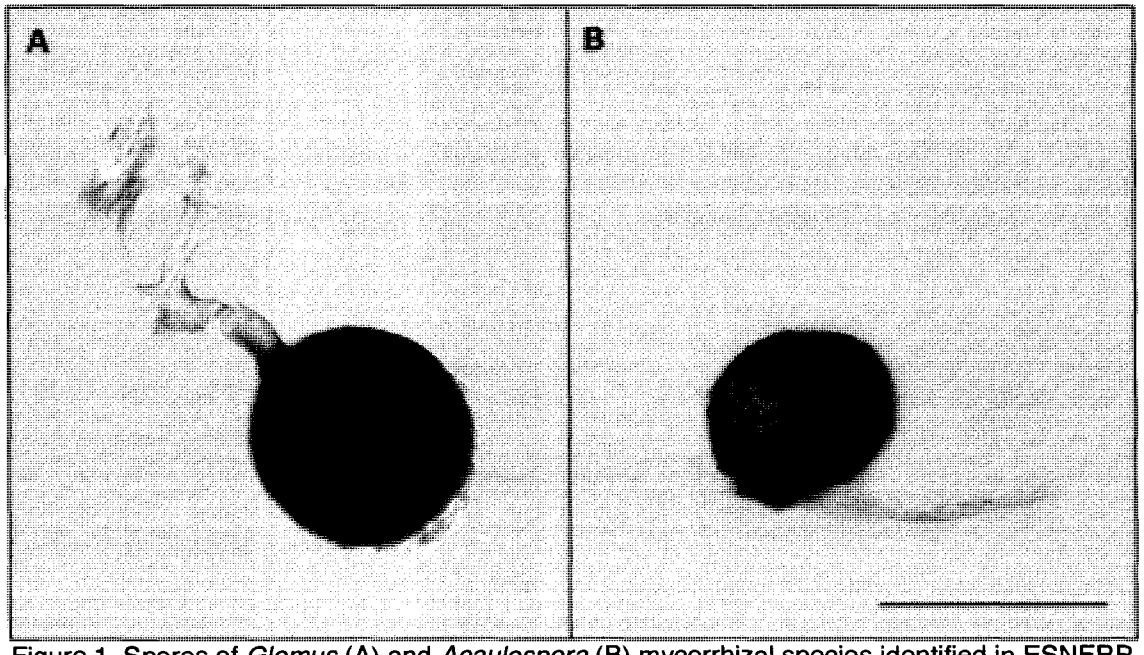

Figure 1. Spores of *Glomus* (A) and *Acaulospora* (B) mycorrhizal species identified in ESNERR field sample. Bar = 100 μm.

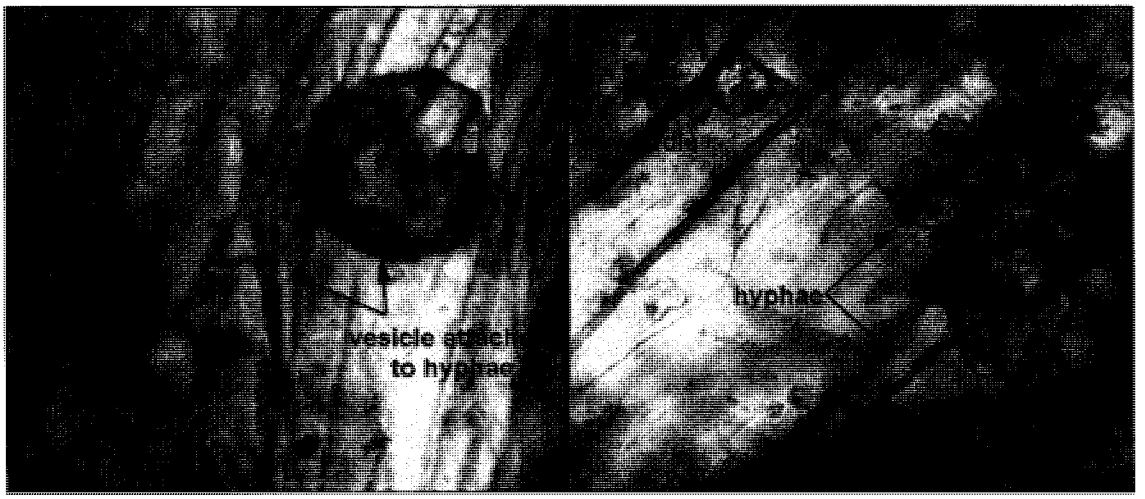

Figure 2: Arbuscular mycorrhizal vesicles and attached hyphae. This colony of mycorrhizae was observed in the roots of *Nassella pulchra* in a ESNERR field sample.

Soil Fertility

A soil nutrient analysis (by A & L Western Laboratories, Inc.) of collected field soils for use in the trial revealed very low mean levels of P, moderate levels of $NaHCO_3$-P, and low levels of NO_3-N (Table 1). Soils with low P have been determined to be ideal for the occurrence of AM-plant interactions (Allen et al. 2003).

Table 1. Soil nutrient analysis of ESNERR field samples collected in a coastal prairie to assess existing mineral content, primarily P and N. Mycorrhizal-plant interactions are more likely to occur where soil P and N are low.

Phosphorus (Weak Bray) ppm	Phosphorus ($NaHCO_3$-P) (Olsen Method) ppm	Potassium ppm	Magnesium ppm	Calcium ppm	Sodium ppm	Soil pH
6[1]	11[2]	29	132	680	12	6.7

Nitrogen (NO_3-N)	Sulfur (SO_4-S) ppm	Zinc ppm	Manganese ppm	Iron ppm	Copper ppm	Boron ppm
5[3]	1	1	23	43	0.4	0.6

[1] Level > 30 ppm is preferred for top production in most crops (A & L Western Laboratories, Inc.)
[2] Level > 15 ppm is preferred for top production in most crops (A & L Western Laboratories, Inc.)
[3] Level > 21 ppm is preferred for top production in most crops (Brown et al. 1991)

Seedling Emergence

All inoculated grasses emerged significantly more quickly and in greater numbers in the presence of AM in comparison to grasses that were not planted with inoculum (Table 2). A repeated-measures analysis of variance showed the between-subjects *p* values to be less than 0.05 for all grasses (Table 3). Competition had no effect on seedling emergence in the presence or absence of AM.

Table 2. Cumulative mean number of seedlings per pot that emerged from inoculated and uninoculated soils.

	N. pulchra	*N. pulchra* in competition	*B. carinatus*	*B. carinatus* in competition	*B. hordeaceus*	*B. hordeaceus* (with *B. carinatus*)	*B. hordeaceus* (with *N. pulchra*)
Week 1 + AM	3	6	3	2	6	7	7
Week 1 - AM	1	0	0	0	2	1	3
Week 2 + AM	6	6	5	5	7	8	8
Week 2 - AM	1	2	4	2	4	3	3
Week 3 + AM	6	6	6	6	8	9	9
Week 3 - AM	3	6	5	2	4	5	5

Table 3. Comparison of inoculated and control seedling emergence using a repeated measures general linear model with sphericity assumed.

Between-subjects Effects (inoculated *vs.* control)	Df	Mean Square	F	Sig.
N. pulchra	1	194.400	39.376	0.000
N. pulchra (with *B. hordeaceus*)	1	173.400	21.437	0.000
B. carinatus	1	52.267	5.513	0.031
B. carinatus (with *B. hordeaceus*)	1	147.267	25.988	0.000
B. hordeaceus	1	211.250	15.146	0.001
B. hordeaceus (with *N. pulchra*)	1	380.017	75.196	0.000
B. hordeaceus (with *B. carinatus*)	1	281.667	39.734	0.000

Mycorrhizal Colonization and Dependency

At eight weeks, percent colonization of three grasses was measured from each treatment group using the grid-intersect method (McGonicle et al. 1990). In plants grown independently (multiple plants but one species/pot), the mean percentage of root length colonized by mycorrhizal hyphae was 47.66% for *B. carinatus,* 48.23% for *N. pulchra,* and 49.57% for *B. hordeaceus.* When grown in competition, mean percent colonization was 47.97% for *B. carinatus,* 47.04% for

N. pulchra, 48.12% for *B. hordeaceus* with *B. carinatus,* and 47.64% for *B. hordeaceus* with *N. pulchra.* These infection levels, compared by standard t-test, were not significantly different.

Mycorrhizal dependency (MD) was measured by the following equation, as formulated by Plenchette et al. (1983):

$$\frac{\text{mean dry mass of mycorrhizal treatment - mean dry mass of control}}{\text{mean dry mass mycorrhizal treatment}} * 100$$

Only *N. pulchra* demonstrated dependency (Table 4).

Table 4. Height and dried shoot biomass in grasses grown alone or in competition and in the presence or absence of mycorrhizae.

Plant Species	n	x̄ Biomass + AM (g)	x̄ Biomass - AM (g)	x̄ Height + AM (cm)	x̄ Height - AM (cm)	MD[1]
N. pulchra	40	0.21 ± 0.08[3]	0.12 ± 0.03[2, 3]	36.10 ± 6.26	45.50 ± 6.24[2, 3]	40%
N. pulchra (with *B. hordeaceus*)	32	0.07 ± 0.02	0.04 ± 0.00[2]	25.90 ± 10.13	31.30 ± 4.00	49%
B. carinatus	40	0.14 ± 0.04[3]	0.15 ± 0.04[3]	30.80 ± 2.66	36.20 ± 2.82[2]	-4%
B. carinatus (with *B. hordeaceus*)	40	0.06 ± 0.03	0.09 ± 0.05[2]	25.70 ± 2.41	27.40 ± 6.36	-52%
B. hordeaceus	40	0.11 ± 0.03[3]	0.12 ± 0.04[3]	28.60 ± 1.64[3]	31.40 ± 2.91[2]	-7%
B. hordeaceus (with *N. pulchra*)	40	0.05 ± 0.01	0.12 ± 0.03[2]	24.80 ± 3.29	29.60 ± 3.13[2]	-140%
B. hordeaceus (with *B. carinatus*)	40	0.12 ± 0.06	0.15 ± 0.05	24.00 ± 1.25	29.40 ± 10.85	-23%

[1] MD = mycorrhizal dependency
[2] Significantly different from treated (+ AM) quantity (previous column of table) at $P < 0.05$
[3] Significantly different from species in competition (row beneath in table) at $P < 0.05$

Height and Biomass

After 18 weeks, plants colonized by AM were generally shorter than plants without AM, as measured by the length of the longest tiller of each plant. The exceptions were *N. pulchra* grown in competition, *B. carinatus* grown in

competition, and *B. hordeaceus* grown with *B. carinatus*, for which there were no significant differences between treatments (Table 4).

However, where AM colonization produced shorter plants or where no height effects were observed, significant differences between mean dry aboveground biomass were noted only with *N. pulchra* and not the others. Thus *N. pulchra* produced greater biomass with AM when grown independently (F = 14.341, P = 0.000) and in competition (F = 4.061, P = 0.000), while plant height decreased or showed no statistical difference at all. In the other species, as plant height decreased, biomass remained constant or declined. Thus when the grasses were grown independently, mycorrhizal colonization had no significant effect on the shoot biomass of *B. carinatus* or *B. hordeaceus* but had a significant negative effect on their heights (F = 0.083, P = 0.000; F = 9.383, P = 0.019, respectively). Mycorrhizal colonization also had a significant negative effect on the biomass of *B. hordeaceus* when grown in competition with *N. pulchra* (F = 6.733, P = 0.000) but a positive effect on height (F = 0.078, P = 0.004).

A two-way ANOVA revealed that plants of *N. pulchra* produced more biomass when growing among their own species than in competition, with and without mycorrhizal colonization (F = 5.028, P = 0.028). The same result occurred for *B. carinatus* (F = 66.059, P = 0.000) and *B. hordeaceus* when compared to the mean biomass of *B. hordeaceus* in competition with *N. pulchra* (F = 20.82, P = 0.000). Results from another two-way ANOVA similarly showed

that all three plants grew taller when grown on their own. Adding mycorrhizae to *N. pulchra* and *B. carinatus* in competition produced shorter plants ($F = 14.83$, $P = 0.000$; $F = 6.78$, $P = 0.013$, respectively). When mycorrhizae were added to *B. hordeaceus,* plant heights decreased when in competition with both *N. pulchra* and *B. carinatus,* but plant height was significantly different only with *B. carinatus.*

Seed Production and Viability

By the end of the trial, only *B. hordeaceus* had produced seed. *Nassella pulchra* and *B. carinatus* require a longer period to flower than the duration of my experiment. Flowering of *B. hordeaceus* began at Week 5 for inoculated plants and at Week 8 for the controls. In the presence of mycorrhizae, *B. hordeaceus* produced a significantly larger number of seeds when grown alone and in competition (Figure 2). Competition, however, significantly reduced seed production in the absence of mycorrhizal fungi. Adding AM lessened this effect; that is, seed production was reduced during competition, but not as dramatically as in the absence of mycorrhizae.

When tested for viability, the seeds from plants grown with AM were more successful when grown in competition than alone (Table 5). Competition reduced seed viability in the absence of mycorrhizae. Adding mycorrhizae did not simply lessen this effect; rather, it reversed it.

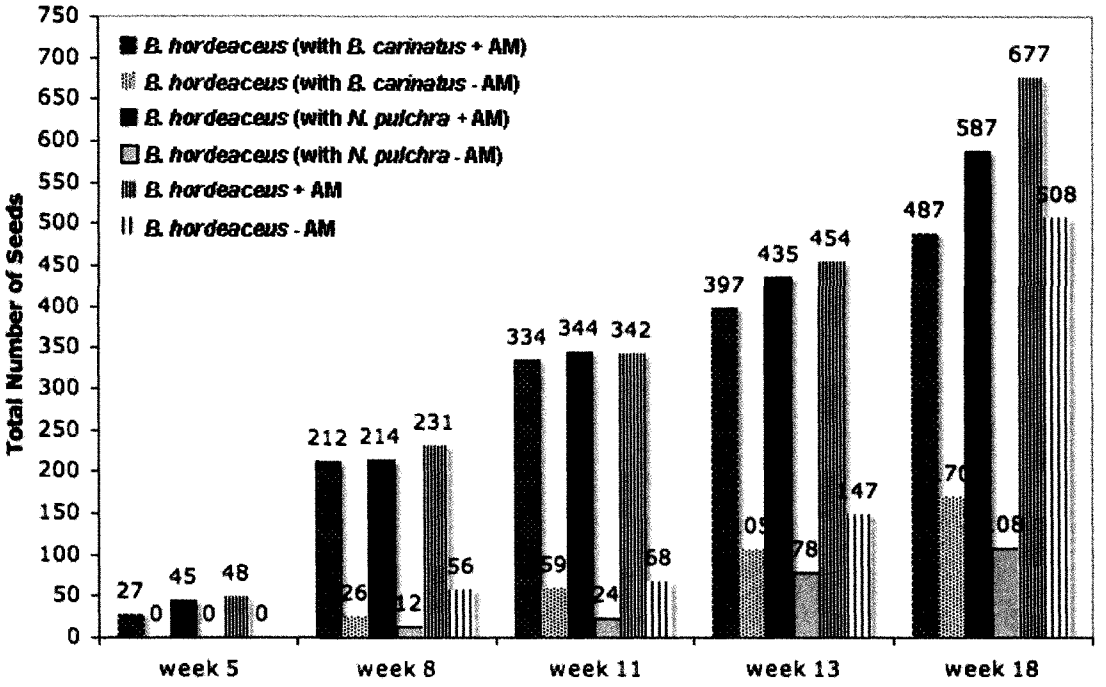

Figure 3. Total number of seeds produced by *B. hordeaceus* plants in a greenhouse over the course of 18 weeks.

Table 5. Percent germination of *B. hordeaceus* after 13 days during seed viability trial.

	With AM (%)	Without AM (%)
B. hordeaceus	50	58
B. hordeaceus (with *N. pulchra*)	60	16
B. hordeaceus (with *B. carinatus*)	88	42

Plant Tissue Mineral Content

A plant tissue analysis was performed (by the Agricultural Analytical Services Lab, Penn State University, University Park, PA) on all plants at the conclusion of the trial. Shaded results in Table 6 show significant differences in

mineral content between inoculated and uninoculated plants. Where results are shaded in gray, inoculated plants contained a significantly higher mineral content. Where results are shaded in black, inoculated plants contained a significantly lower mineral content.

Table 6. Post-experiment plant tissue analysis of *N. pulchra*, *B. carinatus* and *B. hordeaceus* leaves.

	%N	%P	%K	%Ca	%Mg	Mn ppm	Fe ppm	Cu ppm	B ppm	Al ppm	Zn ppm	Na ppm
B. carinatus + AM	0.97	0.17	2.48	0.61[2]	0.27	399	1740[2]	6	116[2]	2951[2]	32[2]	1253
B. carinatus - AM	0.86	0.15	2.43	0.45	0.20	340	326	4	45	585	20	1262
N. pulchra + AM	0.98	0.27	1.37	0.31	0.19	82	402[2]	4	71[2]	742[2]	28	174
N. pulchra - AM	0.99	0.28	1.42	0.29	0.17	102	191	5	29	262	40[1]	549[1]
B. hordeaceus + AM	1.20	0.21	3.19[2]	0.63	0.33[2]	333	454	6	71	778	34	3993[2]
B. hordeaceus - AM	1.01	0.13	1.84	0.53	0.18	382	665	6	74	1021[1]	43	2814
B. carinatus + AM in competition	1.20	0.21	2.49	0.58	0.26	335	644[2]	10[2]	53	1117[2]	30	1147[2]
B. carinatus - AM in competition	1.25	0.20	2.65	0.43	0.21	355	246	6	54	409	26	697
N. Pulchra + AM in competition	1.21	0.28	1.71	0.33	0.19	72	195	7	49[2]	230	27	206
N. Pulchra - AM in competition	1.11	0.27	1.70	0.34	0.18	130[1]	195	6	36	274	26	247
B. hordeaceus + AM in competition with *N. pulchra*	1.00	0.32	2.62	0.79[2]	0.46	281	541[2]	5	92[2]	930[2]	33	4495
B. hordeaceus - AM in competition with *N. pulchra*	1.05	0.26	3.34[1]	0.50	0.30	469[1]	183	7	67	261	31	4651
B. hordeaceus + AM in competition with *B. carinatus*	1.03	0.29	2.50	0.62	0.38	299	282	7	65	486	37[2]	3131
B. hordeaceus - AM in competition with *B. carinatus*	0.91	0.25	3.29	0.52	0.29	454[1]	401[1]	7	96[1]	725[1]	26	3749

[1] Untreated (- AM) plants significantly greater than treated (+ AM) cell above at $P < 0.05$

[2] Treated (+ AM) plants significantly greater than untreated (-AM) cell below at $P < 0.05$

DISCUSSION

AM inoculation of all three grasses resulted in greater and faster seedling emergence. This result concurs with that of Richter and Stutz (2002), who found that *Sporobolus wrightii* emerged more quickly in the presence of AM, and with that of Salyards et al. (2003), who observed more rapid emergence of *Deschampsia caespitosa* and *Bromus carinatus*. However, these results are contrary to those of Koide (1985), who found that AM caused growth depressions in seedlings of *Helianthus annuus.* Rapid seedling emergence is a desirable quality for grassland management, especially in situations where quick seedling establishment is needed, for example, in erosion control. Application of AM for this purpose may be appropriate; however, the present study demonstrated that more rapid seedling emergence did not necessarily lead to larger or more robust plants.

In this investigation, the effects of AM on plant size and vigor were species specific. Of the three grasses tested, only *N. pulchra* demonstrated a significant positive growth response to inoculation. Generally, inoculated *N. pulchra* had greater biomass but shorter tillers. Considering that the total number of tillers was equal in inoculated and uninoculated treatments, this result indicates that AM inoculation altered this plant's architecture, producing shorter and thicker leaves and thus sturdier plants. Similarly, Koide (1985) observed that AM inoculation of *Helianthus annuus* caused a shift in the plant's carbon allocation

that altered leaf and stem structure in the same way. A study on the effects of shorter, more robust plants in grassland ecosystems would provide more insight into the usefulness of using AM in the restoration of *N. pulchra* stands.

Though positive growth effects were clearly noted in *N. pulchra* as a result of mycorrhizal inoculation, it is not probable that these were due to increased mineral uptake. N and P uptake is essential to the production of plant biomass, and the near equal concentrations of these macronutrients in inoculated and uninoculated plants strongly suggests that vegetative development in this study could not be attributed to mineral uptake. Overall, results from the plant tissue analysis showed no consistent trend in macronutrient content that coincided with the growth data.

Only *Bromus hordeaceus* flowered over the course of the experiment. California native perennial grasses often take longer to flower than exotic annual species and, in the case of *N. pulchra*, may not flower until the second year following seed germination (US Forest Service 2008). Although mycorrhizal inoculation did not affect vegetative growth in *B. hordeaceus,* it had a clear effect on seed production. In general, inoculated plants produced significantly more seed and more viable seed. Plants in competition produced more seed. Contrary to these results, Wilson and Hartnett (1998) and Smith et al. (1998) found no significant mycorrhizal effects on the flowering of inoculated warm-season grasses. An increase in exotic seed production and viability is of concern

to land managers whose goals are to reduce the number of non-native species in California grasslands. Results from this research indicate that mycorrhizal inoculation in the field may enable *B. hordeaceus* to achieve higher reproductive success, thereby increasing non-native populations rather than eradicating them.

B. carinatus and *N. pulchra* suffered when put in competition with the non-native *B. hordeaceus*. Dyer & Rice (1996, 1997) similarly found that *N. pulchra* grew larger and produced more florets when competition was relieved in interior grasslands. In addition, the study described here revealed that, when grown with *B. hordeaceus*, *N. pulchra* had higher biomass in the presence of mycorrhizae than in their absence, whereas *B. hordeaceus* was smaller in the presence of mycorrhiza . Given that the plant tissue analysis did not account for any increase in nitrogen and phosphorus uptake, and as it is unlikely that micronutrient uptake would have such a significant influence over growth, the increase in biomass of *N. pulchra* might be attributable primarily to an increase in water uptake. Alternatively, an indirect effect of mycorrhizae on plant photosynthesis or other physiological functions might explain the change in biomass. In addition, the lack of correlation between the micronutrient levels and plant growth adds to my suspicion that the mycorrhizal effect on *N. pulchra* was mainly in elevating moisture acquisition from the soil and away from *B. hordeaceus*.

Aside from hastening *B. carinatus* seedling emergence, AM had little effect on this native grass. AM, however, had a clear influence on the development of

N. pulchra and may not only be beneficial in assisting seedling emergence but also in its competition with the non-native *B. hordeaceus* or other grasses for moisture. This kind of species-specific information is essential for making informed judgments on the management of restoration programs in any particular community.

The native grasses, *N. pulchra* and *B. carinatus,* analyzed in this study are common California native grasses that are desirable for restoration projects. They often grow in competition with the annual non-native *B. hordeaceus* which is widespread throughout many grassland habitats and very difficult to control. The widespread nature of these grass competitions makes this investigation relevant to land managers who constantly struggle to find effective strategies to remove non-natives and to restore native grasses. Many recent restoration projects have employed mycorrhizal inoculation with the intention of boosting natives with little awareness of the potential negative effects that may ensue.

Throughout this paper I have offered insight into the complexity of mycorrhizal-plant interactions during restoration. In the case of restoring *N. pulchra,* it is clear that the addition of mycorrhizae may aid in this plant's reestablishment by producing fast emerging, robust plants that are better suited to compete against non-natives as well as tolerate low moisture and drought conditions. These benefits may become increasingly important as scientists begin to look at the effects of global warming on native plant populations.

Though vegetatively less robust, the non-native *B. hordeaceus* may become substantially more successful reproductively in the presence of AM, thereby contributing more seed to the non-native seedbank on a restoration site. This addition of non-native seed, however, may be inconsequential where there is already a large non-native seedbank. While an increase in non-native seedling emergence may be observed the second year after AM inoculation, it may not pose a large threat to the natives if a healthy and robust native stand capable of competitive dominance is established. Future studies are needed to weigh the net effects of sexual reproductive success versus vegetative growth under field conditions in order to determine the implications for restoration.

Table 7: Implications for practice

- *Nassella pulchra*, *Bromus carinatus* and *Bromus hordeaceus* seedlings emerged more quickly and in greater numbers in the presence of AM.
- Plant response to AM inoculation appeared to be species-specific.
- The vegetative advantage gained by the native *N. pulchra* as a result of AM inoculation may allow for a more robust and competitive native population.
- The non-native *B. hordeaceus* experienced high reproductive success in the presence of AM causing an increase of non-native seed in the seedbank. It is possible, however, that the vigorous native population established with the help of AM may be able to withstand the competitive pressure of these emerging non-natives.

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