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**EFFECTS OF VESICULAR-ARBUSCULAR MYCORRHIZAE ON
GROWTH, REPRODUCTION AND SURVIVAL IN THREE PLANT
SPECIES IN A SAND DUNE RESTORATION SITE IN
MONTEREY, CALIFORNIA**

**A Thesis Presented to
The Faculty of the Department of Biology
San Jose State University**

**In Partial Fulfillment
of the Requirements for the Degree
Master of Science**

by

Jane E. Holte

December, 1994

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ABSTRACT

EFFECTS OF VESICULAR-ARBUSCULAR MYCORRHIZAE ON GROWTH, REPRODUCTION AND SURVIVAL IN THREE PLANT SPECIES IN A SAND DUNE RESTORATION SITE IN MONTEREY, CALIFORNIA

by Jane Holte

A field study was done at a disturbed dune restoration site to test the effects of fertilizer and inoculation with vesicular-arbuscular mycorrhizal fungi (VAMF) on *Artemisia pycnocephala*, *Camissonia cheiranthifolia*, and *Eriogonum parvifolium*. Host plant growth, survivorship, reproductive output and VAMF colonization were examined. VAMF inoculation and low phosphate fertilizer were further investigated in *Artemisia* in a greenhouse study.

Inoculation was successful in both field and greenhouse studies. Little or no colonization was found in uninoculated plants grown in the disturbed dune soil. Fertilizer inhibited VAMF colonization in both field and greenhouse studies. In the field study, small VAM induced increases were seen in *Artemisia* in above ground growth at 18 weeks (27%) and 31 weeks (44%), and root weight (48%) at 10 weeks. No responses to VAM were seen in reproductive output or survivorship. Application of results to dune ecology and restoration are discussed.

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INTRODUCTION

Vesicular-arbuscular mycorrhizae (VAM) are symbiotic relationships formed between microscopic root fungi and many higher plants. The fungi (VAMF) are characterized by round or oblong vesicles that are believed to function as fungal storage organs, and tree shaped arbuscules that are believed to be the primary site of fungal-plant exchanges (Brown and King, 1982). Arbuscules are ephemeral and form intracellularly in the cortex of actively growing feeder roots (Brown and King, 1982). Vesicles are typically found in the cortex of roots in the late stages of infection, but are also seen in the cortex of non-host species (Brundrett, 1991).

Host plant benefits in response to VAMF infection have been well documented in many greenhouse and growth chamber experiments. Increased host plant phosphorus uptake has been widely reported (Brundrett, 1991). Several studies suggest that VAM formation can be reduced by both high and low soil phosphorus levels, and that host plant benefits are greatest when phosphorus is in an insoluble form (Brundrett, 1991). VAM may also increase micronutrient uptake, particularly Mg, Cu, and Zn (Brundrett, 1991). Other studies have reported increased tolerance to salinity (Rozma et al., 1986) and increased drought resistance (Read and Boyd, 1986) in response to VAM. The VAM fungi have also been shown to increase soil aggregation in both greenhouse and field studies (Koske et al., 1975; Sutton and Sheppard, 1976; Rose, 1988).

Even though host plant benefits have been demonstrated in many greenhouse

and growth chamber experiments, field studies show more variable host plant responses to VAM (Brundrett, 1991). Factors that can influence host plant responses to VAM include: soil nutrient level, nutrient form, climatic and edaphic conditions, fungal grazing, hyperparasitism, and fungal species (Brundrett, 1991).

VAMF infected plants are common in marine sand dunes worldwide (Giovannetti and Nicolson, 1983; Nicolson, 1960; Ernst et al., 1984; Koske, 1988; Koske and Halvorson, 1980; Sylvia and Will, 1988). In the Western U.S., Rose (1988) found VAM common in root segments at Lanphire-Christensen Dune Preserve, north of Arcata. Pickart et al. (1990) found variable levels of VAMF infection in all species investigated at Lanphire-Christensen.

Several greenhouse studies have investigated the effects of VAM on the growth of dune plants native to the East Coast of the United States. Koske and Polson (1984) found dramatic increases in survival in inoculated vs. uninoculated American beachgrass (*Ammophila breviligulata*). Sylvia (1989) found that VAMF inoculated sea oats (*Uniola paniculata*) had greater shoot dry mass, height, root length, and shoot phosphorus and zinc concentrations than uninoculated plants when grown in natural dune soil. However, Sylvia (1989) found that VAM did not increase plant growth when plants were grown in nursery conditions using standard applications of fertilizer and commercial planting mix.

Several field studies have investigated the effects of VAMF on plant growth in sand dunes. Gemma (1987) found that plants from a plot of American beachgrass

that were inoculated with VAMF became established more quickly, showed more vigorous growth and produced more panicles than a plot of uninoculated plants. Sylvia (1989) tested the effects of VAMF inoculation and three levels of fertilizer on growth of sea oats at a Florida beach restoration site. Three months after outplanting, VAMF inoculated plants had greater plant height and % VAMF colonization than uninoculated plants (% colonization is a measure of the level of VAM formation). There was no interaction between fertilizer and % VAMF colonization. Unfortunately, the study site was inundated with sea water during winter storms resulting in low plant survival and variable VAMF colonization. Growth effects due to VAMF inoculations were not seen in later sampling. In a second experiment, Sylvia (1989) tested VAMF inoculation alone. In this study he found significant increases in dry shoot weight, root length, tillers/plant, and plant height in inoculated vs. uninoculated sea oats. After 14 months, VAMF colonization was not significantly different between treatments; however, differences in tiller production and plant height were still observed.

Low densities of VAMF spores have been reported in unvegetated sand dune areas in Florida (Sylvia 1986; Sylvia and Will, 1988) and northern California (Rose, 1988). Sylvia and Will (1988) found that VAM established slowly in sea oats planted in dredged sand in a Florida beach replenishment project. At the end of the two year study there was still a significant difference in % colonization between planted sea oats and those from natural dune areas. Findings such as

these have sparked interest in the use of VAMF inoculation in sand dune revegetation projects.

The objectives of this project were: to investigate the effects of VAMF inoculation on growth, survivorship, and reproductive output in three plant species native to central California coastal dunes in field conditions; to investigate the effects of VAMF inoculation on growth in greenhouse conditions; to investigate the effects of fertilizer on VAMF colonization; and to investigate VAMF colonization and inoculation techniques in disturbed dune soil.

METHODS

FIELD STUDY

A field study was done at a sand dune restoration site to test the effects of VAMF inoculation and fertilizer on survival, growth and reproduction in *Artemisia pycnocephala* (beach sagewort), *Camissonia cheiranthifolia* (beach evening primrose), and *Eriogonum parvifolium* (buckwheat).

Study Site

The site was located in the "Phillips Petroleum Parcel," which is now part of Monterey State Beach. This parcel is located 500 meters southwest of the parking lot at the east end of Sand Dune Drive in Monterey, California (Fig. 1). The area was formerly owned by the Phillips Petroleum Company and housed large oil storage tanks from the late 1920's until the 1970's. The study site was largely unvegetated except for several hummocks consisting of *Artemisia pycnocephala*, *Calystegia soldanella* (beach morning glory), *Eriogonum parvifolium*, *Camissonia cheiranthifolia*, and *Carpobrotus edulis* (hottentot fig).

The planting area was approximately 50 by 40 meters, located on an unvegetated, ocean facing (north-west) ridge. The average slope was 4.5 degrees. The area was located 60 meters behind the mean high tide line; far enough back from the ocean to be protected from normal winter wave action but still receiving a great deal of wind, salt spray, and sand movement. Sand burial over the 17 month study was as much as 20 centimeters.

Plant Propagation

For each species tested, 500 plants were grown from seed in Supercell[®] planters. The seed used was obtained from Marina State Beach personnel and had been collected from a variety of dune sites along the southern Monterey Bay. The planting mixture consisted of 1 part sterile sand and 3 parts Sunshine[®] #3 potting mix. Sand was sterilized by autoclaving (121 c°) twice for one hour, with 8 hours between sterilizations. Plants were given 20-20-20 Miracle-Gro[®] liquid fertilizer at 4 and 7 weeks. Plants were grown in a greenhouse for 10 weeks, moved to an outdoor holding area to harden for 2 weeks, and then transplanted into the study site and subjected to fertilizer and inoculation treatments.

Experimental Design and Inoculation Methods

Four VAMF inoculation and fertilizer treatments were tested in a randomized block design. Treatments were as follows.

A. VAMF Inoculation - No Fertilizer: 30ml of VAMF infected root and sand from a vegetated dune area were added below the root zone as plants were placed into the ground. Studies have shown this method of inoculation to be effective (e.g. Gemma, 1987; Pickart et al., 1990b).

B. No VAMF Inoculation - No Fertilizer: plants were transplanted without inoculum or fertilizer. Plants were given 10 ml of filtrate from the sand and root inoculant prior to transplanting. The filtrate was prepared by washing the sand and root inoculant twice through a 35 μ m filter and once through a 8 μ m

filter. Filtrate was added to restore the non-mycorrhizal soil flora (Linderman and Hendrix, 1982).

C. VAMF Inoculation - Fertilizer: 30 ml of VAMF infected root and sand inoculant was added below the root zone as plants were placed into the ground. Prior to planting, Osmocote® 14-14-14, slow release fertilizer was applied at rate of .022 kg/square meter (190 lbs/acre).

D. No VAMF Inoculation - Fertilizer: slow release fertilizer was applied at rate of .022 kg/meter and raked into the sand prior to planting. Plants were given 10ml of filtrate prior to transplanting.

Four replicate blocks were prepared, each replicate contained 25 plants per species per treatment (300 plants per block). Plants were placed approximately 1/3 meter apart. Each block was 12 x 12 meters with a 2 meter buffer zone between treatments within blocks (Fig. 2).

Outplanting took place on February 12-14 1993, and coincided with the winter rains. Straw plugs were placed into the ground prior to planting to help stabilize the bare sand. To minimize the disruption of removing root and sand from an undisturbed dune community, inoculant was collected from pure stands of the invasive, non-native beach grass, *Ammophila arenaria*. The *Ammophila* stands were located at Zmudowski State Beach, 28 km north of the study site. To prevent the inadvertent spread of *Ammophila*, the root and sand inoculant was sieved through 500 μm screen to remove seed and rhizomes.

Fine roots were removed from the rhizomes and added back into the inoculant. VAMF infection has been reported in *Ammophila arenaria* (Giovannetti 1985; Nicolson, 1959). I observed numerous spores and low levels of VAMF colonization in summer and fall samples of this *Ammophila* population.

Sampling

At ten weeks (4/26/93), five random plants (per replicate, per species) were removed and assessed for % VAMF colonization, root and shoot wet weight, and shoot dry weight. Because initial correlations between wet and dry shoot weights were high ($R = .939$, $n = 15$), I did not continue to calculate dry weights.

At 61 weeks (4/16/94), percent colonization was assessed for *Artemisia* (five plants per replicate). Roots were sub-sampled using cores, rather than removing the entire plant. Results from a paired t-test did not show a difference in percent colonization between core samples and whole root samples taken from the same plants ($t = .1046$, $t_{05(2)3} = 3.182$). Since none of the 10 week *Eriogonum* samples showed colonization, full sampling of *Eriogonum* was not done at 61 weeks. However, 5 root core samples taken at 67 weeks (5/30/94) from the VAMF Inoculation - No Fertilizer treatment in block A, showed VAMF colonization. Subsequently, *Eriogonum* in all treatments was sampled at 76 weeks (8/1/94).

Since several studies have shown seasonal differences in VAMF colonization in sand dune plants (Giovannetti, 1985; Ernst et al., 1984), most sampling was done in the late spring, when VAMF colonization was suspected to be at its highest. Second year *Eriogonum* samples were done in the early summer, perhaps later than the peak colonization period. However, results of a t-test showed no difference in colonization levels between May and August samples from the same plot ($t = .0614$, $t_{0.05(2)4} = 2.776$).

Roots were stained and cleared using the methods of Koske and Gemma (1992). Roots were stored in 35% isopropyl alcohol prior to staining and clearing. The gridline intercept method was used to assess % VAMF colonization (Giovannetti and Mosse, 1980). One hundred counts were made on each sample. VAMF colonization was determined by the presence of arbuscules and vesicles. Vesicles were counted only if arbuscules could be seen in the same samples, since vesicle-like structures can be present in other types of hyphenated root fungi (Nicolson, 1959).

At 72 weeks (7/7/94), root weights of *Artemisia* were measured in unfertilized plots only (5 plants per plot). This was done to investigate trends discovered in the 10 week sampling.

Plant height, length, and width were assessed for six random plants (per replicate, per species) at 4 weeks (3/16/93), 18 weeks (6/23/93), 31 weeks (9/30/93) and 57 weeks (3/25/94). The means of the six plants were used in

statistical analyses.

The numbers of fruit and seed were assessed for five random *Camissonia* plants (per replicate) at 10 weeks. At 31 weeks the number of flower heads were counted for six *Eriogonum* plants (per replicate). Reproductive output for *Artemisia* was not measured. In the first growing season, only a few of the fertilized, *Artemisia* flowered. In the second growing season, root sampling had greatly reduced the number of *Artemisia* in some plots, making later sampling impossible.

Percent survival was recorded at 68 weeks (6/2/94) for *Artemisia* and *Eriogonum*. Percent survival was calculated from the number of plants that survived out of the number of plants in each plot one month after outplanting. This was done to eliminate plant death due to transplanting that occurred before VAM could possibly develop.

After 18 weeks, the above ground parts of the *Camissonia* had died back, making it impossible to measure plant size. This is a normal pattern for *Camissonia cheiranthifolia*, which normally regenerates from a perennial root stock during the rainy season. All plants in the study were marked with colored markers and it was hoped that the *Camissonia* could be found again during the growing season. Unfortunately, the markers became buried under sand and there were many *Camissonia* recruits into the plots, making it difficult to distinguish the original plants. For these reasons, *Camissonia* was

dropped from the study after the 18 week sample.

Correlations were done on growth measurements (plant height, length and width) and total plant wet weight to assess which growth measurements (or combination of measurements) best predicted plant biomass. The best correlation was found with summing the height, length and width. This "sum" was used in the statistical analyses to analyze plant size.

Assessing VAMF colonization usually involves several levels of sampling; however few studies investigate sampling adequacy. A survey of VAMF literature done by Giovannetti and Mosse (1980) found that large numbers of root intersections (400 - 4000 per plant) are often examined to determine VAMF colonization. In many cases, root samples are rearranged and recounted to achieve the high numbers of counts per sample. This method of sampling can lead to recounting the same root section and therefore problems with the assumption of independent sampling required for statistical analyses. To investigate sampling adequacy in this study, cumulative precision (standard error/mean) was plotted against sample number for all levels of sampling. Precision to mean ratios indicate that 100 counts/per plant were adequate for assessing % colonization (Fig. 3). Although greater precision could be achieved by increasing counts, many root samples did not contain enough material to do more than 100 independent counts. Figure 4a shows that six plants was an adequate sample number for plant size data. Figure 4b shows

that little precision was gained for percent colonization data beyond 5 plants, even though the data still showed high variability.

Statistical Analysis

Two factor, randomized blocked Analyses of Variance (model 1) were done on all colonization, plant size (sum), shoot wet weight, root wet weight, survivorship, and reproductive output data. For all data sets, the assumption of equal variances was checked using Cochran's test (Winer, 1971). In all cases where variances were not equal, log transformations were successful in making them so.

GREENHOUSE STUDY

A greenhouse study was designed to test the effects of VAMF inoculation on *Artemisia* growth in greenhouse conditions. Because preliminary results from the field study showed an inhibition of VAMF colonization in the fertilizer treatments, an additional treatment was added to test the effects of low phosphorus fertilizer on VAMF colonization in greenhouse conditions. *Artemisia* was chosen for this study because it was successfully inoculated with VAMF in the field study and, unlike *Camissonia*, it grew well in containers,

Plant Propagation and Inoculation Methods

Thirty plants of *Artemisia pycnocephala* were grown from seed in Supercell[®] containers in dune sand from an unvegetated blow-out area

adjacent to the field study site. The seed was again provided by Marina State Beach. Natural sand was used rather than sterile sand to avoid soil effects due to autoclaving (Menge and Timmer, 1982). Plants were given liquid fertilizer (Miracle Grow[®], 20-20-20) at 7 weeks. After 12 weeks, plants were transplanted into 625 ml Deepot[®] containers filled with dune sand from an unvegetated blow-out area adjacent to the field study site and subjected to the following treatments:

A. VAMF Inoculation - No Fertilizer: 60 ml of inoculant was added directly below the root zone of the transfers. Inoculant was from the same source as the field study.

B. VAMF Inoculant - Low Phosphate Fertilizer: 60 ml of inoculant was added directly below the root zone of the transfers. In addition, 5 grams of 12-5-8 slow release fertilizer pellets were added to each plant. The fertilizer pellets were obtained from Gromax[®] #2 starter packs.

C. No VAMF Inoculation - No Fertilizer: plants were transferred directly into the sand without inoculant or fertilizer.

Experimental Design and Sampling

Eight plants per treatment were randomly arranged and placed inside of a small, wood frame, plastic greenhouse. To avoid contamination of uninoculated plants, individual plant tubes were separated by one inch in all directions by a wooden frame. This minimized splashing and soil mixing during watering. In

addition, to minimize the contamination from draining water, plastic drain bags were attached to bottoms of the deepot tubes. These bags were designed so that the only exit for draining water was through 35 micron filter cloth, which should have screened out most VAMF spores. At 20 weeks, plants were assessed for % VAMF colonization, and root and shoot wet weights. Staining, clearing and colonization assessment were the same as those used in the field study. Variance to mean ratios showed 100 counts/plant and eight plants were adequate to assess VAMF colonization (Figs. 3, 4b).

Colonization data (three inoculation treatment levels) were statistically analyzed using a non-parametric Kruskal-Wallis test because treatment variances greatly differed. T-tests were run on root, shoot and total wet weights for VAMF inoculated plants and uninoculated plants. No size comparisons were planned for VAMF - Fertilizer treatments. For the t-test data, the assumption of equal variances was checked with an F-test.

RESULTS

FIELD STUDY

VAMF Colonization

After 10 weeks, *Artemisia* and *Camissonia* samples from the Inoculation - No Fertilizer plots showed low levels of VAMF colonization. In *Artemisia* mean VAMF colonization was 6.2%, in *Camissonia* it was 3.7% (Table 1). None of the 10 week *Eriogonum* samples showed signs of VAMF colonization. Little or no colonization was seen in samples from other treatments. Statistical differences were found for both inoculation ($P < 0.001$) and fertilizer treatments ($P < 0.001$); however, a significant interaction between fertilizer and inoculation treatments was found also ($P < 0.001$, Table 1). Although conclusions on the statistical significance of treatment effects are not valid when interaction exists (Underwood, 1981), examination of the data show a clear trend: VAMF colonization was increased by inoculation and inhibited by fertilizer treatments in both *Artemisia* and *Camissonia* (Table 1).

Colonization data from 61 week *Artemisia* samples show the same trends as 10 week samples. The mean percent colonization for the Inoculation - No Fertilizer treatments was higher (mean = 21.2%) in the 61 week samples (Table 2). Unlike the 10 week samples, the No Inoculation - No Fertilizer treatments showed low levels of colonization at 61 weeks (mean = 1.3%). Statistical differences were found for both VAMF inoculation ($P < 0.001$) and fertilizer treatments

($P < 0.001$). Again, there was interaction between fertilizer and inoculation treatments ($P < 0.001$, Table 2).

Seventy-six week *Eriogonum* samples from the Inoculation - No Fertilizer treatments showed low and variable levels of colonization (mean = 7.6, S.E. = 3.6, Table 2). VAMF inoculation significantly increased colonization; however, fertilizer did not have a significant effect (Table 2). There were significant interactions (Table 2). These interactions were not ordered as colonization decreased with fertilizer in inoculated treatments, but increased with fertilizer in uninoculated treatments.

Plant Size

At four weeks, there were no significant differences in plant size (height + length + width) associated with VAMF inoculation for any species (Table 3).

At 18 weeks, a trend of increased plant size in VAMF inoculation treatments was marginally significant for *Artemisia* ($P = .066$, Table 4). This difference was seen only in unfertilized plots. VAMF inoculation did not affect growth in *Eriogonum* and *Camissonia* at 18 weeks (Table 4).

At 31 weeks, *Artemisia* plants in the VAMF inoculated treatments were significantly larger than uninoculated plants in the unfertilized plots ($P = .035$, Table 5). Differences between means were seen only in unfertilized plots. No inoculation treatment effects were seen in *Eriogonum* at 31 weeks (Table 5).

At 57 weeks, no inoculation effects were seen in either *Artemisia* or *Eriogonum* (Table 6).

For all sampling dates, plant size was significantly larger in fertilized plots for all species (Tables 3-6). There were no statistically significant interactions between fertilizer and VAMF inoculation for plant size data on any sample date (Tables 3-6). However, differences between inoculation treatments in *Artemisia* were only seen in unfertilized plots (Fig. 5).

Plant Weight

There was no significant difference in shoot wet weight from inoculated and non-inoculated treatments for any species at 10 weeks (Table 7). Fertilized plots showed significantly higher shoot wet weights for all species, and no significant interactions were found (Tables 7).

Fertilized plots showed significantly higher root wet weights for all species at 10 weeks (Tables 8, 9). The *Eriogonum* roots in block C were significantly larger than those of other blocks ($P = .012$, Table 8). Results from a two-way blocked ANOVA showed no significant difference in root wet weight from inoculated and non-inoculated treatments for any species at 10 weeks (Table 9). However, there was an obvious trend of larger root wet weights in inoculated than uninoculated *Artemisia* plants in the unfertilized plots (Fig. 6). Although no significant interactions were found, I felt the F-value could have been affected by differences in plant response to VAMF inoculation between fertilized and unfertilized

treatments. For this reason a one-factor, blocked ANOVA was run on the unfertilized *Artemisia* treatments. The results showed inoculated roots were significantly larger ($P = .045$, Table 9). There was no difference between inoculated and uninoculated *Artemisia* root weights in unfertilized plots at 72 weeks (Table 9).

Reproductive Output

There were no significant differences associated with inoculation treatments in the number of flower + fruits produced by *Camissonia* at 10 weeks, nor were there differences in the number of flower heads produced by *Eriogonum* at 31 weeks (Table 10). However, there were significant increases in the number of reproductive structures in the fertilizer treatments (*Camissonia*: $P = .002$, *Eriogonum*: $P = .007$, Table 10). There were no significant interactions between fertilizer and inoculation treatments.

Survivorship

After 68 weeks, survivorship for *Artemisia* and *Eriogonum* was not effected by either VAMF inoculation or fertilizer treatments (Table 11). Survivorship in *Artemisia* did show a block effect ($P = .025$), with higher survival in block A than in block D.

GREENHOUSE STUDY

The methods used in the greenhouse study were apparently successful as plants in the inoculation treatment had a mean colonization of 7.8%, but there

was no colonization in the uninoculated plants (Table 12). Plants given both inoculate and low phosphate fertilizer showed no colonization. Results from a Krustal-Wallis test show these differences to be significant ($H' = 21.83$, $X^2_{0.05(1)2} = 10.60$, $P(H') < 0.01$). There were no differences between inoculated and uninoculated treatments for either shoot wet weight or root wet weight (shoot: $t = 1.2$, $P(t) = .25$; root: $t = .38$, $P(t) = .71$, $n = 8$).

DISCUSSION

VAMF Colonization

All three species showed VAMF colonization; however, there appeared to be variations between species in colonization levels and time required to become mycorrhizal. *Artemisia* showed a higher percent colonization than *Camissonia* or *Eriogonum*. *Eriogonum* appeared to require a longer period of time for VAMF colonization to become established. It is possible that VAMF colonization in *Eriogonum* occurs later in the growing season than *Artemisia*, causing colonization to be missed in the first sampling. This seems unlikely. *Eriogonum* samples from the second growing season were predominately infected with vesicles, indicating an older infection (Brown and King, 1982). In addition, root samples of *Artemisia* and *Eriogonum* taken from a nearby natural dune on 5/20/94 contained both vesicles and arbuscules, indicating infections of approximately the same age.

By the second growing season, colonization levels in both *Artemisia* and *Eriogonum*, although low, were similar to those found in plants from natural dune areas. At 61 weeks, the average colonization in unfertilized, inoculated *Artemisia* was 21.3% (S.E. = 4.7). This was similar to levels reported by Pickart et al. (1990) for *Artemisia* in northern California (mean = 20%, S.E. = 9.8, n = 3); and levels in *Artemisia* from a natural dune area located 50 meters from the study area (mean = 13.2%, S.E. = 5.76, n = 5). Colonization levels in 76 week unfertilized, inoculated, *Eriogonum* (mean = 7.6%, S.E. = 3.6) were similar

to levels found in plants found in the same nearby natural dune area (mean = 4.6%, S.E. = 3.15, n = 5).

Very little VAMF colonization occurred in uninoculated plots. At 61 weeks, uninoculated *Artemisia* plants showed higher levels of colonization than at ten weeks, but levels were still much lower than the inoculated plants. Uninoculated plants in the greenhouse study showed no colonization. The very low levels of colonization found in uninoculated plots in the field study could have resulted from greenhouse contamination, contamination during outplanting, edge effects caused by contact with roots and fungal hyphae from other treatments, or from very low levels of VAMF propagules in the study site soil. VAMF spores, hyphae, and colonized root fragments can all serve as propagules (Brundrett, 1991). If VAMF propagules were present in the study site soil, they existed at levels far too low to restore VAMF colonization to levels found in natural dune sites during this 17 month study.

Reduction of VAMF propagules in response to disturbance of soil or vegetation has been well documented (Brundrett, 1991). Among the factors that can reduce VAM are intense fire, soil erosion, long fallow periods and cover by non-mycorrhizal vegetation (Brundrett, 1991). The study site had been unvegetated for decades and experienced large amounts of sand movement. It has been the site of intense fires during the oil storage period. At present, one of the dominant species in the few nearby areas of vegetation is *Carpobrotus edulis*

(hottentot fig), a plant in a family that appears to be mostly non-mycorrhizal (Brundrett, 1991). It is not surprising that VAMF colonization was low at this site.

Fertilizer Effects

Fertilizer inhibited VAMF colonization in both *Artemisia* and *Camissonia*. Although fertilizer was not statistically significant in *Eriogonum* colonization, examination of the means shows that only unfertilized, inoculation treatments produced substantial colonization. The statistical analysis for *Eriogonum* was undoubtedly affected by the very low levels of colonization in the fertilized, uninoculated plots. However, these levels were probably too low to be biologically significant.

Reduction of VAMF colonization by high phosphate levels has been reported often (Brundrett, 1991). Levels of fertilizer used in local dune restoration projects can vary both by application rate and formula. For example, a high rate of 500 lbs/acre of 13-13-13 slow release fertilizer was called for in the Marina State Beach Revegetation Plan (1985); while the Asilomar State Beach Revegetation Plan (1986) called for a rate 100 lbs/acre of 17-12-10. The level of fertilizer used in this experiment (190 lbs/acre of 14-14-14) represents a moderate level of fertilization for dune restoration projects. Results from this study clearly show that fertilization, at even this moderate level, can greatly inhibit the development of VAM in dune restoration sites.

It is possible that reducing the phosphate level and/or application rate of fertilizer could alter the inhibitory effects on VAMF colonization. Silvia (1989) found no reduction of VAMF colonization in inoculated sea oats using a low phosphate (18-6-12) slow release fertilizer at rates of 134-401 lbs/acre in a Florida revegetation project. However, results from this greenhouse study show low phosphate fertilizer (12-5-8) can still inhibit VAM development. Comparisons between greenhouse and field study results are confounded by many factors, and it is possible that different results would be obtained using the greenhouse fertilizer regime in field conditions. Also, it is difficult to compare nutrient levels between locations and plant species. Further studies are needed to determine the effects of different fertilizer regimes on VAMF colonization and how environmental factors effect this relationship in dune systems.

Inoculant

The *Ammophila* roots used as inoculant appeared to cause sufficient levels of VAMF inoculation. There are several points to consider in regard to the inoculum used in this study. First, it came from an area 28 km north of the study site. A number of studies have shown different responses to environmental soil factors between geographically isolated VAM fungal clones or species; VAMF may be adapted to local soil conditions (Brundrett, 1991). Even if local inoculum sources are used, the efficacy of native VAMF to colonize and produce host plant responses can be reduced in severely disturbed soil (Stahl et al., 1988). Finally, it

is unknown how well this inoculant approximates the VAM fungal community found in native dune sites. Although there is probably little host species specificity among VAM fungi, changes in the community of VAMF species has been correlated with soil factors and the species composition of the host plant community (Johnson et al., 1992). Since VAMF species differ in their ability to produce host plant effects, differences in the composition of the fungal community could alter VAM efficacy (Brundrett, 1991). Unfortunately, taxonomic studies on VAMF spores were beyond the scope of this project. It is unknown how closely the inoculum used approximates the fungal community found in native dune areas, and how differences in local soil conditions effected the response of those fungi.

Growth Responses

Increases in plant size and root weight in response to VAMF were only seen for *Artemisia* in the field study. Responses were seen only in unfertilized plots and were fairly small: a 27% increase in above ground size at 18 weeks, a 44% increase in above ground size at 31 weeks, and a 48% increase in root weight at 10 weeks. When uninoculated *Artemisia* growth responses are compared in fertilized and unfertilized plots, increases in plant size due to fertilizer were much larger than increases due to VAMF inoculation: a 259% increase in above ground size at 18 weeks, a 285% increase in above ground size at 31 weeks, and a 935% increase in root weight at 10 weeks.

The VAMF induced growth responses in *Artemisia* appear to be short lived; seen first at 10 weeks, but not after 31 weeks. Several authors have suggested that young plants may be more dependant on mycorrhizae than mature perennials (St. John and Coleman, 1982).

Unlike *Artemisia*, *Camissonia* did not show a response to VAMF in either the 10 week root samples or 18 week plant size samples. Since *Camissonia* was not sampled after 18 weeks, it is unknown if growth responses would be seen at a later date. It is possible that the levels of colonization evident at 10 weeks were too low to cause a host plant response.

No growth responses were seen in *Eriogonum* at any sample date. Although *Eriogonum* colonization was low, experimental colonization levels were similar to those in plants from natural dunes. Several studies suggest that early VAMF infection produces greater host plant responses (Smith et al., 1979 ; Black and Tinker, 1979). It is possible that different results would have been obtained if *Eriogonum* colonization had been established more quickly.

No VAMF induced growth responses were seen in the greenhouse studies, even though colonization was well established in the inoculated plants.

Mycorrhizal fungi increase the host plant's ability to obtain nutrients by hyphal absorption of nutrients beyond the nutrient depletion zone (Harley, 1989).

Several studies have shown that small volumes of soil can reduce the effects of VAM in pot studies (Bäåth and Hayman, 1984). Although Sylvia (1989) saw

increased growth in response to VAMF inoculation in sea oats using the same size container as this experiment (625 ml deepot[®] planter), a larger size container may have been a better choice for *Artemisia* plants.

Reproductive Output and Survival

No differences were seen in reproductive output in either *Eriogonum* or *Camissonia*. Reproductive output for *Camissonia* was sampled at 10 weeks. Colonization levels at that time were very low (mean = 3.7%), possibly too low to affect reproduction. Although reproductive output was not analyzed in *Artemisia* the first year, the lack of reproductive plants in any of the unfertilized plots suggests that VAM does not play a large role in reproduction in these plants.

Fertilizer did increase reproductive output in both *Camissonia* and *Eriogonum*. Reproductive *Artemisia* plants were only seen in the fertilized plots in the first year, suggesting that fertilizer may increase reproduction in these plants.

Survivorship was not affected by either VAMF inoculation or fertilizer in either *Artemisia* or *Eriogonum*. These results seem surprising. Many plants were found buried under the sand and little loss to herbivory was observed. Few plants appeared to just die. Most mortality seemed to be related to sand burial. One would expect that larger plant size, especially the size differences of 300 - 900% associated with fertilizer treatments, would be a significant factor in plant survival at this site.

Biological Significance

Harley (1989) proposed that host plant benefits can only be judged in terms of selective advantage. Although both fertilizer, and to a lesser extent, VAMF inoculation produced increases in plant size and weight; it is unclear if these increases were biologically significant to the host plant. Apparently growth differences induced by VAMF and fertilizer did not significantly affect the survivorship of these plants. Increased survivorship would seem to be a selective advantage. Increased reproductive output would also seem to be a selective advantage. There is no evidence from this study that reproductive output was affected by VAMF inoculation, although reproductive output was increased by fertilizer in *Eriogonum* and *Camissonia*. This study investigated a limited number of plant responses in three species over a relatively short period of time. Although it may appear that VAMF inoculation in this study provided little 'benefit' to the plant, many factors including long-term effects, plant competitive interactions, and life history stage differences were not studied.

Restoration Applications

The results of this study support the idea that in disturbed soils VAMF propagules may be limited, and the establishment of VAM slow. The goal of ecological restoration is to return areas disturbed by human impact to their pre-disturbed condition. VAM appear to be a common component in intact dune systems and have the potential, at least for *Artemisia pycnocephala*, to cause

increases in host plant growth. Given the complexity of possible VAM interactions and the lack of knowledge about them, it seems prudent to consider VAM in dune restoration projects.

Many West Coast dune restoration sites are areas potentially devoid of VAMF propagules. Human impact has left many dune areas unvegetated. Other dune restoration areas are almost completely covered by invasive, non-native vegetation such as *Carpobrotus edulis*. If this species, which is able to form vast areas of almost 100% cover, is non-mycorrhizal then VAM establishment may be slow at many dune restoration sites.

Dispersal of VAMF can occur by hyphal contact with uninfected roots; contact between infected and uninfected roots; or movement of spores by wind, water, or animals (Brundrett, 1991). Spore numbers are often poorly correlated with VAM formation and hyphal contact may be the main source of VAMF inoculation (Brundrett, 1991). The proximity of uninoculated plants to native dune areas containing VAMF colonized roots and fungal hyphae is likely to affect the rate VAM development. VAMF inoculation is likely to speed the development of VAM unvegetated dune sites, particularly if they cover a large areas. More research is necessary to determine the length of time for establishment of VAM in disturbed restoration sites and how environmental factors influence this process.

The method used to inoculate plants in the field was inexpensive and

effective. Local roots and sand from nearby dune areas would probably make a more effective inoculant and would eliminate the threat of inadvertent spread of the invasive, non-native *Ammophila*. The potential disruption caused by removing large amounts of root and sand could be mitigated by cultivating the local VAMF in host plants grown in a greenhouse. Silvia (1989) cultivated VAMF inoculant in celery roots. Alternatively, plants could be inoculated in the greenhouse prior to planting. This type of inoculation was successful for *Artemisia* in the greenhouse study. Cultivating VAMF infected plants in greenhouse conditions is potentially more difficult as pot size, nutrient levels, and light levels are critical factors in VAM development.

Finally, the use of fertilizer should be carefully considered when establishment of VAM is desired. Although fertilizer did dramatically increase plant size and reproductive output, even the moderate levels of fertilizer used in this study were capable of inhibiting the development of VAM.

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Table 1. Means and standard errors (n = 4), and results from two factor blocked ANOVA's for percent VAMF colonization in 10 week *Artemisia* and *Camissonia* samples.

	VAMF Fertil.		No VAMF Fertil.		VAMF No Fertil.		No VAMF No Fertil.	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
<i>Artemisia</i>	0.4	(0.2)	0.1	(0.1)	6.3	(0.2)	0.0	(0.0)
<i>Camissonia</i>	0.6	(0.3)	0.0	(0.0)	3.7	(0.4)	0.4	(0.2)

Artemisia

Source:	SS	DF	MS	F	P
Block	0.04	3	0.01	0.10	0.960
Fertilizer	33.96	1	33.96	259.17	<0.001
VAMF	43.20	1	43.20	329.67	<0.001
VAMF x Fertil.	35.14	1	35.14	268.14	<0.001
Error	1.18	9	0.13		

Camissonia

Source:	SS	DF	MS	F	P
Block	1.31	3	0.44	1.60	0.258
Fertilizer	11.39	1	11.39	41.58	<0.001
VAMF	16.61	1	16.61	60.61	<0.001
VAMF x Fertil.	7.16	1	7.16	26.12	0.001
Error	2.47	9	0.27		

Table 2. Means and standard errors (n = 4), and results for two factor ANOVA's for percent VAMF colonization in 61 week *Artemisia* samples and 76 week *Eriogonum* samples.

	VAMF Fertil.		No VAMF Fertil.		VAMF No Fertil.		No VAMF No Fertil.	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
<i>Artemisia</i>	0.0	(0.0)	0.7	(0.7)	21.3	(4.7)	1.3	(0.4)
<i>Eriogonum</i>	1.3	(0.3)	0.4	(0.4)	7.6	(3.6)	0.0	(0.0)

Artemisia

Data were transformed - log(x + 1)

Source:	SS	DF	MS	F	P
Block	0.215	3	0.072	2.804	0.101
Fertilizer	2.250	1	2.250	88.043	<0.001
VAMF	1.323	1	1.323	51.750	<0.001
VAMF x Fertil.	0.723	1	0.723	28.272	<0.001
Error	0.230	9	0.026		

Eriogonum

Data were transformed - log(x + 1)

Source:	SS	DF	MS	F	P
Block	0.370	3	0.123	1.267	0.343
Fertilizer	0.232	1	0.232	2.384	0.156
VAMF	0.759	1	0.759	7.787	0.021
VAMF x Fertil.	0.452	1	0.452	4.635	0.060
Error	0.877	9	0.097		

Table 3. Means and standard errors (n = 4), and results from two factor blocked ANOVA's for plant size data in 4 week *Artemisia*, *Eriogonum* and *Camissonia* samples.

	VAMF Fertil.		No VAMF Fertil.		VAMF No Fertil.		No VAMF No Fertil.	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
<i>Artemisia</i>	26.4	(1.4)	25.0	(1.2)	19.9	(2.6)	18.6	(1.0)
<i>Eriogonum</i>	17.2	(0.8)	19.3	(0.4)	15.8	(1.2)	16.1	(0.9)
<i>Camissonia</i>	21.2	(1.1)	20.7	(1.4)	16.3	(1.7)	17.7	(1.4)

Artemisia

Source:	SS	DF	MS	F	P
Block	8.89	3	2.96	0.22	0.880
Fertilizer	163.20	1	163.20	12.11	0.007
VAMF	7.43	1	7.43	0.55	0.477
VAMF x Fertil.	0.01	1	0.01	0.00	0.984
Error	121.30	9	13.48		

Eriogonum

Source:	SS	DF	MS	F	P
Block	12.35	3	4.12	1.40	0.305
Fertilizer	21.39	1	21.39	7.29	0.024
VAMF	5.88	1	5.88	2.00	0.191
VAMF x Fertil.	3.15	1	3.15	1.07	0.327
Error	26.42	9	2.94		

Camissonia

Source:	SS	DF	MS	F	P
Block	48.34	3	16.11	2.95	0.091
Fertilizer	62.41	1	62.41	11.42	0.008
VAMF	0.72	1	0.72	0.13	0.725
VAMF x Fertil.	3.42	1	3.42	0.63	0.449
Error	49.18	9	5.46		

Table 4. Means and standard errors (n = 4), and results from two factor blocked ANOVA's for plant size data for 18 week *Artemisia*, *Eriogonum* and *Camissonia* samples.

	VAMF Fertil.		No VAMF Fertil.		VAMF No Fertil.		No VAMF No Fertil.	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
<i>Artemisia</i>	46.4	(5.5)	43.1	(3.0)	21.1	(1.1)	16.6	(0.5)
<i>Eriogonum</i>	52.9	(6.3)	56.7	(4.9)	28.0	(1.5)	23.8	(2.2)
<i>Camissonia</i>	78.5	(11.8)	74.7	(15.0)	31.2	(2.7)	27.5	(2.6)

Artemisia

Data were transformed - log (x)

Source:	SS	DF	MS	F	P
Block	0.015	3	0.005	1.354	0.317
Fertilizer	0.555	1	0.555	155.252	<0.001
VAMF	0.016	1	0.016	4.371	0.066
VAMF x Fertil.	0.006	1	0.006	1.573	0.241
Error	0.032	9	0.004		

Eriogonum

Source:	SS	DF	MS	F	P
Block	366.73	3	122.24	2.34	0.142
Fertilizer	3346.62	1	3346.62	63.94	<0.001
VAMF	0.16	1	0.16	0.00	0.957
VAMF x Fertil.	64.80	1	64.80	1.24	0.295
Error	471.07	9	52.34		

Camissonia

Source:	SS	DF	MS	F	P
Block	438.43	3	146.14	0.32	0.811
Fertilizer	8911.36	1	8911.36	19.49	0.002
VAMF	57.00	1	57.00	0.13	0.732
VAMF x Fertil.	0.00	1	0.00	0.00	0.998
Error	4114.87	9	457.21		

Table 5. Means and standard errors (n = 4), and results form two factor blocked ANOVA's for plant size data for 31 week *Artemisia* and *Eriogonum* samples.

	VAMF Fertil.		No VAMF Fertil.		VAMF No Fertil.		No VAMF No Fertil.	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
<i>Artemisia</i>	64.7	(10.5)	54.2	(6.3)	27.4	(1.4)	19.0	(1.0)
<i>Eriogonum</i>	66.6	(9.3)	66.4	(7.2)	39.9	(4.4)	32.0	(5.3)

Artemisia

Data were transformed - log (x)

Source:	SS	DF	MS	F	P
Block	0.015	3	0.005	0.562	0.653
Fertilizer	0.652	1	0.652	73.719	<0.001
VAMF	0.054	1	0.054	6.111	0.035
VAMF x Fertil.	0.007	1	0.007	0.769	0.403
Error	0.080	9	0.009		

Eriogonum

Source:	SS	DF	MS	F	P
Block	813.56	3	271.19	1.73	0.230
Fertilizer	3727.10	1	3727.10	23.78	0.001
VAMF	64.80	1	64.80	0.41	0.536
VAMF x Fertil.	58.52	1	58.52	0.37	0.556
Error	1412.01	9	156.89		

Table 6. Means and standard errors (n = 4), and results from two factor blocked ANOVA's for plant size data for 57 week *Artemisia* and *Eriogonum* samples.

	VAMF Fertil.	No VAMF Fertil.	VAMF No Fertil.	No VAMF No Fertil.
	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)	Mean (S.E.)
<i>Artemisia</i>	83.9 (4.4)	90.4 (8.0)	38.9 (5.4)	35.6 (2.8)
<i>Eriogonum</i>	60.9 (1.4)	70.5 (1.9)	39.9 (4.5)	36.3 (5.4)

Artemisia

Source:	SS	DF	MS	F	P
Block	25.46	3	8.49	0.05	0.983
Fertilizer	9960.04	1	9960.04	63.16	<0.001
VAMF	9.92	1	9.92	0.06	0.808
VAMF x Fertil.	96.04	1	96.04	0.61	0.455
Error	1419.31	9	157.70		

Eriogonum

Source:	SS	DF	MS	F	P
Block	230.79	3	76.93	1.62	0.252
Fertilizer	3049.8	1	3049.8	64.39	<0.001
VAMF	35.11	1	35.11	0.74	0.412
VAMF x Fertil.	174.9	1	174.9	3.69	0.087
Error	426.29	9	47.37		

Table 7. Means and standard errors (n = 4), and results of two factor blocked ANOVA's for shoot wet weight for 10 week *Artemisia*, *Eriogonum* and *Camissonia* samples.

	VAMF Fertil.		No VAMF Fertil.		VAMF No Fertil.		No VAMF No Fertil.	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
<i>Artemisia</i>	14.4	(2.3)	15.9	(2.1)	1.8	(0.3)	1.7	(0.3)
<i>Eriogonum</i>	11.3	(2.7)	15.4	(2.9)	2.2	(0.5)	1.9	(0.2)
<i>Camissonia</i>	34.8	(12.3)	42.7	(13.4)	2.9	(0.3)	2.9	(0.3)

Artemisia

Source:	SS	DF	MS	F	P
Block	44.02	3	14.67	1.80	0.218
Fertilizer	718.24	1	718.24	87.86	<0.001
VAMF	1.96	1	1.96	0.24	0.636
VAMF x Fertil.	3.06	1	3.06	0.38	0.556
Error	73.57	9	8.18		

Eriogonum

Source:	SS	DF	MS	F	P
Block	56.59	3	18.86	1.21	0.361
Fertilizer	511.89	1	511.89	32.84	<0.001
VAMF	15.02	1	15.02	0.96	0.352
VAMF x Fertil.	19.14	1	19.14	1.23	0.297
Error	140.29	9	15.59		

Camissonia

Source:	SS	DF	MS	F	P
Block	539.33	3	179.78	0.47	0.709
Fertilizer	5137.31	1	5137.31	13.48	0.005
VAMF	63.60	1	63.60	0.17	0.692
VAMF x Fertil.	62.02	1	62.02	0.16	0.696
Error	3428.85	9	380.98		

Table 8. Means and standard errors (n = 4), and results from two factor blocked ANOVA's for root wet weights for 10 week *Eriogonum* and *Camissonia* samples.

	VAMF Fertil.		No VAMF Fertil.		VAMF No Fertil.		No VAMF No Fertil.	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
<i>Eriogonum</i>	5.4	(0.6)	6.5	(1.7)	3.9	(1.0)	2.9	(0.6)
<i>Camissonia</i>	5.4	(1.4)	5.6	(1.0)	1.7	(0.3)	1.6	(0.4)

Eriogonum

Source:	SS	DF	MS	F	P
Block	39.01	3	13.00	6.61	0.012
Fertilizer	25.76	1	25.76	13.09	0.006
VAMF	0.00	1	0.00	0.00	0.986
VAMF x Fertil.	4.31	1	4.31	21.88	0.173
Error	17.71	9	1.97		

Camissonia

Source:	SS	DF	MS	F	P
Block	1.35	3	0.45	0.11	0.952
Fertilizer	59.68	1	59.68	14.48	0.004
VAMF	0.03	1	0.03	0.01	0.933
VAMF x Fertil.	0.08	1	0.08	0.02	0.895
Error	37.09	9	4.12		

Table 9. Means and standard errors (n = 4), and ANOVA results for root wet weights in 10 and 72 week *Artemisia* samples. Both two factor and one factor ANOVA's were run on 10 week samples.

	VAMF Fertil.		No VAMF Fertil.		VAMF No Fertil.		No VAMF No Fertil.	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
10 weeks	7.7	(0.9)	6.4	(1.3)	3.1	(0.3)	2.1	(0.2)
72 weeks					8.1	(1.7)	8.7	(1.4)

10 week - Two Factor ANOVA

Source:	SS	DF	MS	F	P
Block	11.19	3	3.73	1.61	0.254
Fertilizer	78.32	1	78.32	33.87	0.000
VAMF	5.29	1	5.29	2.29	0.162
VAMF x Fertil.	0.16	1	0.16	0.07	0.798
Error	20.81	9	2.31		

10 week - One Factor ANOVA

Source:	SS	DF	MS	F	P
Block	0.77	3	0.26	1.52	0.371
VAMF	1.81	1	1.81	10.72	0.047
Error	0.51	3	0.17		

72 Week - One Factor ANOVA

Source:	SS	DF	MS	F	P
Block	6.69	3	2.23	0.14	0.932
VAMF	0.55	1	0.55	0.03	0.866
Error	49.04	9	16.35		

Table 10. Means and standard errors (n = 4), and results from two factor blocked ANOVA results for reproductive output in 31 week *Eriogonum* and 10 week *Camissonia* samples.

	VAMF Fertil.		No VAMF Fertil.		VAMF No Fertil.		No VAMF No Fertil.	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
<i>Eriogonum</i>	29.7	(13.7)	27.0	(9.3)	2.5	(1.0)	1.0	(0.8)
<i>Camissonia</i>	75.4	(20.1)	88.7	(21.8)	10.8	(1.7)	10.0	(1.6)

Number of Flower Heads - *Eriogonum*

Source:	SS	DF	MS	F	P
Block	1131.86	3	377.29	0.36	0.785
Fertilizer	20506.24	1	20506.24	19.42	0.002
VAMF	156.25	1	156.25	0.15	0.709
VAMF x Fertil.	198.81	1	198.81	0.15	0.675
Error	9501.96	9	1055.77	0.19	

Number of Fruit and Flowers - *Camissonia*

Source:	SS	DF	MS	F	P
Block	1.69	3	1.69	0.01	0.934
Fertilizer	1194.56	1	398.19	1.70	0.007
VAMF	2830.24	1	2830.24	12.08	0.236
VAMF x Fertil.	17.64	1	17.64	0.08	0.790
Error	2108.05	9	234.23		

Table 11. Means and standard errors (n = 4), and results from two factor blocked ANOVA's for survivorship at 68 weeks for *Artemisia* and *Eriogonum*.

	VAMF Fertil.		No VAMF Fertil.		VAMF No Fertil.		No VAMF No Fertil.	
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)
<i>Artemisia</i>	87.5	(5.2)	87.5	(3.7)	90.8	(1.9)	90.0	(2.0)
<i>Eriogonum</i>	83.0	(4.2)	94.5	(2.2)	88.3	(6.3)	76.8	(9.2)

Survivorship - *Artemisia*

Source:	SS	DF	MS	F	P
Block	362.19	3	120.73	5.11	0.025
Fertilizer	33.06	1	33.06	1.40	0.267
VAMF	0.56	1	0.56	0.02	0.881
VAMF x Fertil.	0.56	1	0.56	0.02	0.811
Error	212.56	9	23.62		

Survivorship - *Eriogonum*

Source:	SS	DF	MS	F	P
Block	294.75	3	98.25	0.60	0.632
Fertilizer	156.25	1	156.25	0.95	0.355
VAMF	0.00	1	0.00	0.00	<1.000
VAMF x Fertil.	529.00	1	529.00	3.22	0.106
Error	1477.75	9	164.19		

Table 12. Means and standard errors (n = 8) for greenhouse studies on the effects of VAMF inoculation and fertilizer on *Artemisia*.

	VAMF No Fertilizer		No VAMF No Fertilizer		VAMF Fertilizer	
	Mean	(S.E.)	Mean	(S.E)	Mean	(S.E.)
Colonization	7.88	(1.82)	0.00	(0.00)	0.00	(0.00)
Shoot Weight	1.44	(0.12)	1.64	(0.11)		
Root Weight	4.34	(0.28)	4.53	(0.41)		

Figure 1. Location of the field study site: Monterey State Beach, Monterey, California.

Figure 2. Layout for experimental blocks used in field study.
Not drawn to scale.

Monterey Bay

Figure 3. Mean precision (stand error/mean) of percent colonization data in response to the number of counts made for greenhouse and field studies. Means were calculated from three randomly selected plants.

Figure 4. Relationship between precision (standard error/mean) and sample size.
A. Precision of plant size data in response to number of plants for field study.
B. Precision of colonization data in response to number of plants for both field and greenhouse studies.

Figure 5. Mean plant size (height + length + width) for *Artemisia* over time. Error Bars represent standard error (n = 4).

Figure 6. Mean root weight for 10 week *Artemisia* (n = 5). Error bars represent standard error.