

Slender-horned Spineflower (*Dodecahema leptoceras*)
Microhabitat Characterization of Mycorrhizal Associations

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ABSTRACT

The Slender-horned Spineflower (*Dodecahema leptoceras*) is a federally and state listed endangered annual plant. This study hypothesized that Spineflower is excluded from adjacent habitat into current microhabitat by soil-born antagonistic fungi in the form of arbuscular-mycorrhizal fungi, total fungal community, or pathogenic fungi. Spineflower is not expected to form mycorrhizae based on its classification in the plant family Polygonaceae in which annuals are typically nonmycorrhizal. Spineflower was grown in the greenhouse with *Glomus clarum* to determine its mycorrhizal status. Occupied habitat was compared to adjacent, suitable but unoccupied habitat for AM fungi and total fungal hyphal density. Root infection and spore counts were used to measure the presence of AM fungi. A europium-based stain was used to determine total fungal hyphal density. Potentially pathogenic fungi were cultured from roots of Spineflower and grasses (*Bromus* sp.) from unoccupied habitat. Soil moisture was characterized for suitability to pathogens. Spineflower was found to form mycorrhizae, although the relationship was likely not mutualistic. Hyphae and vesicles were found, and no autofluorescing (a response to unwelcome, invasive fungi) was observed in pot culture roots. Conversely, no arbuscules were found and root systems were substantial. No differences were found between occupied and unoccupied habitat for any of the response variables. Results suggested that AM and pathogenic fungi were not the limiting factor for Spineflower in adjacent suitable but unoccupied habitat. Spineflower's association with AM fungi was possibly commensal, where the fungus acquired carbon as evidenced by vesicles, and the plant showed no cost or gain.

BACKGROUND

The Slender-horned Spineflower (*Dodecahema leptoceras*) (Figure 1) is a federally and state listed endangered plant species. It is listed not only because of habitat loss to development, but also because it exhibits rarity, a highly patchy population distribution, and narrow habitat range. It is a small-statured annual typically found in Alluvial Fan Sage Scrub habitat with populations that are spatially disjunct from one another (Allen 1996) (Figure 2). Numerous populations have become extirpated while others appear barely able to maintain their current levels (Allen 1996). Spineflower's ability to establish new individuals or populations outside of its currently occupied microhabitat is considered to be vital to its survival and recovery. Preservation of biodiversity and natural resources is a goal of listing species as endangered.

The Spineflower normally occurs on flood deposited alluvium, between 200 m and 700 m in elevation, in drainage systems of adjacent foothills to the Transverse and Peninsular Ranges of Southern California (Hickman 1993). It grows in regions with an annual precipitation range of 36-44 cm, and mean annual temperature of 18°C (Allen 1996).

The objective of our research was to explore the possibility that soil fungi influence Spineflower habitat selection of relatively open patches of soil. Although Spineflower grows with native and non-native grasses and forbs, as well as cryptogamic crusts, it is seldom found among dense vegetation. Antagonistic fungi may be a limiting factor excluding Spineflower from suitable habitat adjacent to its current microhabitat. In theory, natural selection excludes species from habitats for which they are not suited. Spineflower may simply be excluded from all but a very narrow suitable habitat where it may avoid antagonists. The better understood the mechanisms by which Spineflower chooses its habitat, the more likely new populations could be successfully established in adjacent suitable habitat or restored to historical locations.

INTRODUCTION

An often neglected aspect of characterizing habitat or plant communities is the below ground biota. Possibly one of the more important biological interactions governing plant community structure is that of a mycorrhiza, the symbiosis between a plant and mycorrhizal fungus. Typically, this relationship is described as mutualistic, where plant and fungus exchange resources, benefiting both symbionts. This mutualism provides the plant increased access to water and nutrients in exchange for providing basic carbon compounds to the fungus.

Mycorrhizal associations are generally considered to fall into two broad groups: (1) the ectomycorrhizas of woody Angiosperms and Gymnosperms, characterized by a mycelial sheath covering the surface of short lateral roots, and (2) the endomycorrhizas, characterized by internal root mycelium growth and internal cellular fungal proliferation. Among these are several groups of which the most common one by far is the Zygomycetes found often in shrubs, perennials, and forbs of most (> 80%) terrestrial plant taxa (Gianinazzi-Pearson 1996). This group is called arbuscular-mycorrhizal (AM) fungi because of their ability to differentiate hyphae into internal cellular haustoria, known as arbuscules (Gianinazzi-Pearson 1996). These structures establish a large surface area of contact with plant cell protoplasts (without penetrating the plasma membrane) for they are highly divided. This interface is considered to function as the site of resource exchange between the plant and fungus. AM fungi also are often characterized by carbon storage structures called vesicles, hence the name vesicular-arbuscular mycorrhizal (VAM) fungi seen in earlier literature.

The typical portrayal of mycorrhizal fungi has been one of benevolent symbiont because of the mutual exchange model initially employed to describe their symbioses with plants. However, the degree of exchange between plant and fungus is considered to be unique for each plant species-fungal species combination (Francis and Read 1995). Francis and Read (1995) provide a model depicting a continuum to describe these symbioses with mutualism at the positive end and competition at the negative end. Each mycorrhiza falls

within the two extremes. Hence, AM fungi may at times act as antagonists, for example toward a nonmycotroph (a plant that does not form mutualistic associations with mycorrhizal fungi) (Allen et al. 1989). This model describes a differential plant response toward AM fungi.

The reported mycorrhizal status of plant taxa suggests that the Slender-horned Spineflower is a nonmycotroph. It is a member of the family Polygonaceae, in which annuals are typically nonmycotrophs (Tester et al. 1987; Johnson 1998). Coastal Sage Scrub, a plant community of which Alluvial Fan Sage Scrub is a type, is considered mycorrhizal with most member species forming mycorrhizae, specifically with AM fungi (Allen 1991). Such a system could be antagonistic toward a nonmycotroph, and might limit it to areas where mycorrhizae are less dense or not present at all (Allen and Allen 1990).

Nonmycotrophs have been shown to play a role in the early seral stages of succession, establishing populations following a disturbance, when mycorrhizal inoculum has been found to be reduced (Allen and Allen 1992). Competition from mycotrophs (plants that do form mutualistic associations with mycorrhizal fungi) replaces them as succession advances (Allen and Allen 1984, 1990; Johnson 1998). Since Spineflower habitat may be less exposed to disturbance with current flood control in place to protect development and agriculture, some small populations presently in decline may be in such a state because of succession without interruption by disturbance. Pathogenic fungi also have been hypothesized to exclude plants from habitat by compromising fecundity, growth, and survival, or by unbalancing competitive interactions (Van der Putten and Peters 1997). Spineflower may only survive where it is dispersed to sites free of AM or pathogenic fungi.

In this research we tested the hypothesis that Spineflower is a nonmycotroph persisting in a microhabitat lacking (or with low densities of) AM and pathogenic fungi. We further tested this hypothesis by comparing adjacent suitable but unoccupied habitat as defined by Dr. Edith Allen (1996) for the presence of AM and pathogenic fungi which in turn

may exclude Spineflower from these adjacent habitats. We also tested the subhypotheses: a) water relations in both occupied and unoccupied Spineflower habitat support soil-borne pathogens, and b) total soil fungal hyphae may be less dense in Spineflower microhabitat than in unoccupied habitat, reflecting the expected absence or low density of AM fungi in Spineflower microhabitat.

METHODS

Research sites were four distinct geographic locations (Figure 3). These included Bee Canyon Creek (Los Angeles county), Santa Ana River (San Bernardino county), San Jacinto River (Riverside county), and Bautista Creek (Riverside county). A fifth location, Santa Ana River, was sampled for plant material in June of 1998 before study sites were chosen. All sites were selected with the assistance and advice of the California Department of Fish and Game. Sampling of soil was conducted monthly from August 1998 to July 1999. Sampling of plant material was conducted in June and July 1998 and again in March, April, and June 1999. This study had both a field evaluation and a greenhouse growth experiment component.

Field Evaluation

Overview of Experimental Design

Experimental Plots. The study compared soil fungi present in both habitats occupied by Spineflower and habitat suitable but unoccupied by Spineflower. Suitable but unoccupied plots were defined as similar in appearance and adjacent to (within 10 m) occupied habitat but without Spineflowers (Allen 1996). These plots were further defined as similar in edaphic factors to occupied habitat (Allen 1996). Although plots used for the current study were not the same ones used in Allen's (1996), her finding of no significant difference in edaphic factors between occupied and adjacent suitable unoccupied habitat was used as a model for the delineation between habitat types. Approximate population boundaries for each site were located using maps provided by CDFG. These boundaries were used as reference points for sampling occupied and unoccupied habitat. Plant roots and soil cores were sampled from occupied and unoccupied habitat at each of the four sites.

Soil Sampling. Soil cores were collected monthly and analyzed for percent moisture and in May and June for spore extractions. Sample size was $n = 5$ to minimize the impact on Spineflower populations.

Non-destructive root sampling. A non-destructive root sampling method was employed to access fungi present in Spineflower roots while at the same time minimizing harm to individual plants. The procedure was previously used on Mount St. Helens to protect the few invading plants (Allen and MacMahon 1988). Root sampling of Spineflower was from five randomly chosen plants at Orange Street and San Jacinto sites in June 1998, at San Jacinto only in July 1998, and at each of the four study sites in March and April of 1999. A hand trowel was used to expose a wall of soil, one-half inch away from the plant's basal rosette and parallel to the Spineflower's primary root. A probe was used to pick soil away from the wall until Spineflower primary roots were visible while the basal rosette was securely held in place by the crusty top layer of soil. A primary root was searched along its upper two inches for secondary roots. When found, up to two secondary roots per plant were plucked using tweezers and stored in a sterile bag. Roots were then transported to the soil lab at San Diego State University and refrigerated until cultured or stained.

Five plants sampled for their roots at the San Jacinto site were flagged in both June 1998 and April 1999. Plants were observed during subsequent visits to determine if the non-destructive sampling method altered development.

Root sampling. In addition to non-destructive sampling, CDFG granted permission (July 1998) to take five Spineflower plants from the San Jacinto site, providing a necessary addition of root material for analysis. Grass roots were also sampled in the same manner -- by using a trowel to dislodge entire plants from the substratum, maintaining intact root and shoot systems.

Fungi Isolation. Fungi present in roots were identified using two methods, culturing fungi from plant roots on Potato Dextrose Agar and direct observation following staining plant roots with Trypan Blue. Staining does not reveal all fungi present, and yet, not all fungi are easily cultured (especially mycorrhizal fungi); therefore, the combination of both approaches increases the likelihood of capturing key fungi present. Cultures were

used to identify pathogenic fungi, such as *Fusarium*, or other pathogens possibly present that could be cultured. Staining was used to observe mycorrhizal fungi, and the pathogenic fungi *Pythium* and *Phytophthora*. In addition, AM spores were viewed for identification to genus.

Spore Counts

AM spores were extracted from soil cores (2 cm x 5 cm) collected from under randomly chosen Spineflowers and grasses (*Bromus sp.*) in occupied habitat, and grasses (*Bromus sp.*) in unoccupied habitat. Five cores were taken for each of the three sampling categories at all four study sites in May and June, 1999. Spores were isolated from soil samples using the sucrose method where approximately 5 g of soil was centrifuged for 10 minutes in 15 mls of 2 M sucrose/calgon (sodium hexa meta phosphate) solution (Allen et al. 1979). Spores were electrostatically attracted to the sucrose and thus collected in the supernatant. The supernatant was then poured through a 20 μ m fine sieve. The inside of the sieve containing the spores was rinsed with deionized water. Water and spores were captured and stored in small sample bottles. Each sample was filtered through a 47 mm grid filter paper with a 0.45 μ m pore size. The filter paper with collected spores was placed in a covered petri dish and refrigerated to prevent spores from germinating until analysis. Each filter paper was observed under a dissecting scope for intact AM spores, which were then mounted in PVLG on a permanent slide for count and identification to genus. Identification was based on novel morphological features among 6 possible genera of AM fungi (Schenck and Perez 1990).

Hyphal Density

Hyphal density created by all soil fungi present in occupied and unoccupied habitat was measured by staining living fungal DNA with a Europium-based fluorescent stain (Morris et al. 1997). Resulting fluorescent fungal material viewed at 400x magnification was measured using imaging analysis computer software. The procedure isolated fungi from additional soil cores (2 cm x 5 cm) collected from under randomly chosen Spineflowers and grasses in unoccupied habitat in June 1999. Five cores were taken for each of the two categories per site. Bacteria were excluded from samples by a filtering system that allowed the small-sized prokaryotes to pass while retaining the eukaryotic fungi. Soil arthropods were left behind in dilutions by micropipetting diluted soil samples onto filters.

Water Relations

Five soil cores were randomly sampled per site from August 1998 through February 1999. Five soil cores were sampled from under randomly chosen Spineflowers in occupied habitat and grasses (*Bromus sp.*) in unoccupied habitat March through July 1999, when Spineflowers were present. September and January were excluded from sampling.

Five grams of soil from each soil core were weighed into small tins and oven dried. After drying, each tin with soil was re-weighed to calculate percent moisture. Percent moisture values were calculated by recording the weight of a small tin, the tin + 5g of soil sample (before drying), and the tin + 5 g of soil sample (after drying) and applying values to the formula:

$$\frac{\text{Wet Wt.} - \text{Dry Wt.}}{\text{Dry Wt.}}$$

In April 1999, additional one-time 50 g soil samples were collected from each site and used to generate soil moisture-energy curves that reflect a relationship between percent moisture and water potential (Brady 1974). Soil water potentials of soils with known

water content were determined using a Decagon Thermocouple Psychrometer SC-10A. The psychrometer measured soil water suction (water potential) in relation to critical values for water content remaining in the soil (percent moisture), governed by soil structure. This water potential is largely the matric potential component, Ψ_m , of water potential, Ψ , arising from the attraction between water and soil particles (Campbell 1977) as these soils had negligible salinity (Allen 1996). Data produced by the psychrometer were fit with a curve and defined by a log function equation generated in the statistics software SYSTAT8 (1998). Percent moisture values measured in the field were plugged into respective equations by site as x-values and generated corresponding y-values, or water potentials.

Pathogenic Fungi Identification

Cultures. Plant root systems were collected from both occupied and unoccupied habitat to identify potential pathogens. Fungi were cultured from seven Spineflower root systems. Two plants were collected from Orange Street in June 1998 and five plants from San Jacinto in July 1998. Five grass (*Bromus* sp.) root systems were collected from unoccupied habitat at all four sites in July 1999. Plant roots brought back to the soils lab at SDSU were surface sterilized in 70% ethanol and cut into 1 cm segments from which five pieces were randomly selected and placed, equally spaced apart, onto Potato Dextrose Agar (PDA) plates. This protocol was applied to each root system. Plates were then incubated at room temperature under ambient light until spore production occurred. Transferring spore-bearing hyphae onto PDA plates using flame-sterilized tweezers soaked in ethanol isolated resulting colonies of non-yeast fungi. Plates were again incubated as described above. Once growth was observed plates were refrigerated to retard growth until identification was made.

Permanent specimen slides of each isolate were made to show reproductive structures by mounting spore-bearing hyphae, transferred as described above, in polyvinyl lactoglycerol (PVLG) on a microscope slide and covering. Observation of specimens was at 200x and 400x magnification. *Illustrated Genera of Imperfect Fungi* was consulted for morphological features of hyphae and spores (Barnett and Hunter 1972).

Root percent infection. Remaining Spineflower root samples were stained with Trypan Blue, cut into 1 cm pieces, mounted in PVLG to create permanent microscope slides, and observed at 200x magnification. Non-mycorrhizal fungi were identified by regularly occurring septae (McGonigle 1990), and quantified by adding the number of observations of fungal hyphae and reproductive structures intersecting the microscope horizontal gridline and then dividing by the total number of observations. Among the non-mycorrhizal potential fungi pathogens were identified by reproductive structures (Domsch et al. 1980; Gisi 1983), and quantified. If non-mycorrhizal fungi were not identified as a pathogen they were considered benign.

Root Percent Infection (AM)

Spineflower root samples were stained with Trypan Blue, cut into 1 cm pieces, mounted in PVLG to create permanent microscope slides, and observed at 200x magnification. Observations were made along the entire length of root pieces in order to maximize data from small samples. Percent infection was calculated by adding the number of observations of fungal hyphae and structures, such as vesicles and arbuscules, intersecting the microscope horizontal gridline, and then dividing by the total number of observations (McGonigle 1990).

Growth Experiment

Pot Cultures

Seed was obtained with the permission of the California Department of Fish and Game from Rancho Santa Ana Botanic Garden in Claremont, California. This seed was obtained from plants raised in a greenhouse in 1991. Seed was also obtained from Nancy Ferguson at University of California, Riverside, who collected from natural habitats during August 1998 under contract with the California Department of Fish and Game. Seeds were cleaned and placed in cold strata (refrigeration at 2°C) for 28 days while contained in large petri dishes lined with continually dampened filter paper. Petri dishes

were kept in a completely dark environment. Upon germination, seeds were removed from the petri dishes and planted in flats of steam sterilized soil consisting of 2 parts perlite, 1 part vermiculite, 1 part peat, and 2 parts sand (Gordon-Reedy 1997). Soil had been steam sterilized for two four-hour periods twenty-four hours apart. Flats were placed in the Soil Ecology and Restoration Group (SERG) greenhouse under an automatic daily watering regime with temperatures set at 26°C by day and 22°C by night.

The first ten two-week old Spineflower seedlings to emerge were transplanted from germination flats into sterilized soil (consisting of 1 part native soil collected from the San Jacinto site and 2 parts sand) inoculated with *Glomus clarum*, an arbuscular-mycorrhizal fungus (provided by INVAM at West Virginia University). Soil was contained in glass pots that allowed observation of root systems. Pots were assembled using two pieces of glass, 12.5 cm x 30.5 cm, joined together with silicon caulking which provided a 1 cm wide space between plates for soil and plants. Each pot received two Spineflower seedlings and was replicated 4 times for a total of 5 experimental pots (Figure 4). The control consisted of 5 identical treatments with blue corn (*Zea mays*), a known mycotroph, grown in place of Spineflower seedlings. All pots were placed in the SERG greenhouse at 22°C by day and 19°C by night, and kept at an angle of approximately 35° to encourage root growth along the glass for observation. Roots were protected from direct light by tin foil wrapped around each pot. Plants and surrounding soil were watered every other day with approximately 50 mls of water. Remaining seedlings to emerge were transplanted into pots containing 1:1:1 soil mixture of sand, potting soil, and vermiculite.

Autofluorescence. Interaction between plant and fungus was observed and characterized by the presence or absence of fungal hyphae and by the presence or absence of autofluorescing root chemical compounds typically produced in defense against unwanted, invasive fungi (Allen et al. 1989). Root systems were observed under blue light at 100x magnification after 10, 15, and 36 days. Roots were read from root tips toward aboveground vegetation since mycorrhiza formation typically occurs behind root tips. Once root systems reached the bottom of the glass pots (50 days) plants were

harvested. Roots were stained and mounted in PVLG on permanent slides to determine percent root infection.

Root percent infection. All root systems were harvested from glass pots and stained with Trypan Blue. Fifty random 1 cm root pieces from each root system were evaluated for presence of mycorrhizal hyphae, vesicles, arbuscules and non-mycorrhizal hyphae. Two random observations per 1 cm segment were observed to yield 100 observations per sample. Percent infection was calculated by adding the number of random observations (200x magnification) of fungal hyphae and structures intersecting the microscope horizontal gridline, and then dividing by the total number of observations (McGonigle et al. 1990).

Cryptogamic Crusts

Identification. Five additional soil samples per site were collected from occupied habitat by filling a hand trowel with crusted soil, keeping cryptogamic soil crusts intact. Crusts were stored in sterile bags and transported to University of California, Riverside for identification. Crusts were placed in petri dishes filled with water and incubated in ambient light to allow non-vascular plants to grow and reproduce.

RESULTS

Field Evaluation

Overview of Experimental Design

Non-destructive root sampling. None of the ten flagged plants died or exhibited any altered development.

Spore Counts

AM spores were present in both occupied and unoccupied habitat. Spore counts resulting from individual soil cores ranged from 0 to 380 per 5g of soil, and showed high variance among treatments (i.e. Cone Camp May 1999 sample: occupied #1 = 14, occupied #2 = 380). Figure 5 shows data summarized into averages per sample category (each bar = average of five samples). Spore counts resulting from Spineflower soils showed no pattern. However, two patterns did appear: a) soil sampled from grassy areas in occupied habitat appeared to have lower spore counts than grassy areas in unoccupied habitat at all sites during both months with the exception of Cone Camp sampling in May 1999, and b) soil sampled from grassy areas in unoccupied habitat appeared to have the greatest spore counts of all sampling categories at all sites in June and at Bee Canyon in May.

Analysis of variance (ANOVA) was performed on log transformed spore count data for May and June. Contrasts were conducted using a Fisher's LSD pairwise comparison. The residuals of log transformed data appeared normally distributed about zero, necessary to meet the normality assumption of the ANOVA (Ott 1993). Data were log transformed because the residuals of the raw data were skewed about zero, and results from an F-max test for homogeneity of variance showed the raw data to be heterogeneous: $F_{\max} 0.05(12,4)$ for $\alpha(a, n-1) = 51.4$, test statistic = 564.4 (Sokal and Rohlf 1995). Figure 5 summarizes raw data with + standard error, which does not accurately reflect variance for standard error assumes normality. See Appendix 1 for range of raw data.

Neither trend in Figure 5 was statistically significant. Results of the ANOVA showed no significant difference between sampling categories at $p = 0.163$ (Figure 6). However, it did show significant difference between sites with Bee Canyon and San Jacinto showing lower spore counts than Bautista Creek and Santa Ana River ($p < 0.004$). Analyses were performed using SYSTAT8 (1998).

Spores were identified as *Glomus sp.* (Schenck and Perez 1990) and shown in Figure 7. Other genera of AM fungi were not found. No suspensor cells or attached vesicles typical of other genera were observed.

Hyphal Density

Relative measurements of active hyphal density (cm/g) were compared between soil samples taken from occupied and unoccupied habitat (Appendix 2). Data were summarized in Figure 8 and showed no trend among sampling categories. Summarized data showed Bautista Creek to have the greatest hyphal density followed by Santa Ana River, San Jacinto, and Bee Canyon, respectively. An ANOVA was performed on data to test for differences between sampling categories. Results of the ANOVA showed no significant difference. Analyses were performed using SYSTAT8 (1998).

Water Relations

Percent moisture data were generated from soil samples taken from all four sites per month (Appendix 3). A unique soil moisture curve was generated for each of the four sites and defined by log equations with the accuracy of R^2 values ranging from 0.997 to 1.000 (Figure 9). Resulting water potential values computed by applying percent moisture values measured in the field to soil moisture curves were averaged by site per month and graphed over a calendar year (Figure 10). Bautista Creek showed the highest values followed by San Jacinto, Santa Ana River, and Bee Canyon, respectively. Water potentials were then compared with corresponding monthly precipitation data from California Data Exchange Commission (CDEC) weather stations (Figure 10). Precipitation data showed late rains in 1999 (over three inches in April), typical of a La

Niña weather pattern. Water potential data for Bautista Creek showed a peak in April 1999, while the other three sites showed peaks in May 1999.

Water potentials rarely reached values as high as -0.15 to -0.3 MPa, water potentials favorable for the groups of pathogenic fungi investigated for this study (Gisi 1983). MPa is the abbreviation for mega Pascals, the resulting expression of units from the van't Hoff equation, $\Psi_s = -RTc_s$ (Taiz and Ziegert 1999). Water potentials favorable for pathogens were observed at San Jacinto and Bautista in August and October 1998. This was not seen again until 30 April 1999, a rainy day, at Bautista and at Santa Ana River in May 1999. Some values appeared very low (i.e. Bee Canyon July 1999 sample: occupied #4 measured $\Psi_m = -98.5$).

Pathogenic Fungi Identification

Cultures. Root material cultured on PDA generated 88 unique isolates of fungi. No plant pathogens were found.

Root percent infection. Percent infection of Spineflower roots by non-mycorrhizal fungi was quantified (Appendix 4). Of these, possible *Phytophthora* was found ranging from 3 to 15 percent infection in six Spineflower primary roots collected from the Orange Street and San Jacinto sites in June 1998. It was identified by reproductive structures (Gisi 1983) shown in Figure 11. A number of non-mycorrhizal hyphae were observed, but not identified because they lacked storage or reproductive structures.

Root Percent Infection (AM)

Percent AM infection of Spineflower roots sampled from the field ranged from 0% to 45% (Appendix 4). Zero percent infection occurred mostly among small samples of root material. AM structures typical of a functioning mycorrhiza were included in quantifying percent infection. Vesicles (Figure 12) were often found. However, no arbuscules, structures considered to be the site of symbiotic resource exchange, were found.

Growth Experiment

Pot Cultures

Seed germination began after approximately 28 days with radicals and/or cotyledons visible. Old seed from Rancho Santa Ana Botanical Garden collected in 1991 germinated more quickly than seed gathered in 1998. Germination was approximately 30% for all seeds. Seedlings needed to be approximately two weeks old to survive transplanting.

Of the remaining plants grown in the greenhouse that were not used in growth experiments thirteen survived to produce flower-stalks. Only three of the thirteen fully matured to produce flowers. No seed was produced.

Pot culture plant roots grew along the inside surface of the glass planters, and were fully visible (Figure 13). Root systems appeared substantial where two plants colonized an entire planter, 12.5 cm x 30.5 cm x 1 cm. Under a dissecting scope extensive hyphae were observed through the glass entering and linking roots in both experimental and control treatments. Hyphae were distinguished from root hairs by a smaller diameter and by branching.

Autofluorescence. No autofluorescing roots were observed through the glass planters under blue light at 200x magnification, signature of root chemical compounds typically produced in defense against unwanted, invasive fungi. Resolution could not be obtained through the glass to observe internal colonization or structures.

Root Percent Infection. Spineflower showed colonization by AM fungi similar to that of a known mycotroph, blue corn (Appendix 5). Spineflower percent infection averaged 69% compared to 63% in blue corn. Of the two often definitive structures of mycorrhizae, only vesicles, the carbon-storage structures, were found. Arbuscules were

not found in either Spineflower or blue corn roots. Seven of the ten Spineflowers survived, three perished. All ten blue corn plants survived.

Data were described in two different ways: (1) by the raw data, and (2) by averaged data per pot to avoid pseudoreplication as two plants were grown per pot. Distributions of both sets of data, where $n = 5$ for both species and $n = 7$ for Spineflower and $n = 10$ for blue corn, were compared (Figure 14). Parametric statistical tests were applied to both sets of data comparing percent infection of Spineflower and blue corn. Data appeared normally distributed. Results of a t-test showed no difference between distributions of percent infection per planter ($n = 5$). To expose any possible differences lost in averages, a t-test was applied to the uneven samples of $n = 7$ and $n = 10$. Results showed no difference.

Cryptogamic Crusts

Identification. The cryptogamic soil crusts were dominated by moss, exhibiting a typically green vegetative mat. No sporulating structures, essential for identification, were expressed.

DISCUSSION

Occupied vs. Unoccupied

Spore counts were a response variable employed to reveal AM fungi presence in occupied and unoccupied habitat. Counts might also have revealed any major differences in AM fungi density and activity between habitat types. The sampling design controlled for differential responses that Spineflower and *Bromus sp.* might have toward AM fungi by also sampling *Bromus sp.* in occupied habitat, hence the three sampling categories: Spineflower, grasses occupied, and grasses unoccupied. Note that sampling was not random across the habitat, but from underneath randomly selected plants. The biology of AM fungi is such that if present in a habitat they will be found concentrated at the base of plants (Allen 1991).

Spore counts were taken from two months of soil samples, May and June 1999, to account for trends regardless of temporal variation. May and June were ideal for spore counts because plant growth is reaching its end and fungal sporulation is at a maximum.

Presence of AM fungi was observed in all sampling categories at all four sites. A subtle trend appeared at three of the four sites in May and at all four sites in June where grasses in occupied habitat showed lower spore counts than grasses in unoccupied habitat. No statistical significance was found for these differences, $p = 0.163$, however this p value is low and suggests that there is an 84% chance that spore densities are in fact different. Further, variance among the data was high with counts ranging from 0 to 380, typical of AM spore counts (St. John and Koske 1988). These results lend little evidence to suggest any differences in AM presence, density or activity between occupied and unoccupied habitat, however if there exists an 84% chance that altering mycorrhizae could affect the survival of this species, the question of difference between occupied and unoccupied habitat should not be dismissed.

Counts may not have statistically differed by sampling category, but they did statistically differ by site. Bee Canyon and San Jacinto showed significantly lower spore counts than the Bautista Creek or Santa Ana River sites. However, these results cannot be related to the number of Spineflower individuals per site. Bee Canyon had only 30 individuals in 1999, the smallest population of all four sites, where the opposite was observed at San Jacinto which had the largest number of individuals in 1999 (upwards of 300-500) with the most vigorous plants (most flowers produced) of all four sites. AM fungi density was not predictive of population size.

Spores were identified as *Glomus sp.* Diversity of mycorrhizal fungi species has been found to be significantly lowered by disturbances, either mammalian or mechanical (Allen 1991). *Glomus sp.* have been found to be the most persistent group under such soil disturbances, characteristic of the study sites (Allen 1991).

Europium staining allowed for the estimation of hyphal density for the soil-born fungal community at large. This parameter was measured in occupied and unoccupied habitat to compare with spore counts and to determine if the soil fungal community differed between habitat types. As with spore counts, resulting densities did not statistically differ by habitat. Unlike spore counts, densities did not statistically differ by site. The fungal community at large appeared to behave differently than AM fungi with only AM fungi statistically differing by site. These results suggest that AM fungi and the fungal community at large play unique ecological roles, reflecting that AM fungi are a unique group.

Water relations data were employed to characterize the soil moisture of Spineflower microhabitat and to determine if resultant soil moisture provided suitable habitat for pathogenic fungi, organisms that could potentially exclude Spineflower from otherwise suitable habitat. September and January were excluded from sampling because characterizing water relations under a Mediterranean climate in semi-arid soils does not require data from each month over a calendar year. Such environments have two seasons, rainy winters and hot dry summers, and thus many months have similar soil

moisture. Characterization of water relations was easily achieved with ten months of data.

This study was not of the scope to identify all soil-born pathogenic fungi present in Spineflower microhabitat. Rather it was designed, primarily, to identify common fungal plant pathogens (of the groups *Fusarium*, *Pythium*, and *Phytophthora*), and secondarily, to identify fungal plant pathogens that could be cultured or stained, as well as identified. The rhizosphere contains an inexplicable variety of fungi, with an equally inexplicable variety of life strategies. However, known fungal plant pathogens accompanied by protocols for isolation and recorded traits that could be keyed out, were searched for in both occupied and unoccupied habitats. It is for these groups of pathogenic fungi that soils with water potentials lower than -0.15 and -0.3 MPa are inhospitable (Gisi 1983). It is not that they cannot exist in these soils, for fungi have very clever survival strategies with dormant life stages, but they cannot persist and thrive without adequate soil moisture.

Some soil Ψ_m values may have appeared exceptionally low (i.e. Bee Canyon July 1999, occupied #4, measured $\Psi_m = -98.5$ MPa) because desorption curves were built on percent moisture data higher than measured in the field. The further field percent moisture values were from the psychrometer settings used to achieve critical water potential values, the less accurate the curves, and hence the more extreme Ψ_m values appeared. However, the psychrometer correlated percent moisture values with water potentials crucial for pathogen success, and therefore, were suitably accurate for critical values. Additionally, curves appeared to match theoretical curves for silt soils (Campbell 1977).

Average soil water potentials occasionally reached relatively high values ideal for plant pathogen success, $\Psi_m = -1.5$ to -3.0 , as seen at San Jacinto and Bautista in August and October 1998, Bautista in April 1999, and Santa Ana River in May 1999. However, predominantly low water potentials throughout all sites persisted throughout the year, suggesting little threat to Spineflower populations from pathogenic fungi.

No significant plant pathogens were cultured from occupied or unoccupied habitat. The only evidence of soil-born plant pathogens was reproductive structures belonging to possible *Phytophthora* that were found infecting several root systems sampled from the field. These structures were most likely sporangiophores, which develop and release zoospores upon maturity (Gisi 1983). *Phytophthora* are virulent pathogens that cause alterations in metabolic activities of the plant. However, they require more soil moisture to flourish and achieve virulence than generally occurs in Spineflower habitat (Gisi 1983). An El Niño weather pattern was present in 1998 bringing considerable rainfall to Southern California's semi-arid environments. This may account for the presence of *Phytophthora* in the June 1998 samples. Further, the rapid shift from high to low water potential values indicates that the soil does not have a high water holding capacity, a property important to pathogens. A lack of water holding capacity is related to low soil organic matter. A lack of pathogens, along with the low water potentials typical of semi-arid soils, suggests that pathogens are probably not a limiting factor preventing Spineflower presence in unoccupied habitat or individual plant survival.

Other non-mycorrhizal hyphae present in Spineflower roots may be dark septate endophytes (DSE), identified by their regular septae and lack of reproductive structures (this group is sterile). These fungi often function in the same way that mycorrhizal fungi function, as symbionts that contribute to a hyphal network that mobilize and move nutrients (Jumpponen and Trappe 1998). DSE or other fungi may have assisted Spineflower in nutrient and water uptake.

Results of analyses comparing occupied and unoccupied habitat lend no evidence to suggest any differences in fungal density or activity between habitat types. Likely, some factor other than fungal presence -- AM, pathogenic, or other -- has a greater effect on Spineflower success, such as soil moisture (Ferguson et al. 1996).

Generally, the differences among sites can be related to trends in moisture. Of the four sites the lowest spore counts (significant), the lowest hyphal density, the fewest number of individuals, and the lowest soil moisture measurements all converged on Bee Canyon.

These results are reflected in Allen's (1996) research, which reported that soils sampled at Bee Canyon had a higher percentage of sand than any other Spineflower location. A higher content of sand diminishes the water holding capacity of soil. Water drains through sand more easily than it drains through clay or silt because of its comparatively large particle size. Further, this site was reported to have had very little rain the winter of 1998-1999 (Mary Meyer of CDFG, personal communication), although the California Data Exchange Commission's (CDEC) closest weather station at Saugus did not provide values that appeared lower than the other sites. It is possible that Saugus had more precipitation than Bee Canyon, and that parameters measured at Bee Canyon may have been the lowest simply because of a lack of adequate soil moisture. This may have been confounded by late precipitation, typical of a La Niña weather pattern reported for the winter and spring of 1999, which may account for fewer numbers of individuals at all study sites in 1999 than in 1998. Water remains a limiting factor for plants and fungi alike in semi-arid soils. AM fungi are obligate biotrophs (Gianinazzi-Pearson 1996) and if plants do not thrive then fungi have limited access to carbon sources, and they in turn do not thrive.

The only fungal pathogen found was possibly a *Phytophthora*, and at a time when soil water potentials were relatively high.

San Jacinto's significantly lower AM spore counts cannot be related to soil moisture in the same way as Bee Canyon because it showed the second highest water potential values. Low spore counts at this site may be accounted for by the high volume of off-road vehicle traffic it incurs. Mechanical disturbance diminishes both diversity and density of AM fungi (Allen 1991).

Also note that Bee Canyon has been reported to support thousands of Spineflowers in recent years. Annuals exhibit a life strategy where the number of individuals per year vary greatly (Pake and Venable 1996). Pake and Venable (1996) suggest that germination of annuals is particularly sensitive to subtle environmental cues. This ensures that conditions will be ideal for successful growth, and prevents poor conditions

from heavily impacting the population. Environmental cues were reported to have little effect on Spineflower germination with the exception of precipitation (Ferguson et al. 1996). If indeed Bee Canyon had poor rainfall in the winter of 1998-1999, Pake and Venable's model would predict few individuals.

Mycorrhizal Status

The mycorrhizal status of the Slender-horned Spineflower was determined by percent root infection measured in the field and by a growth experiment (e.g. the culturing of mycorrhizae) that provided two response variables: a) autofluorescence of plant defense chemicals under blue light and, b) again, percent root infection.

Evidence equivocal in both support of and against our original hypothesis that the Slender-horned Spineflower is a non-mycotroph emerged from our research.

Evidence against non-mycorrhizal status was first, high colonization of Spineflower roots by AM fungi in both the field and in the greenhouse pot cultures: 45% (field) and 68% (pot cultures), similar to 63% (blue corn control, a known mycotroph). Secondly, vesicles, signature carbon-storage structures of mycorrhizae, were observed inside Spineflower root cortical cells, suggesting that fungi were receiving carbon from the plant. Thirdly, no autofluorescence of characteristic plant defense chemicals was observed on roots of either Spineflower or blue corn, a known mycotroph. These results suggest that AM fungi colonized Slender-horned Spineflower roots and formed carbon-storage structures without initiating common plant defenses.

Coupled with reports of a non-mycorrhizal status for annuals among the Polygonaceae, evidence for the non-mycorrhizal status of Spineflower was first, the lack of arbuscules, signature resource exchange structures of a mycorrhiza. These structures are ephemeral; however, with the high percent infection measured in the large pot culture root samples, finding an arbuscule was believed to be likely. Finding no arbuscules in the pot culture

control weakened this evidence slightly, however arbuscules were not found in Spineflower field samples either. Arbuscules have been reported to be present in blue corn, the control. Because arbuscules are ephemeral and difficult to see, the most powerful evidence to support a lack of arbuscules in AM colonized Spineflowers is the lack of arbuscules in Spineflower roots sampled from the field. Secondly, zero percent infection was found in roots sampled from the field, indicative of plant survival without AM fungi. Individuals either never encountered AM fungi or they did not require the benefits of AM symbiosis. Although microhabitat soils appear to lack enough organic material to prevent substantial water holding capacity, they were measured as silty soils (Allen 1996) which hold some nutrients for which mycotrophs may not always need the benefits of symbiosis to acquire. If silty soils contain enough organic matter to provide nutrients, but exhibit poor water holding capacity, then it seems that symbiosis would be necessary for obtaining water. However, Spineflowers grown in glass chambers exhibited substantial root systems, also possibly indicative of no need for AM symbiosis to obtain soil moisture. Some roots quickly grew the 30-cm length of the pots and might have gone further given the opportunity. Spineflowers may be able to reach water sources deeper than what was measured in the top 5 cm of soil for this study. Finally, three Spineflowers died in the pot cultures possibly from either transplantation shock or stress from AM fungi while all of the blue corn survived. These combined results suggest that Spineflower survived in the field without AM fungi symbiosis. Further, there was no evidence of mutualism with a lack of arbuscules, and possible seedling death in the pot cultures. Adult plants appear to tolerate AM fungi.

Mycorrhizae were established between Spineflower and AM fungi. AM fungi benefited as evidenced by the presence of vesicles. However, with the lack of arbuscules and possible seedling death, this symbiosis may be short of mutualistic. Roots without arbuscules and simultaneously with high colonization eliciting no defense chemicals, suggest that mycorrhizae established between Spineflower and AM fungi would probably fall somewhere in the middle of Francis and Read's (1995) model for describing mycorrhizal symbioses. Possibly, as a commensal relationship (0, +) where Spineflower can, in other words, take or leave AM fungi, would describe this symbiosis.

Cryptogamic Crusts

Cryptogamic soil crusts were sampled for identification, only. Identification contributes to answering the question of whether crusts can cause surface soil to be inhospitable to Spineflower seedling establishment. Further study is needed to see what role crusts play. Eldridge and Rosentreter (1999) argue that the morphological groupings of microphytic crusts in arid landscapes provide a crucial monitoring aspect if the goal is characterizing ecosystem properties, but not in determining the biodiversity of a system (e.g. plant community make-up). Cryptogamic soil crusts were identified as mosses, however without reproductive structures further identification was problematic. Crusts are indicative of surface water as mosses have swimming sperm essential to reproduction. Soils in Spineflower habitat have been characterized as slightly hydrophobic (Wood and Wells 1996). The nature of the soil may provide standing or sheeting water necessary for non-vascular plant reproduction.

CONCLUSIONS

- Results suggest that AM fungi (or the soil-born fungal community) are not limiting factors for Spineflower in suitable habitat adjacent to extant populations. Results provide little evidence to suggest a difference in fungal activity between the microhabitats supporting Spineflower and adjacent suitable but unoccupied habitat. However a difference among spore densities of $p = 0.163$ suggests an 84% chance that altering mycorrhizae could affect the survival of this species. The question of difference between occupied and unoccupied habitat should not be dismissed.
- Pathogenic fungi are not likely limiting factors for Spineflower in adjacent suitable habitat. Surface soils are typically too dry to successfully support fungal pathogens.
- Spineflower does not appear to show a strong preference for AM fungi. Mycorrhizae are present, but apparently not very important to nutrient uptake since no arbuscules were observed within roots. Mycorrhizae may not be important for water uptake either since deep and extensive root systems demonstrated by greenhouse-grown Spineflowers may suggest that these plants have deeper roots facilitating water uptake in the field. Additionally, some roots collected from the field were found with zero percent colonization. The likely nature of the symbiosis is not mutualistic, but at best commensal where the fungi receive carbon and the plant shows no effect, cost or gain.
- Spineflowers may also depend on upwelling zones both for water and nutrients. Upwelling is difficult to detect, as it requires an understanding of the hydrology and root depth activity, both difficult parameters to measure in the rocky condition of these locations. However, it may be that the difference between occupied and unoccupied habitat is not a surface feature, but related to unseen depth features. These are the conditions that upstream development, dam building, roads, and compaction are likely to affect. Finally, we have no idea how upwelling would relate to competitive interactions between Spineflower and exotic grasses. Additional research on this would be both valuable and important.

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Figure 1. A Slender-horned Spineflower (*Dodecahema leptoceras*) in bloom. This plant was one of thirteen grown in the Soil Ecology and Restoration Group greenhouse that never produced seed.

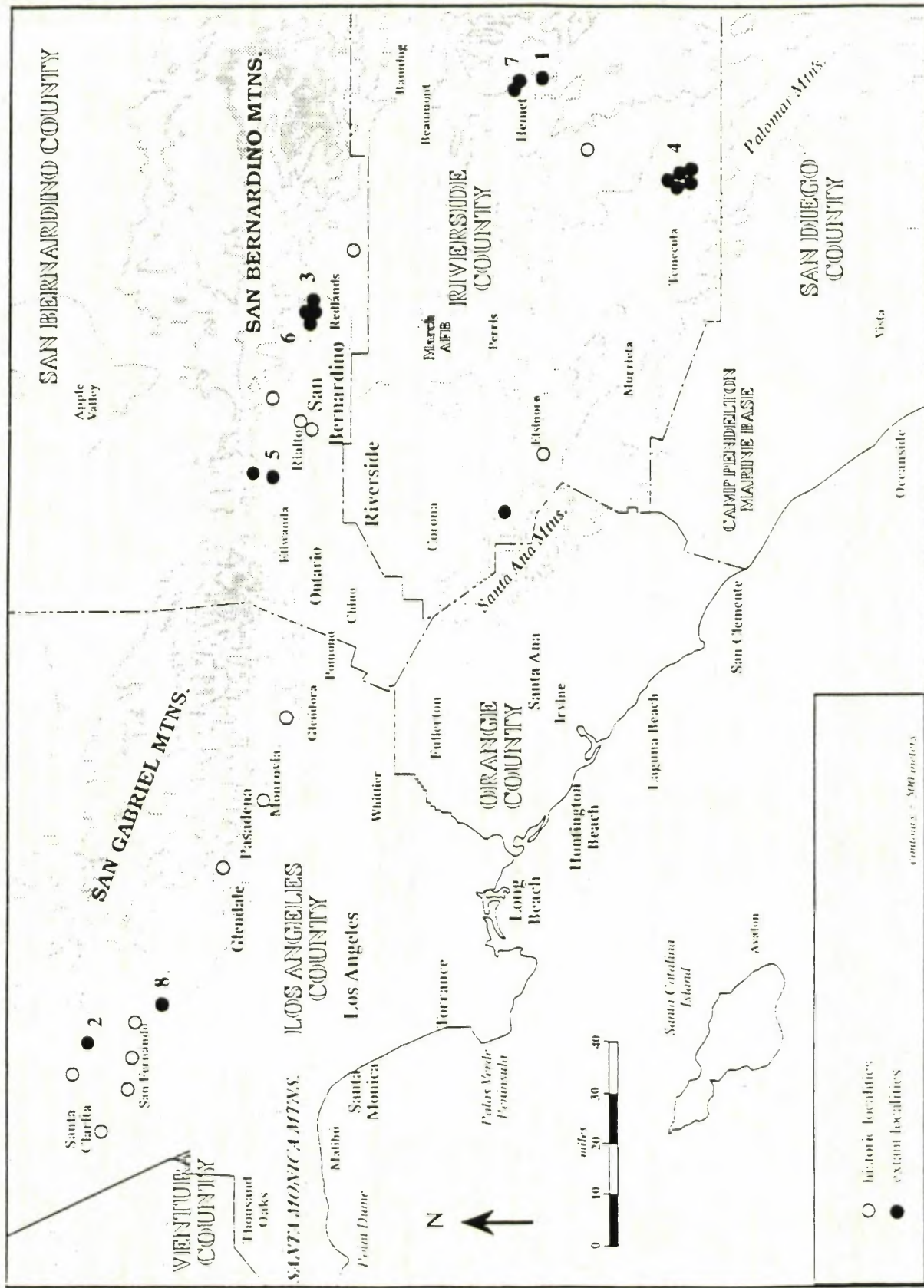


Figure 2: A map showing extant and historical distribution of Spineflower populations (provided by the California Department of Fish and Game): 1) Bautista Creek, 2) Bee Canyon Creek, 3) Cone Camp on the Santa Ana River, 4) Dripping Springs on Arroyo Seco Creek, 5) Lytle Creek, 6) Orange St. site on the Santa Ana River, 7) San Jacinto Wash, 8) Big Tujunga Wash.



Figure 3a: Bee Canyon site, the most northern location of Spineflower populations. This view is north toward Highway 14 in northern Los Angeles county.



Figure 3b. Santa Ana River site characterized by silty shallow depressions at the bottom of a wash (top) and a close up of these depressions with Spineflowers growing in sparsely vegetated microhabitat (bottom).

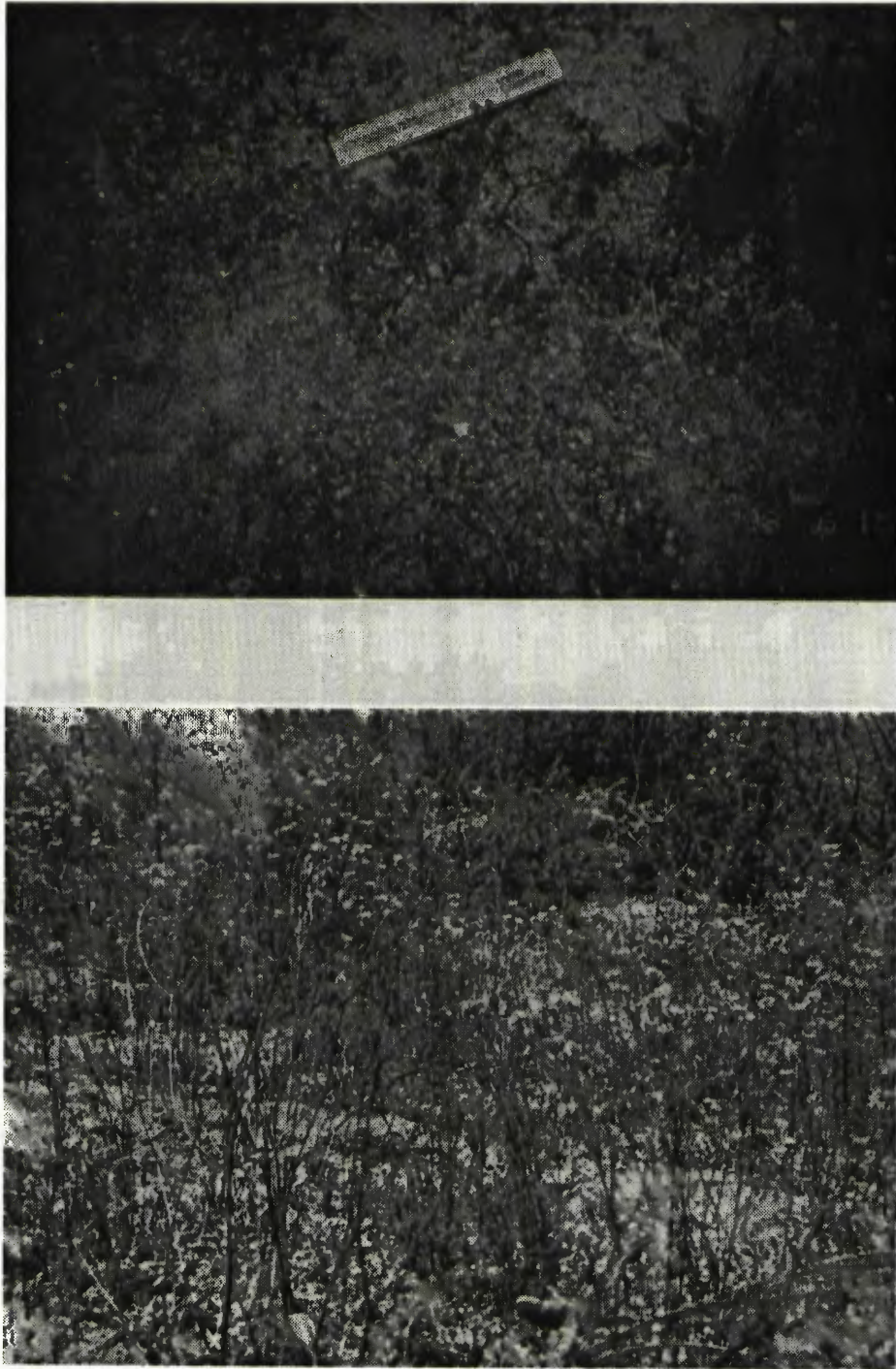


Figure 3c. Spineflowers at San Jacinto site characterized by very large plants (top) and heavy off-road vehicle use as evidenced by numerous trails (bottom).



Figure 3d. Spineflowers at Bautista Creek, with plants appearing among grasses (top) and on typical sparsely vegetated patches of soil (bottom).

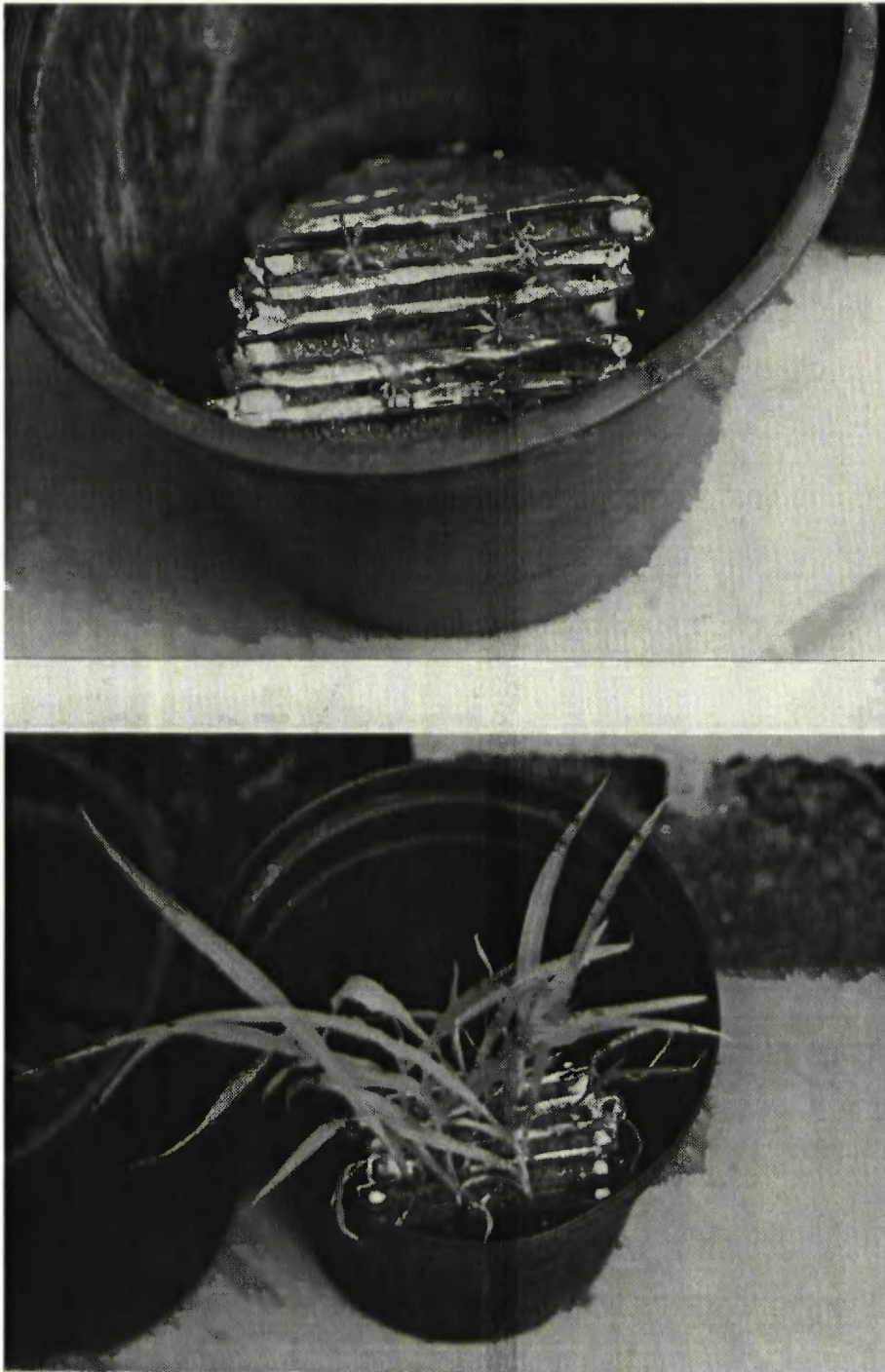
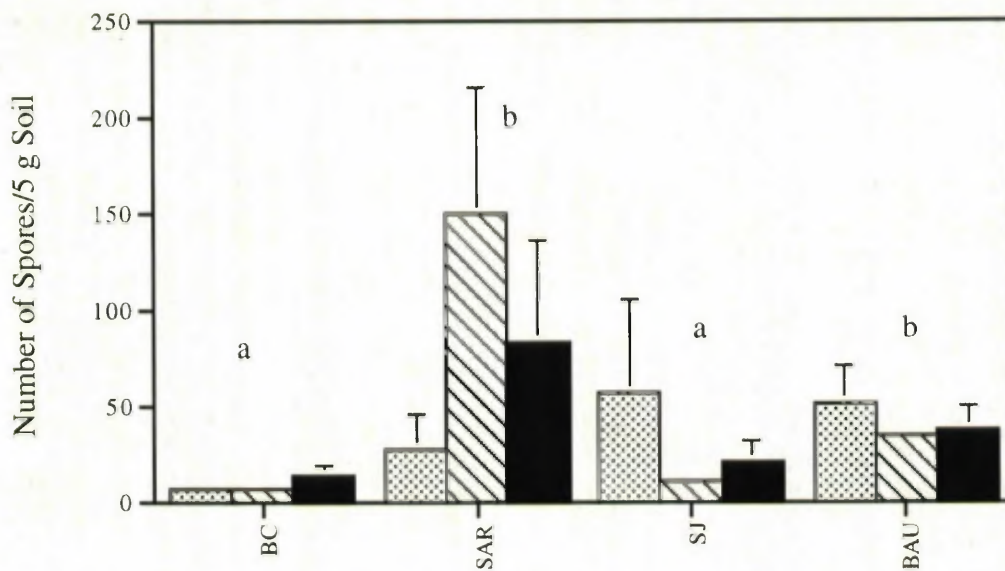


Figure 4. Pot cultures with Spineflower (top) and blue corn, the control (bottom).

May 1999 Spore Count Data



June 1999 Spore Count Data

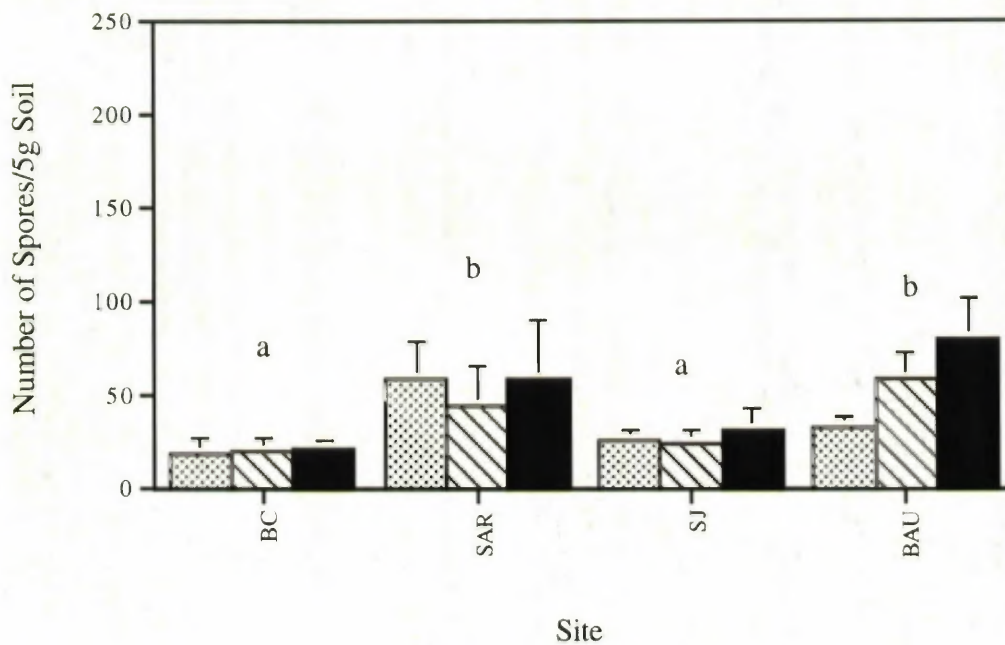


Figure 5: Mean number of spores (per 5 g of soil) from five soil cores per sample category. Study sites sampled were Bee Canyon (BC), Santa Ana River (SAR), San Jacinto (SJ), and Bautista Creek (BC). Sampling categories, SPINEFLOWER (stippled), OCCUPIED (hatched) (control for plant species differences), and UNOCCUPIED (solid), consisted of soil cores sampled from beneath Spineflowers and grasses (*Bromus sp.*) in occupied and unoccupied habitat, respectively, in May and June. Log transformed data differed significantly by month, May > June (ANOVA, $p = 0.04$). Differing letters indicate significant differences among sites (ANOVA, Fisher's LSM < 0.004). Error bars = + standard error.

Figure 6: Summary of Analysis of Variance on mean spore counts from five soil cores per sample category at all four study sites.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Month	4.888	1	4.888	4.354	0.040
Category	4.147	2	2.074	1.847	0.163
Site	43.816	3	14.605	13.010	0.000
Month*Category	1.154	2	0.577	0.514	0.600
Month*Site	6.121	3	2.040	1.818	0.149
Category*Site	2.512	6	0.419	0.272	0.894
Month*Category*Site	10.769	6	1.795	1.599	0.156
Error	106.646	95	1.123		

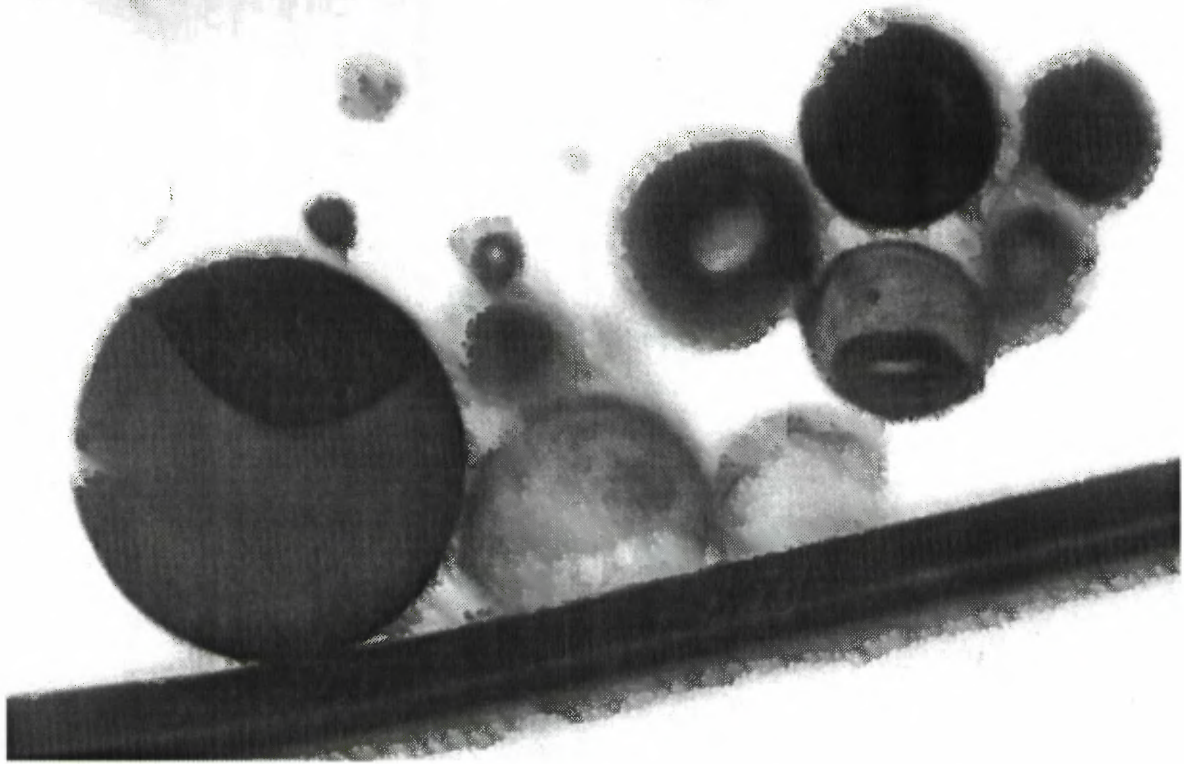


Figure 7. Variety of *Glomus* spores extracted from Cone Camp soil core viewed at 100x magnification.

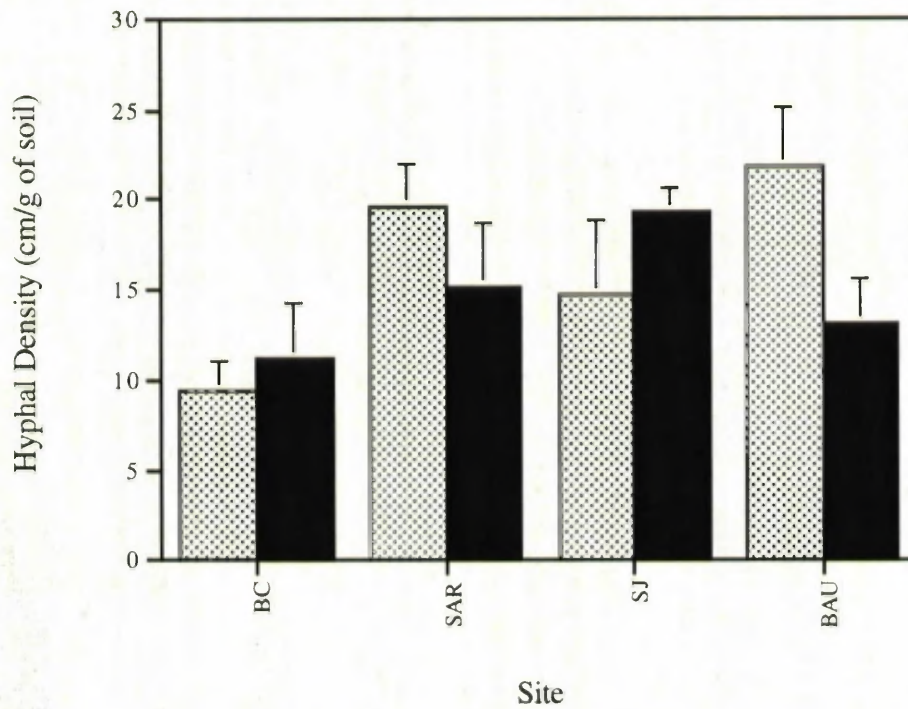
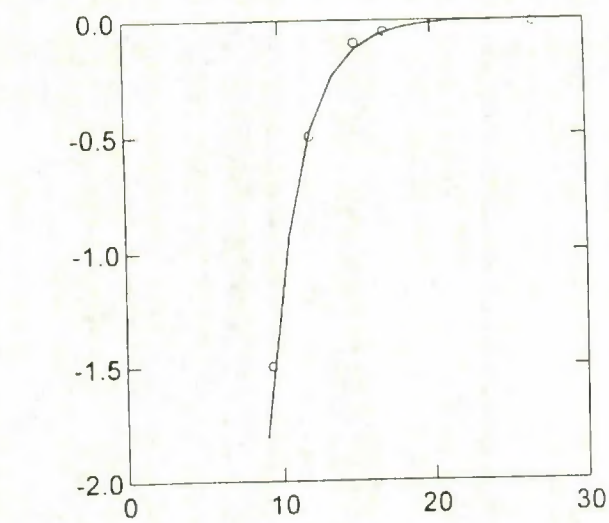
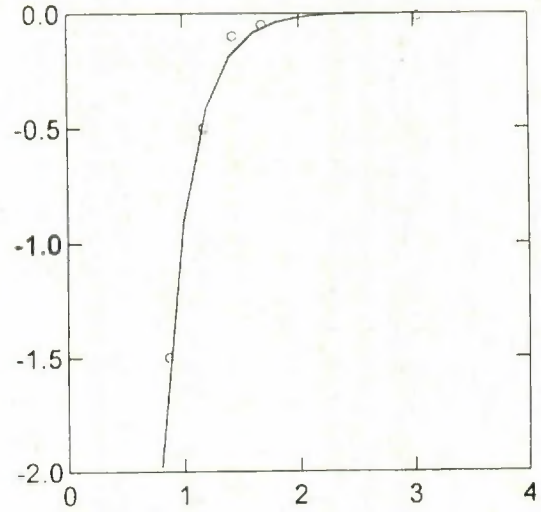


Figure 8: Mean active hyphal length (europium) of soil-born fungi (cm/g of soil) measured in occupied (stippled) and unoccupied (solid) habitat by site: Bee Canyon (BC), Santa Ana River (SAR), San Jacinto (SJ), and Bautista Creek (BAU). No significant difference was found between hyphal densities in occupied and unoccupied habitats. Error bars = + standard error.

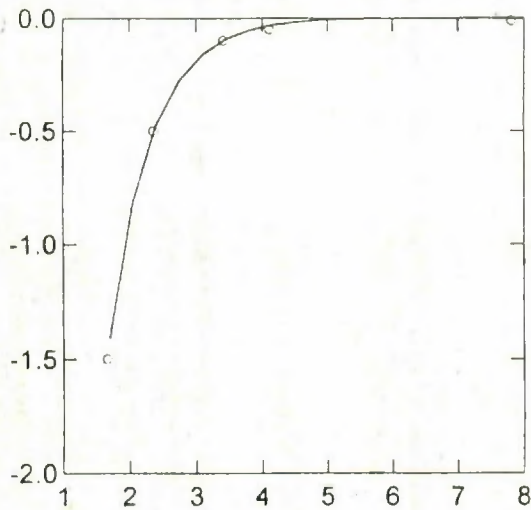


$$y = -99.053 * e^{-0.445x} \quad (R^2 = 0.999)$$

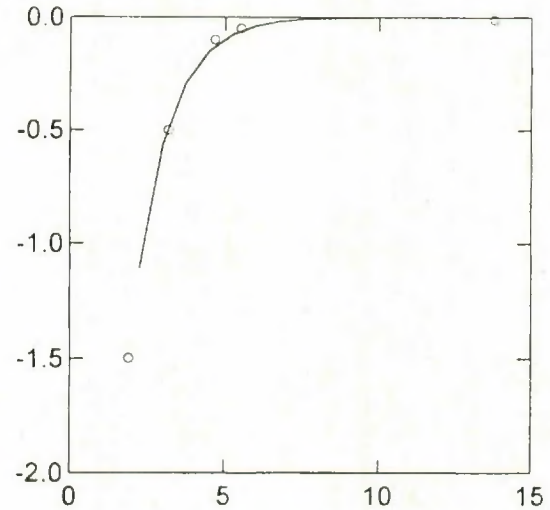


$$y = -45.248 * e^{-3.911x} \quad (R^2 = 0.997)$$

ψ_m (MPa)



$$y = -19.819 * e^{-1.555x} \quad (R^2 = 1.0)$$



$$y = -8.244 * e^{-0.891x} \quad (R^2 = 0.999)$$

Percent Moisture

Figure 9: Soil moisture curves with defining equations and corresponding R^2 values: A = Bee Canyon, B = Santa Ana River, C = San Jacinto, D = Bautista Creek. Psychrometer measured soil ψ_m (-MPa) (y-axes) from corresponding critical soil moisture values (x-axes).

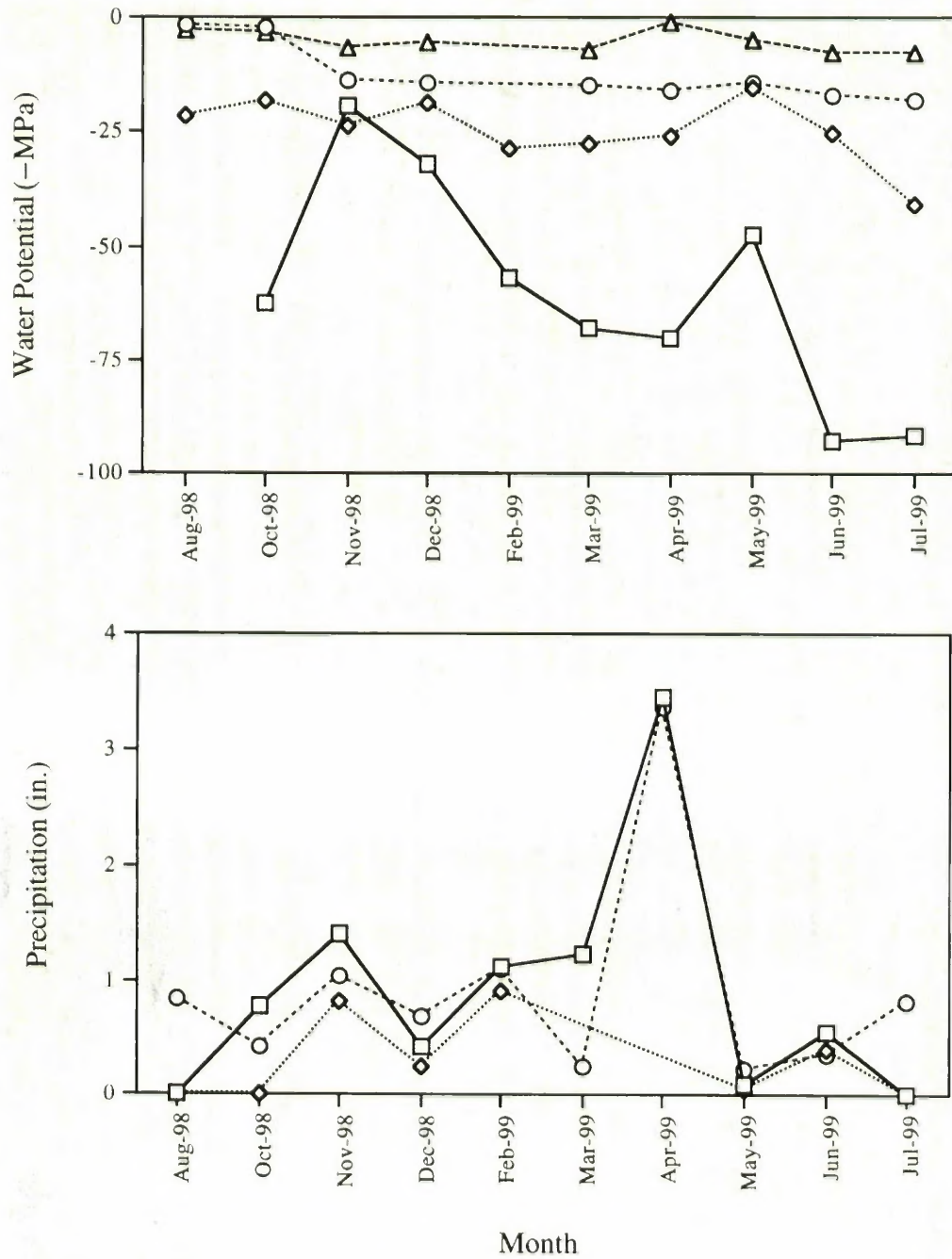


Figure 10: Mean soil water potential (Ψ_m) by site over a calendar year (top): Bee Canyon (squares), Santa Ana River (diamonds), San Jacinto (circles), and Bautista Creek (triangles). Corresponding monthly precipitation data from California Data Exchange Commission (CDEC) weather stations (bottom): Saugus - L.A. co. (squares), San Bernardino Medical Center - S.B. co. (diamonds), and Hemet - Riverside co. (circles). (Note missing data: BC Aug 98, SJ Feb 99, BAU Feb 99, and S.B. Mar and Apr.)

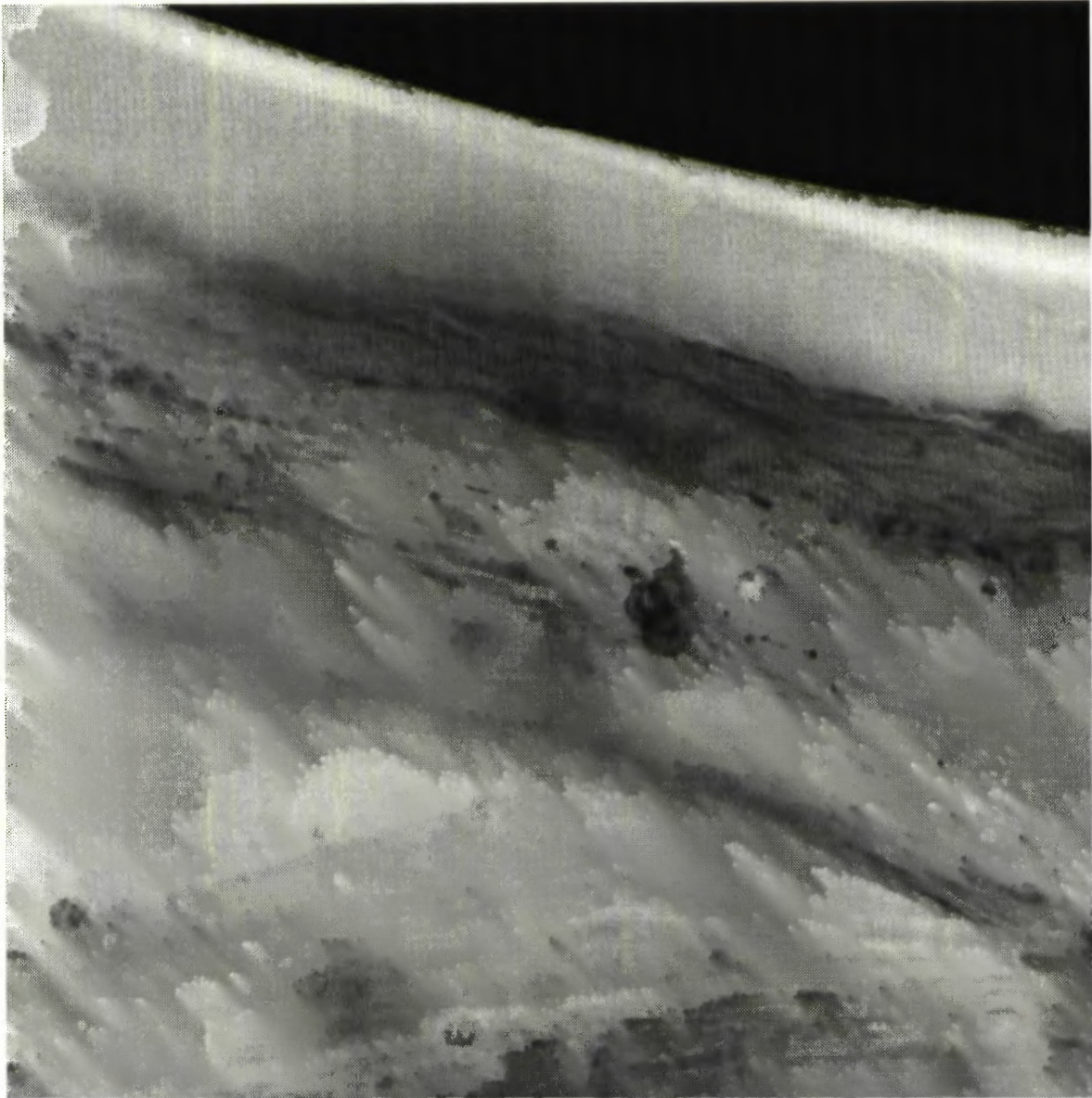


Figure 11. Reproductive structures possibly belonging to *Phytophthora* found on Spineflower root from the San Jacinto site.

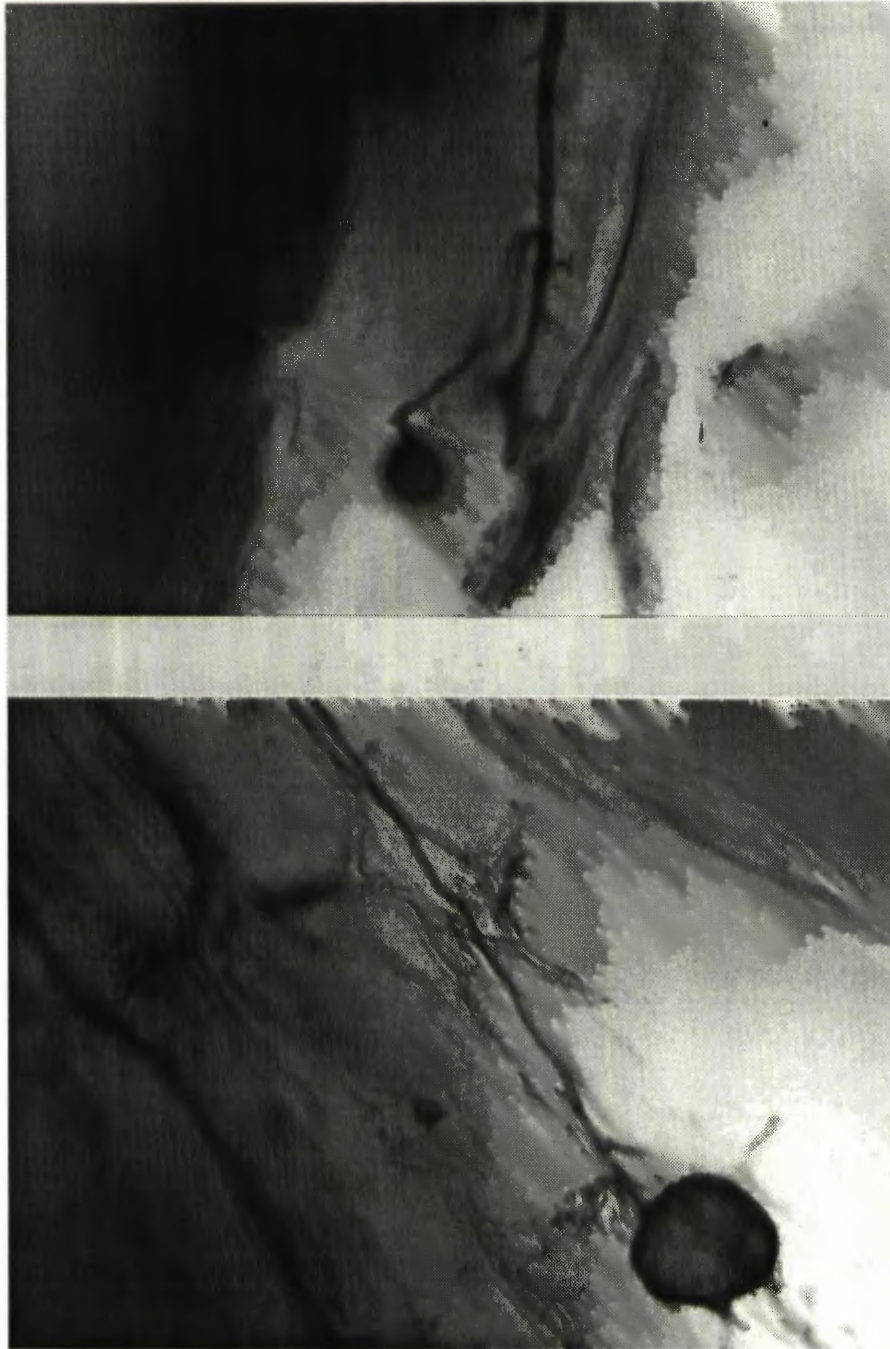


Figure 12. AM fungal hyphae and vesicles present within Spineflower root cortex cells at 200x magnification.

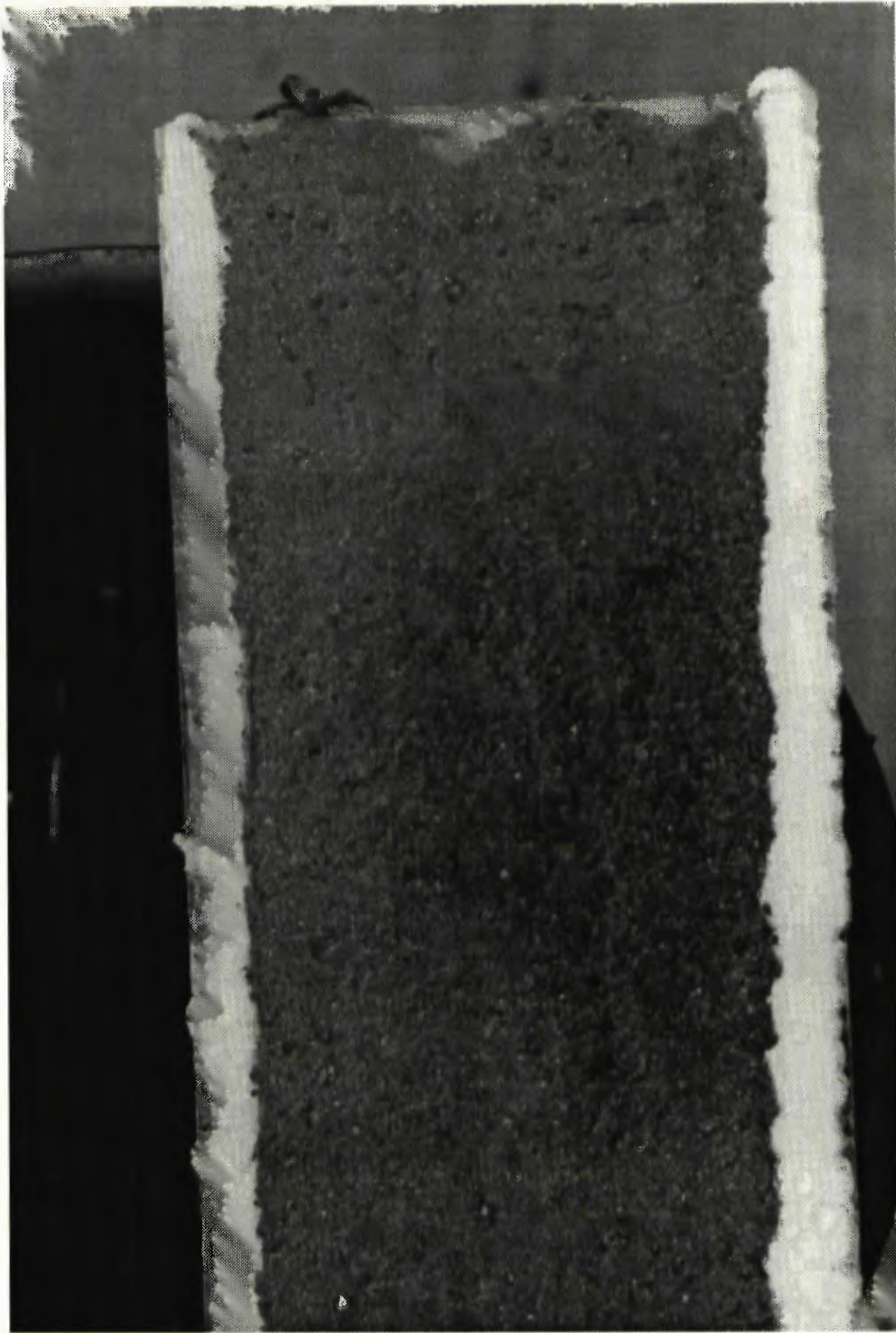
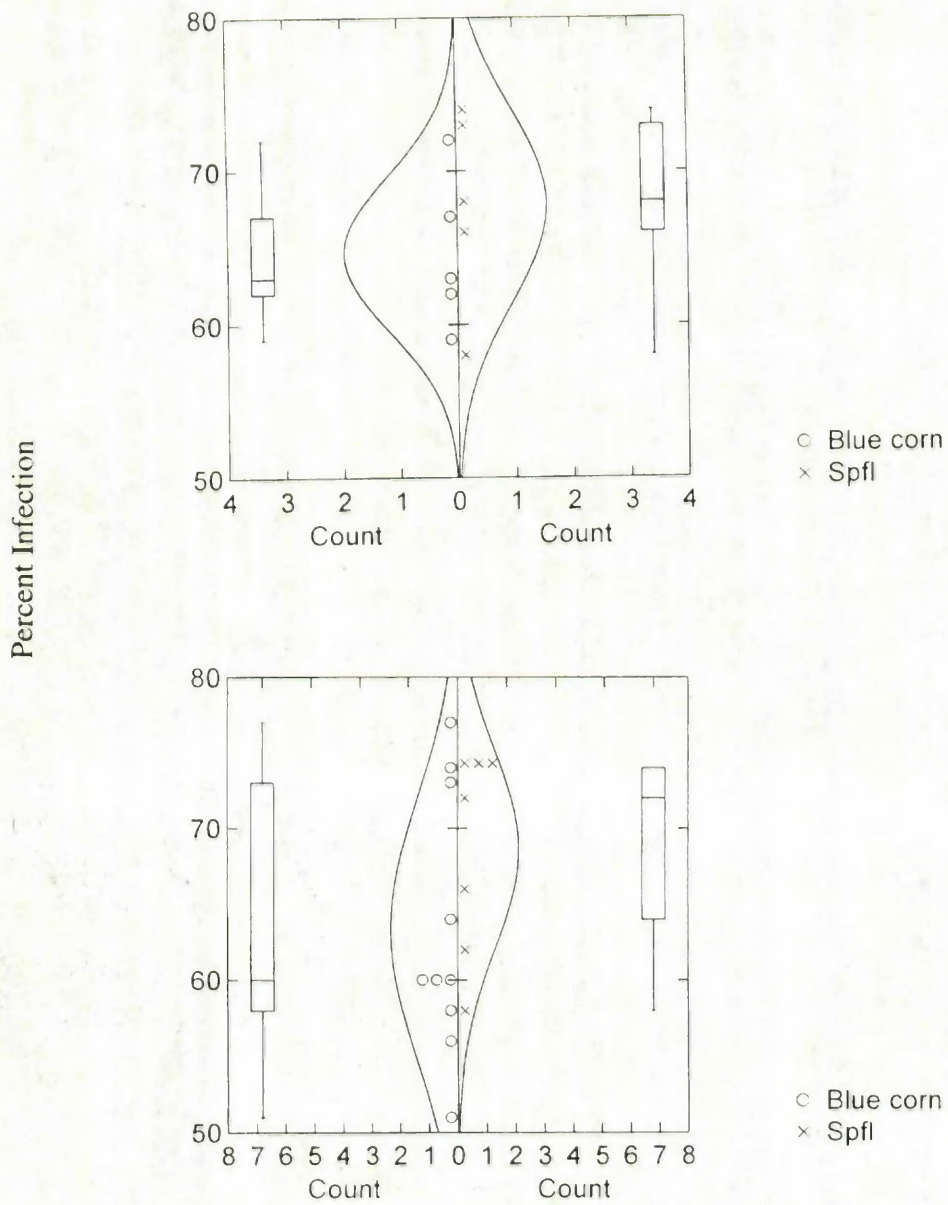


Figure 13. Spineflower roots growing against a glass growth chamber constructed for pot cultures.



Number of Samples Per Y-Value

Figure 14: Percent infection data from pot cultures. Distributions of data for Spineflower $n = 5$ and blue corn $n = 5$, avoiding pseudoreplication, compared (top), and distributions of raw data for Spineflower $n = 7$ and blue corn $n = 10$ compared (below). No statistical difference was found for either set of distributions. Blue corn = o, Spineflower = x.

Appendix 1: Raw spore count data (5g of soil) for May and June 1999. Five soil samples were gathered from each site from beneath Spineflowers and grasses, occupied and unoccupied.

Site	Sample #	Spineflower		Occupied		Unoccupied	
		May	June	May	June	May	June
Bee Canyon	#1	2	4	7	23	12	29
	#2	4	33	2	7	5	34
	#3	1	42	18	9	13	10
	#4	9	2	5	47	35	7
	#5	16	10	2	12	6	23
Santa Ana River	#1	25	9	14	13	0	45
	#2	4	94	380	110	19	9
	#3	5	110	208	9	39	183
	#4	5	61	62	85	63	20
	#5	98	15	87	2	292	34
San Jacinto	#1	14	20	7	12	15	35
	#2	2	7	20	14	61	12
	#3	15	43	5	45	10	34
	#4	251	34	10	10	13	7
	#5	1	23	15	37	1	69
Bautista	#1	19	21	32	43	58	52
	#2	116	19	31	31	7	68
	#3	65	41	46	45	72	149
	#4	15	36	23	54	25	97
	#5	42	46	38	118	27	31

Appendix 2: Raw data for fungal hyphal density (cm/g soil) determined from europium stain. Five soil samples were gathered from beneath Spineflowers and grasses unoccupied per site in June 1999.

Site	Occupied	Unoccupied
Bee Canyon	13.66	6.65
	4.37	12.90
	11.86	21.77
	7.13	10.00
	9.66	4.88
Santa Ana River	20.37	9.58
	25.76	20.54
	12.89	26.37
	15.60	11.66
	23.28	7.40
San Jacinto	25.39	22.05
	13.06	21.98
	20.11	16.75
	14.35	15.58
	0.57	19.95
Bautista Creek	20.89	10.78
	15.90	13.42
	14.20	12.60
	30.84	21.76
	27.36	7.28

**APPENDIX 3: Percent moisture data determined by: Wet Wt. - Dry Wt./Dry Wt.
Water potential values determined by respective soil moisture curves.**

Percent Moisture August 1998

Sample	Sample #	Tin	Tin + Wet	Tin + Dry	Moisture	% Moisture	H ₂ O Potential**
SAR 12/2	#1	1.573	6.6066	6.5935	0.0026093	0.26093019	-16.30807709
SAR 12/2	#2	1.673	6.8497	6.8386	0.00214883	0.21488307	-19.52607542
SAR 12/2	#3	1.6826	6.8767	6.8666	0.0019483	0.19483025	-21.11909008
SAR 12/2	#4	1.5948	6.6326	6.625	0.00151087	0.15108743	-25.05956332
SAR 12/2	#5	1.6993	6.7105	6.7035	0.00139882	0.1398825	-26.18215359
SJ 11/25	#1	1.5919	6.7636	6.5392	0.04535807	4.53580741	-0.017136588
SJ 11/25	#2	1.0626	6.262	6.142	0.02362484	2.36248376	-0.503072406
SJ 11/25	#3	1.7826	6.8732	6.7847	0.01769257	1.76925691	-1.265473772
SJ 11/25	#4	1.5873	6.6066	6.5237	0.01679361	1.67936148	-1.455331886
SJ 11/25	#5	1.6002	6.6556	6.6024	0.01063532	1.06353205	-3.791829962
Bau 11/25	#1	1.5829	6.6452	6.576	0.01385913	1.38591256	-2.398006034
Bau 11/25	#2	1.5975	6.8197	6.6374	0.03617135	3.61713526	-0.328446323
Bau 11/25	#3	1.5892	6.7816	6.6719	0.02158302	2.15830169	-1.204950946
Bau 11/25	#4	1.685	6.7885	6.7671	0.00421086	0.42108577	-5.664950975
Bau 11/25	#5	1.5959	6.8563	6.8038	0.01008084	1.00808387	-3.357789708

* Bee Canyon not selected as study site until 15 September 1998.

Percent Moisture October 1998

Sample	Sample #	Tin	Tin + Wet	Tin + Dry	Moisture	% Moisture	H ₂ O Potential**
BC 1/5	#1	1.07	6.13	6.09	0.00796813	0.79681275	-69.48239333
BC 1/5	#2	1.56	6.78	6.74	0.00772201	0.77220077	-70.24756999
BC 1/5	#3	1.56	6.69	6.65	0.00785855	0.78585462	-69.82204256
BC 1/5	#4	1.57	6.58	6.52	0.01212121	1.21212121	-57.75795686
BC 1/5	#5	1.75	6.93	6.84	0.01768173	1.76817289	-45.09710822
SAR 1/5	#1	1.58	6.6	6.58	0.004	0.4	-9.466492612
SAR 1/5	#2	0.93	6.07	6.06	0.00194932	0.19493177	-21.11070595
SAR 1/5	#3	1.6	6.15	6.14	0.00220264	0.22026432	-19.11942293
SAR 1/5	#4	1.5	6.71	6.7	0.00192308	0.19230769	-21.32847609
SAR 1/5	#5	1.55	6.18	6.17	0.0021645	0.21645022	-19.40676417
SJ 1/5	#1	1.48	6.82	6.6	0.04296875	4.296875	-0.024847465
SJ 1/5	#2	1.54	6.68	6.57	0.02186879	2.18687873	-0.661030467
SJ 1/5	#3	1.48	6.8	6.72	0.01526718	1.52671756	-1.845214022
SJ 1/5	#4	1.58	6.64	6.58	0.012	1.2	-3.06681963
SJ 1/5	#5	1.55	6.64	6.59	0.00992063	0.99206349	-4.23753759
Bau 1/5	#1	1.56	6.66	6.61	0.00990099	0.99009901	-3.412030079
Bau 1/5	#2	1.53	6.93	6.82	0.02079395	2.07939509	-1.29271498
Bau 1/5	#3	1.39	6.57	6.48	0.01768173	1.76817289	-1.705816053
Bau 1/5	#4	1.47	6.62	6.6	0.00389864	0.38986355	-5.824756768
Bau 1/5	#5	1.4	7.01	6.96	0.00899281	0.89928058	-3.699607268

** H₂O Potential was determined from applying percent moisture to soil moisture curve equations seen in

Figure 13.

APPENDIX 3 (continued)

Percent Moisture November 1998

Sample	Sample #	Tin	Tin + Wet	Tin + Dry	Moisture	% Moisture	H ₂ O Potential**
BC 12/2	#1	1.5982	6.7745	6.533	0.04893815	4.89381535	-11.22234891
BC 12/2	#2	1.539	6.6352	6.153	0.10450802	10.4508019	-0.946530781
BC 12/2	#3	1.0379	6.6939	6.2408	0.08708605	8.70860482	-2.055102895
BC 12/2	#4	1.4997	6.7836	6.687	0.0186224	1.86224047	-43.24830537
BC 12/2	#5	1.6075	6.7404	6.6367	0.02061958	2.06195816	-39.57049154
SAR 12/2	#1	1.5376	6.596	6.5917	0.00085079	0.08507944	-32.44066663
SAR 12/2	#2	1.6939	7.1	7.0922	0.0014449	0.14448993	-25.71458514
SAR 12/2	#3	1.5515	6.7106	6.6834	0.00530018	0.53001812	-5.693115258
SAR 12/2	#4	1.5861	6.9077	6.9006	0.00133597	0.13359676	-26.83377963
SAR 12/2	#5	1.0396	6.1917	6.1855	0.00120484	0.12048427	-28.24579209
SJ 11/25	#1	1.529	6.6354	6.6243	0.00217848	0.21784782	-14.1241728
SJ 11/25	#2	1.6132	6.6331	6.6197	0.00267652	0.26765205	-13.07160254
SJ 11/25	#3	1.5329	6.655	6.6433	0.00228945	0.2289449	-13.88253755
SJ 11/25	#4	1.0294	6.2152	6.2042	0.00212569	0.2125686	-14.24059798
SJ 11/25	#5	1.0164	6.1717	6.1598	0.00231364	0.23136447	-13.83040359
Bau 11/25	#1	1.4988	6.5575	6.5469	0.0020998	0.20997999	-6.837303917
Bau 11/25	#2	1.6897	6.8346	6.8236	0.00214262	0.21426206	-6.811267089
Bau 11/25	#3	1.028	6.1346	6.1234	0.00219806	0.2198061	-6.777704181
Bau 11/25	#4	1.685	6.721	6.7117	0.00185012	0.18501204	-6.991114176
Bau 11/25	#5	1.5094	6.7119	6.6975	0.00277558	0.27755826	-6.437763871

Percent Moisture December 1998

Sample	Sample #	Tin	Tin + Wet	Tin + Dry	Moisture	% Moisture	H ₂ O Potential**
BC 1/5	#1	1.5391	6.6344	6.5105	0.02492256	2.4922557	-32.67470636
BC 1/5	#2	1.5968	6.989	6.832	0.0299893	2.99893032	-26.07901763
BC 1/5	#3	1.6129	6.6955	6.5622	0.0269331	2.69331017	-29.87828723
BC 1/5	#4	1.694	6.8155	6.6651	0.03025487	3.02548732	-25.77263331
BC 1/6	#5	1.0388	6.138	6.0495	0.0176622	1.76622029	-45.13631043
SAR 1/5	#1	1.534	6.7323	6.7139	0.00355219	0.35521921	-11.27847599
SAR 1/5	#2	1.0172	6.1318	6.1173	0.00284308	0.28430815	-14.88314393
SAR 1/5	#3	1.5873	6.6652	6.6566	0.00169649	0.16964867	-23.30487402
SAR 1/5	#4	1.5393	6.5544	6.548	0.00127778	0.12777767	-27.45147815
SAR 1/5	#5	1.5097	6.6644	6.651	0.00260634	0.26063447	-16.32694905
SJ 1/5	#1	1.4995	6.5761	6.566	0.00199349	0.19934866	-14.53637172
SJ 1/5	#2	1.0305	6.2478	6.2332	0.00280624	0.28062352	-12.81058133
SJ 1/5	#3	1.6902	6.8825	6.8726	0.00191031	0.19103118	-14.72560161
SJ 1/5	#4	1.0282	6.0867	6.0732	0.00267592	0.26759167	-13.07282985
SJ 1/5	#5	1.685	6.7421	6.7336	0.00168364	0.16836351	-15.25390934
Bau 1/5	#1	1.5295	6.6202	6.5975	0.00447908	0.44790845	-5.531169292
Bau 1/5	#2	1.0386	6.0608	6.0372	0.00472132	0.4721322	-5.413067163
Bau 1/5	#3	1.4984	6.5284	6.504	0.00487454	0.48745405	-5.339671334
Bau 1/5	#4	1.5517	6.7103	6.671	0.00767683	0.76768308	-4.159854249
Bau 1/5	#5	1.6071	6.869	6.8528	0.00308824	0.30882437	-6.260895018

** H₂O Potential was determined from applying percent moisture to soil moisture curve equations seen in

Figure 13.

APPENDIX 3 (continued)

Percent Moisture February 1999

Sample	Sample #	Tin	Tin + Wet	Tin + Dry	Moisture	% Moisture	H ₂ O Potential**
BC 2/28	#1	1.4912	6.5998	6.545	0.01084333	1.08433258	-61.13760001
BC 2/28	#2	1.0218	6.1147	6.0561	0.01164015	1.16401486	-59.00772978
BC 2/28	#3	1.5887	6.6724	6.5977	0.01491316	1.49131563	-51.00989351
BC 2/28	#4	1.6059	6.8986	6.8388	0.0114277	1.14276978	-59.56823766
BC 2/28	#5	1.5217	6.6803	6.6061	0.01459366	1.4593659	-51.74031337
SAR 2/28	#1	1.686	6.7425	6.7366	0.00116818	0.1168178	-28.65374183
SAR 2/28	#2	1.5304	6.8055	6.8003	0.00098674	0.0986736	-30.76094987
SAR 2/28	#3	1.4904	6.5832	6.5791	0.00080571	0.08057068	-33.0177916
SAR 2/28	#4	1.0309	6.1075	6.1022	0.0010451	0.10450969	-30.06678475
SAR 2/28	#5	1.5015	6.5022	6.4918	0.00208404	0.2084043	-20.02715767

** H₂O Potential was determined from applying percent moisture to soil moisture curve equations seen in

Figure 13.

*** Data missing for San Jacinto and Bautista sites

APPENDIX 3 (continued)

Percent Moisture March 1999

Sample	Sample #	Tin	Tin + Wet	Tin + Dry	Moisture	% Moisture	H ₂ O Potential**
BC Unoc 3/28 #1		1.501	6.7	6.6794	0.00397806	0.39780627	-82.98262672
BC Unoc 3/28 #2		1.5888	6.5956	6.5659	0.00596733	0.59673304	-75.95255422
BC Unoc 3/28 #3		1.5217	6.6695	6.596	0.01448476	1.44847565	-51.99166386
BC Unoc 3/28 #4		1.6792	6.959	6.9238	0.00671167	0.67116653	-73.47799349
BC Unoc 3/28 #5		1.0207	6.1349	6.0854	0.00977353	0.97735305	-64.1185012
BC OC 3/28 #1		1.683	6.7988	6.7495	0.00973058	0.97305832	-64.24115867
BC OC 3/28 #2		1.4914	6.9067	6.8584	0.00899944	0.8999441	-66.36567199
BC OC 3/28 #3		1.031	6.1616	6.1327	0.00566478	0.56647784	-76.98206105
BC OC 3/28 #4		1.5798	6.7271	6.6741	0.01040378	1.04037846	-62.34519753
BC OC 3/28 #5		1.5304	6.7938	6.7388	0.01055986	1.05598648	-61.91367495
SAR Unoc 3/2 #1		1.6063	6.6798	6.665	0.00292565	0.29256528	-14.41019074
SAR Unoc 3/2 #2		1.5243	6.7781	6.7656	0.0023849	0.23849045	-17.80398515
SAR Unoc 3/2 #3		1.0072	6.3254	6.3186	0.00128027	0.12802651	-27.42477477
SAR Unoc 3/2 #4		1.4901	6.9731	6.9663	0.00124174	0.1241737	-27.84114997
SAR Unoc 3/2 #5		1.0304	6.1899	6.1835	0.00124197	0.12419709	-27.83860342
SAR OC 3/24 #1		1.5299	6.5663	6.5606	0.00113304	0.11330431	-29.05019826
SAR OC 3/24 #2		1.599	6.912	6.9085	0.0006592	0.06591958	-34.96498039
SAR OC 3/24 #3		1.0215	6.0717	6.0675	0.00083234	0.08323424	-32.67562341
SAR OC 3/24 #4		1.5431	6.6359	6.6323	0.00070738	0.07073803	-34.31223587
SAR OC 3/24 #5		1.6871	6.7173	6.7108	0.00129387	0.12938671	-27.27926962
SJ Unoc 3/24 #1		1.6063	6.6239	6.6148	0.00181691	0.18169113	-14.9410338
SJ Unoc 3/24 #2		1.5243	6.6147	6.6071	0.00149524	0.14952388	-15.70739206
SJ Unoc 3/24 #3		1.5307	6.6499	6.6395	0.0020357	0.20357031	-14.44125789
SJ Unoc 3/24 #4		1.0205	6.1997	6.1903	0.00181825	0.18182522	-14.93791876
SJ Unoc 3/24 #5		1.4907	6.6414	6.6332	0.00159456	0.15945552	-15.46667553
SJ OC 3/24 #1		1.5301	6.5962	6.588	0.00162123	0.16212262	-15.40266274
SJ OC 3/24 #2		1.0305	6.2269	6.2188	0.00156121	0.1561205	-15.54709327
SJ OC 3/24 #3		1.5435	6.6267	6.6192	0.00147763	0.14776287	-15.75046377
SJ OC 3/24 #4		1.687	6.8509	6.841	0.00192084	0.19208382	-14.70151774
SJ OC 3/24 #5		1.5016	6.5366	6.5262	0.00206982	0.20698165	-14.36485513
Bau Unoc 3/24 #1		1.008	6.1159	6.0901	0.00507664	0.50766415	-5.244379341
Bau Unoc 3/24 #2		1.682	6.8712	6.8593	0.0022985	0.22984954	-6.7173231
Bau Unoc 3/24 #3		1.4917	6.6095	6.6018	0.00150682	0.15068198	-7.208263069
Bau Unoc 3/24 #4		1.5993	6.7131	6.7048	0.0016257	0.16256978	-7.132315891
Bau Unoc 3/24 #5		1.0315	6.2069	6.1966	0.00199415	0.19941531	-6.901968252
Bau OC 3/24 #1		1.5792	6.7691	6.7598	0.00179516	0.17951589	-7.025434111
Bau OC 3/24 #2		1.0219	6.2747	6.2715	0.00060957	0.06095703	-7.808188209
Bau OC 3/24 #3		1.5895	6.8853	6.8797	0.00105856	0.10585611	-7.501986618
Bau OC 3/24 #4		1.5216	6.7199	6.7147	0.00100133	0.10013287	-7.540340025
Bau OC 3/24 #5		1.6789	6.9866	6.9793	0.00137725	0.13772545	-7.291959355

** H₂O Potential was determined from applying percent moisture to soil moisture curve equations seen in

Figure 13.

APPENDIX 3 (continued)

Percent Moisture April 1999

Sample	Sample #	Tin	Tin + Wet	Tin + Dry	Moisture	% Moisture	H ₂ O Potential**
BC Unoc 4/21 #1		1.49022	6.65423	6.64077	0.002613	0.26133131	-88.17845208
BC Unoc 4/21 #2		1.52503	6.81167	6.79647	0.002883	0.28834626	-87.12474789
BC Unoc 4/21 #3		1.52988	6.6093	6.58634	0.004541	0.45407261	-80.93066076
BC Unoc 4/21 #4		0.09967	6.00252	5.91193	0.015586	1.55860199	-49.50517329
BC Unoc 4/21 #5		1.02992	6.2459	6.2274	0.003559	0.35594173	-84.54306018
BC OC 4/21 #1		1.68664	6.79369	6.7394	0.010745	1.07446227	-61.40672448
BC OC 4/21 #2		1.67832	6.73301	6.6809	0.010417	1.0416625	-62.3095838
BC OC 4/21 #3		1.50079	6.60866	6.5661	0.008402	0.84022498	-68.15298405
BC OC 4/21 #4		1.03089	6.13196	6.092	0.007896	0.78955012	-69.7073147
BC OC 4/21 #5		1.52039	7.07371	6.9789	0.017369	1.73692088	-45.72866153
SAR Unoc 4/2 #1		1.49168	6.55525	6.55277	0.00049	0.0490013	-37.35677838
SAR Unoc 4/2 #2		1.58964	6.70464	6.6904	0.002792	0.27917408	-15.18500804
SAR Unoc 4/2 #3		1.68385	6.71424	6.70068	0.002703	0.2702902	-15.72188178
SAR Unoc 4/2 #4		1.60705	6.95545	6.95292	0.000473	0.04732625	-37.60231027
SAR Unoc 4/2 #5					***	***	
SAR OC 4/21 #1		1.52487	6.95897	6.9542	0.000879	0.08785614	-32.09027709
SAR OC 4/21 #2		1.02072	6.21575	6.19999	0.003043	0.30428999	-13.76432819
SAR OC 4/21 #3		1.68284	6.70371	6.6969	0.001358	0.13581808	-26.60166908
SAR OC 4/21 #4		1.58899	6.68029	6.6748	0.001079	0.10794741	-29.66524513
SAR OC 4/21 #5					***	***	
SJ Unoc 4/21 #1		1.53012	6.73491	6.72551	0.001809	0.18092963	-14.95873628
SJ Unoc 4/21 #2		1.50109	6.81631	6.79519	0.003989	0.39893466	-10.65786026
SJ Unoc 4/21 #3		1.02217	6.40991	6.40156	0.001552	0.15522206	-15.56882893
SJ Unoc 4/21 #4		1.67895	6.77201	6.76168	0.002032	0.20323724	-14.44873938
SJ Unoc 4/21 #5		1.0312	6.0933	6.08992	0.000668	0.06681532	-17.86320927
SJ OC 4/21 #1		1.0208	6.09261	6.0772	0.003048	0.30476228	-12.33863906
SJ OC 4/21 #2		1.49398	6.59136	6.5883	0.000601	0.0600669	-18.05164917
SJ OC 4/21 #3		0.99687	6.15645	6.1538	0.000514	0.05138716	-18.29694372
SJ OC 4/21 #4		1.5299	6.73944	6.7364	0.000584	0.05838855	-18.09882237
SJ OC 4/21 #5		1.57938	6.6107	6.6068	0.000776	0.07757458	-17.56683238
Bau Unoc 4/30 #1		1.54256	6.76731	6.49689	0.054583	5.45825571	-0.063684112
Bau Unoc 4/30 #2		1.49824	6.66713	6.55895	0.021376	2.13764472	-1.227333855
Bau Unoc 4/30 #3		1.02034	6.12686	5.97657	0.030323	3.03234515	-0.553035068
Bau Unoc 4/30 #4		1.52073	6.97656	6.71876	0.049596	4.95957122	-0.09931166
Bau Unoc 4/30 #5		1.00689	6.45858	6.25439	0.038912	3.89118628	-0.257287456
Bau OC 4/30 #1		1.0297	6.06948	6.0429	0.005302	0.53020027	-5.140124091
Bau OC 4/30 #2		1.5982	6.6315	6.4216	0.043517	4.35170212	-0.17069432
Bau OC 4/30 #3		1.48935	6.65329	6.5784	0.014716	1.47159096	-2.221756531
Bau OC 4/30 #4		1.5298	6.83157	6.7077	0.023923	2.39228259	-0.978202945
Bau OC 4/30 #5		1.60676	6.87765	6.6412	0.046966	4.69664948	-0.125527676

** H₂O Potential was determined from applying percent moisture to soil moisture curve equations seen in

Figure 13.

*** Missing data

APPENDIX 3 (continued)

Percent Moisture April 1999

EXTRA DATA

Sample	Sample #	Tin	Tin + Wet	Tin + Dry	Moisture	% Moisture	H ₂ O Potential**
SJ Unoc 4/30	#1	1.57957	7.0286	6.88161	0.027723	2.77232914	-0.26598091
SJ Unoc 4/30	#2	1.4945	7.19171	6.9283	0.048476	4.84762045	-0.010552329
SJ Unoc 4/30	#3	1.59886	6.66935	6.52489	0.029326	2.93258466	-0.207312478
SJ Unoc 4/30	#4	1.56349	6.745	6.48978	0.051808	5.18077498	-0.006285788
SJ Unoc 4/30	#5	1.68656	6.74888	6.73	0.003743	0.37434767	-11.07322963
SJ OC 4/30	#1	1.56328	6.67331	6.2662	0.086565	8.65653679	-2.82541E-05
SJ OC 4/30	#2	1.49843	6.87906	6.5131	0.072978	7.29778829	-0.000233712
SJ OC 4/30	#3	1.54238	6.54477	6.5371	0.001536	0.15356216	-15.6090662
SJ OC 4/30	#4	1.00756	6.15229	5.9865	0.033298	3.32982522	-0.111778376
SJ OC 4/30	#5	1.49126	6.99771	6.9479	0.009128	0.91283281	-4.793141591

** H₂O Potential was determined from applying percent moisture to soil moisture curve equations seen in

Figure 13.

APPENDIX 3 (continued)

Percent Moisture May1999

Sample	Sample #	Tin	Tin + Wet	Tin + Dry	Moisture	% Moisture	H ₂ O Potential**
BC Unoc 5/30	#1	1.5544	6.8792	6.8653	0.0026173	0.26172588	-48.89816871
BC Unoc 5/30	#2	1.5884	6.6625	6.647	0.0030641	0.30640889	-44.11590904
BC Unoc 5/30	#3	1.6547	6.9164	6.8855	0.0059073	0.59073182	-97.81533464
BC Unoc 5/30	#4	1.7613	7.0662	7.0299	0.0068899	0.68898759	-88.15436377
BC Unoc 5/30	#5	1.6943	6.8483	6.8312	0.0033289	0.33288559	-42.65861828
BC OC 5/30	#1	1.5764	6.6126	6.6004	0.0024283	0.24283439	-38.97768389
BC OC 5/30	#2	1.078	6.1284	6.1077	0.0041156	0.41155536	-17.09670764
BC OC 5/30	#3	1.6235	7.0364	7.0186	0.0032993	0.32992901	-46.96245417
BC OC 5/30	#4	1.5735	6.7948	6.7732	0.0041541	0.41540858	-24.00440631
BC OC 5/30	#5	1.6933	6.9876	6.9563	0.0059472	0.59471784	-24.45230465
BC OC 5/30	#6	1.756	6.8266	6.7968	0.0059118	0.591176	-97.27237343
SAR Unoc 6/3	#1	1.0756	6.3652	6.2826	0.0158633	1.5863261	-0.09145
SAR Unoc 6/3	#2	1.0678	6.2102	6.1184	0.0181761	1.81760583	-0.037012561
SAR Unoc 6/3	#3	1.0663	6.3765	6.375	0.0002826	2.83E-02	-40.5141457
SAR Unoc 6/3	#4	1.5953	6.8391	6.8254	0.0026195	2.62E-01	-16.24346196
SAR Unoc 6/3	#5	1.5754	6.947	6.8472	0.0189309	1.89309154	-0.027550832
SAR OC 6/3	#1	1.5669	6.823	6.7151	0.0209588	2.09587817	-0.012465118
SAR OC 6/3	#2	1.6625	7.1024	6.8958	0.039478	3.94779585	-8.91593E-06
SAR OC 6/3	#3	1.7598	7.1556	7.0666	0.0167709	1.67709354	-0.064122859
SAR OC 6/3	#4	1.0484	6.3612	6.1972	0.0318521	3.1852082	-0.000175971
SAR OC 6/3	#5	1.664	6.7758	6.62	0.0314366	3.14366425	-0.000207016
SJ Unoc 5/30	#1	1.5475	6.9466	6.9444	0.0004076	0.04076414	-18.60169735
SJ Unoc 5/30	#2	1.5991	6.6555	6.6531	0.0004749	0.04748714	-18.40824335
SJ Unoc 5/30	#3	1.6055	6.6857	6.6841	0.000315	0.03150475	-18.8714688
SJ Unoc 5/30	#4	1.0307	6.1822	6.176	0.001205	0.12049832	-16.43258391
SJ Unoc 5/30	#5	1.0599	6.6412	6.6259	0.0027488	0.27488322	-12.92544265
SJ OC 5/30	#1	1.7599	6.9925	6.9863	0.0011863	0.1186285	-16.48043226
SJ OC 5/30	#2	1.5687	6.8498	6.8467	0.0005873	0.05873437	-18.08909245
SJ OC 5/30	#3	1.6557	6.8373	6.8305	0.0013141	0.13140604	-16.15621288
SJ OC 5/30	#4	1.755	6.81	6.7792	0.0061303	0.61303292	-7.639826411
SJ OC 5/30	#5	1.5908	6.7543	6.7471	0.0013964	0.13963501	-15.9507944
Bau Unoc 6/3	#1	1.0435	6.3442	6.3201	0.0045673	0.4567335	-5.487847555
Bau Unoc 6/3	#2	1.5618	6.8793	6.849	0.0057308	0.57308216	-4.947435977
Bau Unoc 6/3	#3	1.0308	6.5826	6.4522	0.0240528	2.40528277	-0.966937631
Bau Unoc 6/3	#4	1.6593	7.0579	6.8183	0.0464431	4.64431091	-0.13152013
Bau Unoc 6/3	#5	1.0643	6.3455	6.2285	0.022656	2.26559777	-1.095091641
Bau OC 6/3	#1	1.7553	7.2227	7.202	0.0038005	0.38004663	-5.875928631
Bau OC 6/3	#2	1.6584	6.871	6.8207	0.0097437	0.97437189	-3.46017896
Bau OC 6/3	#3	1.7483	6.8725	6.8656	0.0013484	0.13483673	-7.310751964
Bau OC 6/3	#4	1.5934	6.8839	6.8702	0.0025963	0.25962705	-6.541444185
Bau OC 6/3	#5	1.09	6.1375	6.1195	0.0035789	0.35788846	-5.993089449

** H₂O Potential was determined from applying percent moisture to soil moisture curve equations seen in

Figure 13.

APPENDIX 3 (continued)

Percent Moisture June 1999

Sample	Sample #	Tin	Tin + Wet	Tin + Dry	Moisture	% Moisture	H ₂ O Potential**
BC Unoc 6/25	#1	1.5808	6.7206	6.6816	0.0076459	0.76458595	-83.91135486
BC Unoc 6/25	#2	1.779	6.9036	6.8607	0.0084421	0.84420568	-93.56054219
BC Unoc 6/25	#3	1.0539	6.2747	6.2534	0.0040965	0.40965477	-95.32746478
BC Unoc 6/25	#4	1.7832	7.0276	7.0056	0.0042126	0.42126225	-93.97968525
BC Unoc 6/25	#5	1.088	6.2928	6.2749	0.003451	0.34510016	-83.86096051
BC OC 6/25	#1	1.0537	6.2461	6.2363	0.0019909	0.18909428	-95.36555089
BC OC 6/25	#2	1.7897	7.0755	7.0648	0.0020284	0.20283976	-94.30709727
BC OC 6/25	#3	1.073	6.1217	6.0885	0.0066195	0.66194796	-94.53460502
BC OC 6/25	#4	1.6298	7.1308	7.099	0.0058144	0.58143787	-96.01112584
BC OC 6/25	#5	1.7815	7.369	7.3038	0.0118067	1.18066748	-97.35447798
SAR Unoc 6/25	#1	1.6822	6.7774	6.7641	0.0026171	0.26171314	-16.25821606
SAR Unoc 6/25	#2	1.6087	6.6974	6.6785	0.003728	0.37279577	-10.52921958
SAR Unoc 6/25	#3	1.6225	6.2308	6.2249	0.0012819	0.12819399	-27.40681738
SAR Unoc 6/25	#4	1.612	6.8399	6.8354	0.0008615	0.08615078	-32.30502393
SAR Unoc 6/25	#5	1.5886	6.8424	6.8362	0.0011815	0.11814925	-28.5049215
SAR OC 6/25	#1	1.656	6.9142	6.8946	0.0037415	0.37414576	-10.47377367
SAR OC 6/25	#2	1.1147	6.2802	6.2758	0.0008525	0.08525314	-32.41863544
SAR OC 6/25	#3	1.7737	6.8548	6.8492	0.0011033	0.11033396	-29.38964389
SAR OC 6/25	#4	1.7828	6.8396	6.8343	0.0010492	0.10491933	-30.01865336
SAR OC 6/25	#5	1.6132	6.7529	6.7493	0.0007009	0.07009209	-34.3990275
SJ Unoc 6/25	#1	1.091	6.2386	6.2366	0.0003887	0.03886816	-18.6566208
SJ Unoc 6/25	#2	1.099	6.4594	6.4552	0.0007841	0.0784138	-17.54392284
SJ Unoc 6/25	#3	1.6813	6.797	6.7954	0.0003129	0.03128605	-18.87788747
SJ Unoc 6/25	#4	1.6883	6.8114	6.8084	0.0005859	0.05859261	-18.09308049
SJ Unoc 6/25	#5	1.09	6.1115	6.1081	0.0006775	0.06775473	-17.83713414
SJ OC 6/25	#1	1.7194	6.9083	6.8819	0.0051138	0.51138015	-8.948133362
SJ OC 6/25	#2	1.7168	6.7962	6.7924	0.0007487	0.074868	-17.64092241
SJ OC 6/25	#3	1.5912	6.9616	6.959	0.0004844	0.04843698	-18.38107451
SJ OC 6/25	#4	1.5975	6.6377	6.6339	0.0007545	0.07545072	-17.62494461
SJ OC 6/25	#5	1.6794	6.7095	6.7025	0.0013936	0.13935617	-15.95771198
Bau Unoc 6/25	#1	1.6853	7.0816	7.0773	0.0007975	0.07974777	-7.678547456
Bau Unoc 6/25	#2	1.6	6.8474	6.8439	0.0006674	0.06674422	-7.768029787
Bau Unoc 6/25	#3	1.6477	6.6562	6.6542	0.0003995	0.03994807	-7.955726111
Bau Unoc 6/25	#4	1.5895	6.642	6.6383	0.0007328	0.07328474	-7.722892414
Bau Unoc 6/25	#5	1.6187	6.7539	6.7516	0.0004481	0.04480898	-7.921343808
Bau OC 6/25	#1	1.0686	6.1911	6.1887	0.0004687	0.04687408	-7.906781854
Bau OC 6/25	#2	1.6163	6.7966	6.7951	0.0002896	0.02896424	-8.033967786
Bau OC 6/25	#3	1.101	6.1486	6.1415	0.0014086	0.14085904	-7.27162841
Bau OC 6/25	#4	1.08	6.4051	6.4025	0.0004885	0.04884922	-7.892879339
Bau OC 6/25	#5	1.5714	6.8462	6.8151	0.0059309	0.59309266	-4.860008077

** H₂O Potential was determined from applying percent moisture to soil moisture curve equations seen in

Figure 13.

APPENDIX 3 (continued)

Percent Moisture July 1999

Sample	Sample #	Tin	Tin + Wet	Tin + Dry	Moisture	% Moisture	H ₂ O Potential**
BC Unoc 7/29	#1	1.0296	6.2578	6.25601	0.0003425	0.03424933	-87.86130379
BC Unoc 7/29	#2	1.5014	6.88273	6.86827	0.0026943	0.26942828	-91.11395098
BC Unoc 7/29	#3	1.6782	6.74788	6.73838	0.0018774	0.18773962	-92.32922016
BC Unoc 7/29	#4	1.6827	6.93267	6.92439	0.0015796	0.15796492	-93.76807637
BC Unoc 7/29	#5	0.9959	6.0177	6.01152	0.0012321	0.12321483	-86.61861604
BC OC 7/29	#1	1.4908	6.615	6.5996	0.0030144	0.30143888	-91.50646362
BC OC 7/29	#2	1.5303	6.73945	6.73019	0.0017808	0.17807966	-92.46945473
BC OC 7/29	#3	1.4911	6.60401	6.59612	0.0015455	0.15455436	-87.47500903
BC OC 7/29	#4	1.0294	6.15952	6.14523	0.0027933	0.27933015	-91.85065638
BC OC 7/29	#5	1.4937	6.60718	6.59852	0.0016964	0.16964293	-98.55873688
SAR Unoc 8/5	#1	1.4906	6.56179	6.56122	0.0001124	0.0112413	-43.30178092
SAR Unoc 8/5	#2	1.5301	6.68164	6.67998	0.0003223	0.03223351	-39.88870539
SAR Unoc 8/5	#3	1.5636	6.7449	6.74352	0.0002664	0.02664118	-40.77074543
SAR Unoc 8/5	#4	1.021	6.27886	6.27817	0.0001313	0.01312503	-42.98393623
SAR Unoc 8/5	#5	1.0309	6.41136	6.41077	0.0001097	0.01096687	-43.34828089
SAR OC 8/5	#1	1.6868	6.70009	6.69827	0.0003632	0.03631683	-39.25674713
SAR OC 8/5	#2	1.5015	6.61425	6.61244	0.0003541	0.03541416	-39.39558252
SAR OC 8/5	#3	1.0215	6.37716	6.37623	0.0001737	0.01736773	-42.27658121
SAR OC 8/5	#4	1.6066	6.7033	6.7005	0.0005497	0.05496738	-36.49521063
SAR OC 8/5	#5	1.5635	6.61382	6.61184	0.0003922	0.03922089	-38.81340071
SJ Unoc 8/5	#1	1.5791	6.97392	6.97317	0.000139	0.01390416	-19.39509324
SJ Unoc 8/5	#2	1.5297	6.78094	6.77914	0.0003429	0.03428911	-18.78993787
SJ Unoc 8/5	#3	1.4938	6.62218	6.62084	0.0002614	0.02613584	-19.02967967
SJ Unoc 8/5	#4	1.4895	6.64954	6.64833	0.0002345	0.02345498	-19.10917499
SJ Unoc 8/5	#5	1.5882	6.69937	6.6988	0.0001115	0.0111532	-19.47823804
SJ OC 8/5	#1	1.5014	6.58079	6.57835	0.0004806	0.04806054	-18.39183715
SJ OC 8/5	#2	1.6787	6.68077	6.67586	0.0009826	0.09825581	-17.01088256
SJ OC 8/5	#3	0.9964	6.29449	6.28965	0.0009144	0.09143738	-17.19220265
SJ OC 8/5	#4	1.5897	6.84769	6.84475	0.0005595	0.0559465	-18.16768123
SJ OC 8/5	#5	1.6823	6.8551	6.85044	0.0009017	0.09016784	-17.22617602
Bau Unoc 7/29	#1	1.6861	6.87155	6.87109	8.872E-05	0.00887169	-8.179090704
Bau Unoc 7/29	#2	1.4987	6.61292	6.6088	0.0008063	0.08062512	-7.672547376
Bau Unoc 7/29	#3	1.6059	6.92357	6.9194	0.0007848	0.0784792	-7.687231442
Bau Unoc 7/29	#4	1.5984	6.88301	6.87974	0.0006192	0.06191634	-7.801517023
Bau Unoc 7/29	#5	1.5242	6.81086	6.80303	0.0014833	0.14832888	-7.223391842
Bau OC 8/5	#1	1.5797	6.94193	6.93343	0.0015877	0.158769	-7.156510382
Bau OC 8/5	#2	1.031	6.30812	6.30122	0.0013092	0.13092308	-7.336289488
Bau OC 8/5	#3	1.5248	6.65676	6.64946	0.0014245	0.14244792	-7.261341326
Bau OC 8/5	#4	1.5983	6.61564	6.60425	0.0022753	0.2275306	-6.731216567
Bau OC 8/5	#5	1.5292	6.71656	6.71439	0.0004185	0.04184956	-7.942258711

** H₂O Potential was determined from applying percent moisture to soil moisture curve equations seen in

Figure 13.

Appendix 4: Summary of percent infection data from Spineflower roots sampled in the field.

June 1998										
Sample ^a	No. Observations	1°/2° Root ^b	AM ^c	Arbuscules	Vesicles	%AM ^c	%NM ^d	% <i>Phytophthora</i> ^e		
Orange St. 1	100	1°	9	0	2	15	15	0		
Orange St. 2	70	1°	12	0	0	17	16	3		
San Jacinto 1	8	2°	0	0	0	0	8	0		
San Jacinto 2	58	2°	0	0	0	0	43	3		
San Jacinto 3	8	2°	0	0	0	0	8	0		
San Jacinto 4	92	1°	1	0	0	1	35	13		
San Jacinto 5	34	2°	1	0	0	3	20	0		
San Jacinto 6	100	1°	5	0	2	7	12	15		
July 1998										
San Jacinto 1	46	1°	16	0	4	43	0	0		
San Jacinto 2	79	1°	30	0	0	38	5	2		
San Jacinto 3	20	1°	0	0	0	0	7	0		
San Jacinto 4	58	1°	12	0	5	29	7	2		
San Jacinto 5	25	1°	5	0	0	20	2	0		
Bautista Creek	45	1°	4	0	4	18	2	0		
April 1999 Two secondary roots per plant x five plants/site = 1 sample										
Bee Canyon	20	2°	3	0	0	15	0	0		
Santa Ana River	63	2°	18	0	9	43	3	0		
San Jacinto	82	2°	25	0	12	45	5	0		
Bautista Creek	8	2°	2	0	0	25	0	0		

^aSamples were labeled by site and number. April 1999 samples were combined by site.

^bBoth thicker primary (1°) roots and thinner (2°) roots were sampled.

^cArbuscular-mycorrhizal fungi (AM) were identified by aseptate or irregularly septate hyphae that form internal structures.

^dNon-mycorrhizal fungi (NM) were identified by regularly septate hyphae present in root cortical cells but with no internal structures.

^e*Phytophthora* is a group of soil-borne pathogenic fungi included in NM, identified by reproductive structures external to root cortical cells.

Appendix 5: Root percent infection results of Spineflower and Blue Corn grown in pot cultures with the AM fungus, *Glomus clarum*. Data were summarized per individual plant (n = 7 Spineflowers, n = 10 Blue Corn) and per growth chamber (n = 5 Spineflowers, n = 5 Blue Corn) to avoid pseudoreplication of data.

Pot No.	Plant Species	% Infection per plant	Plant Species	% Infection per chamber (avoids pseudoreplication)
Pot #1	Spineflower	62	Spineflower	68
Pot #1	Spineflower	74	Spineflower	--
Pot #2	Spineflower	72	Spineflower	72
Pot #3	Spineflower	74	Spineflower	74
Pot #4	Spineflower	66	Spineflower	70
Pot #4	Spineflower	74	Spineflower	--
Pot #5	Spineflower	58	Spineflower	58
Pot #6	Blue Corn	51	Blue Corn	63
Pot #6	Blue Corn	74	Blue Corn	--
Pot #7	Blue Corn	60	Blue Corn	67
Pot #7	Blue Corn	73	Blue Corn	--
Pot #8	Blue Corn	60	Blue Corn	59
Pot #8	Blue Corn	58	Blue Corn	--
Pot #9	Blue Corn	77	Blue Corn	67
Pot #9	Blue Corn	56	Blue Corn	--
Pot #10	Blue Corn	60	Blue Corn	62
Pot #10	Blue Corn	64	Blue Corn	--

-- signifies no data because preceding value = average of data for two plants.



Figure 1. A Slender-horned Spineflower (*Dodecahema leptoceras*) in bloom. This plant was one of thirteen grown in the Soil Ecology and Restoration Group greenhouse that never produced seed.

original
figures



Figure 3a: Bee Canyon site, the most northern location of Spineflower populations. This view is north toward Highway 14 in northern Los Angeles county.



Figure 3b. Santa Ana River site characterized by silty shallow depressions at the bottom of a wash (top) and a close up of these depressions with Spineflowers growing in sparsely vegetated microhabitat (bottom).



Figure 3c. Spineflowers at San Jacinto site characterized by very large plants (top) and heavy off-road vehicle use as evidenced by numerous trails (bottom).



Figure 3d. Spineflowers at Bautista Creek, with plants appearing among grasses (top) and on typical sparsely vegetated patches of soil (bottom).



Figure 4. Pot cultures with Spineflower (top) and blue corn, the control (bottom).

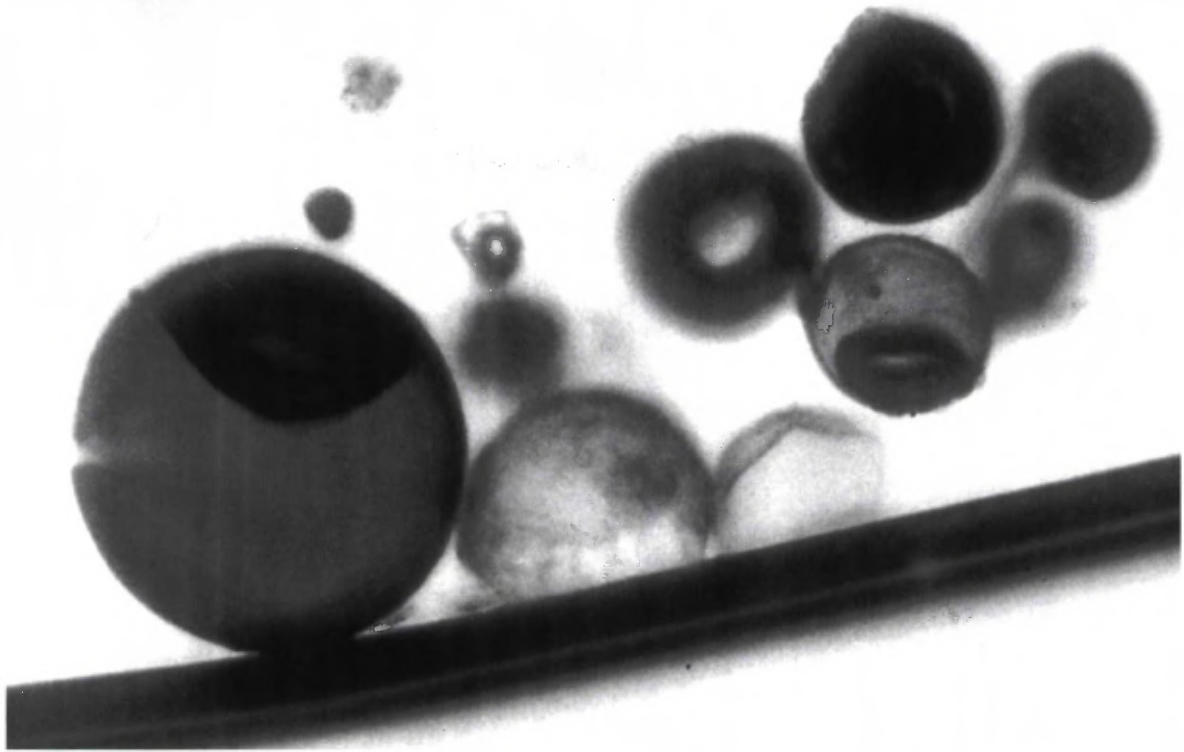


Figure 7. Variety of *Glomus* spores extracted from Cone Camp soil core viewed at 100x magnification.

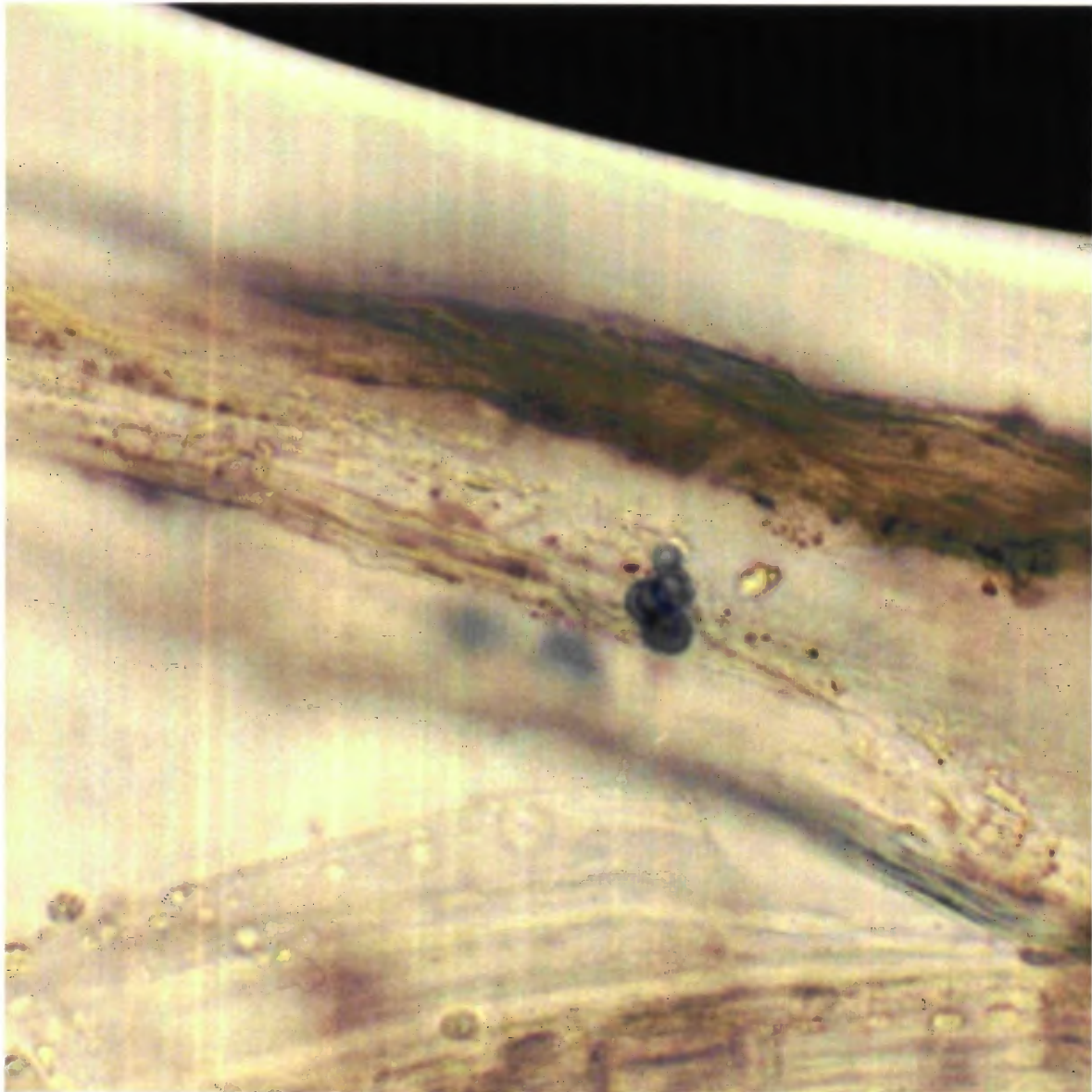


Figure 11. Reproductive structures possibly belonging to *Phytophthora* found on Spineflower root from the San Jacinto site.

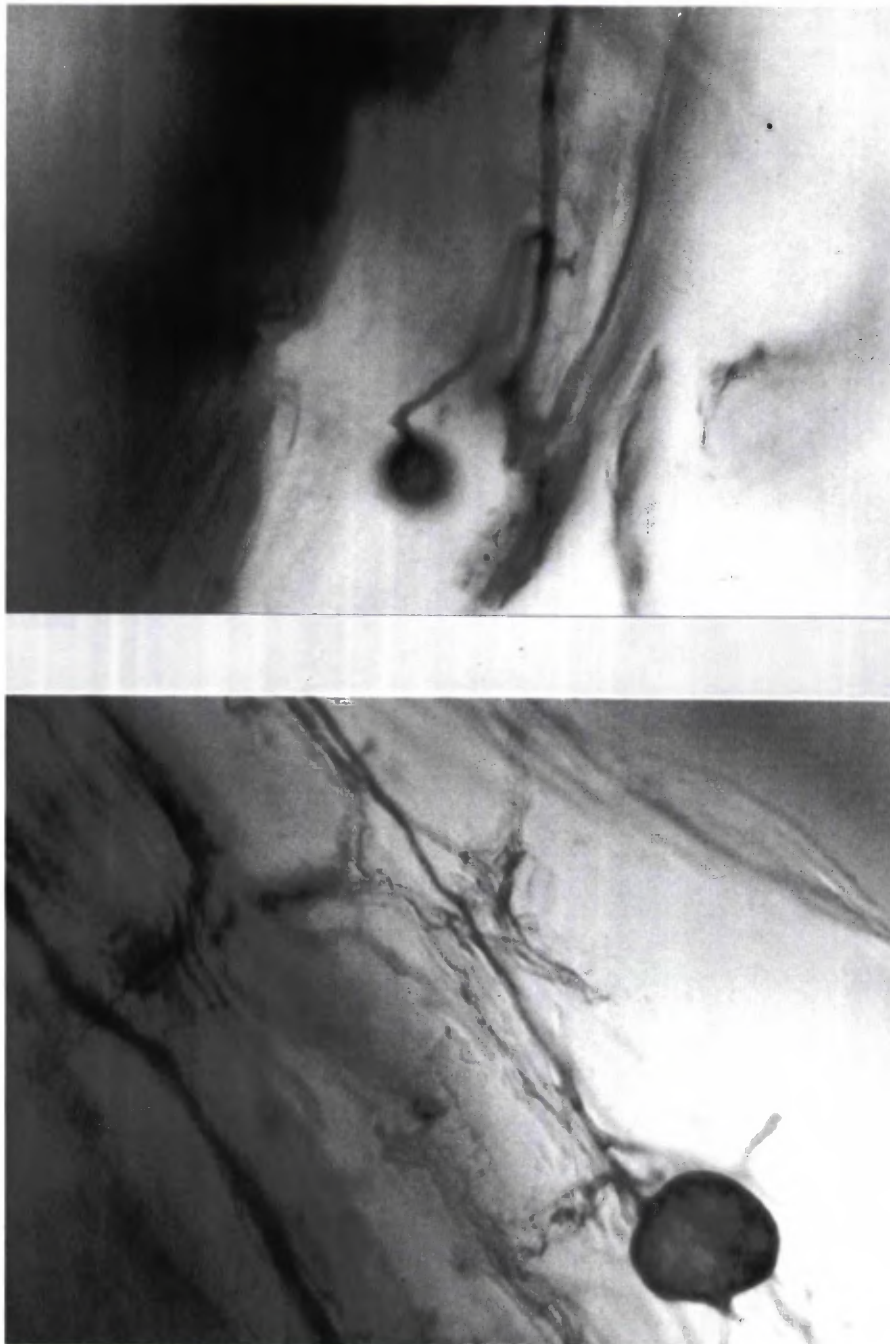


Figure 12. AM fungal hyphae and vesicles present within Spineflower root cortex cells at 200x magnification.

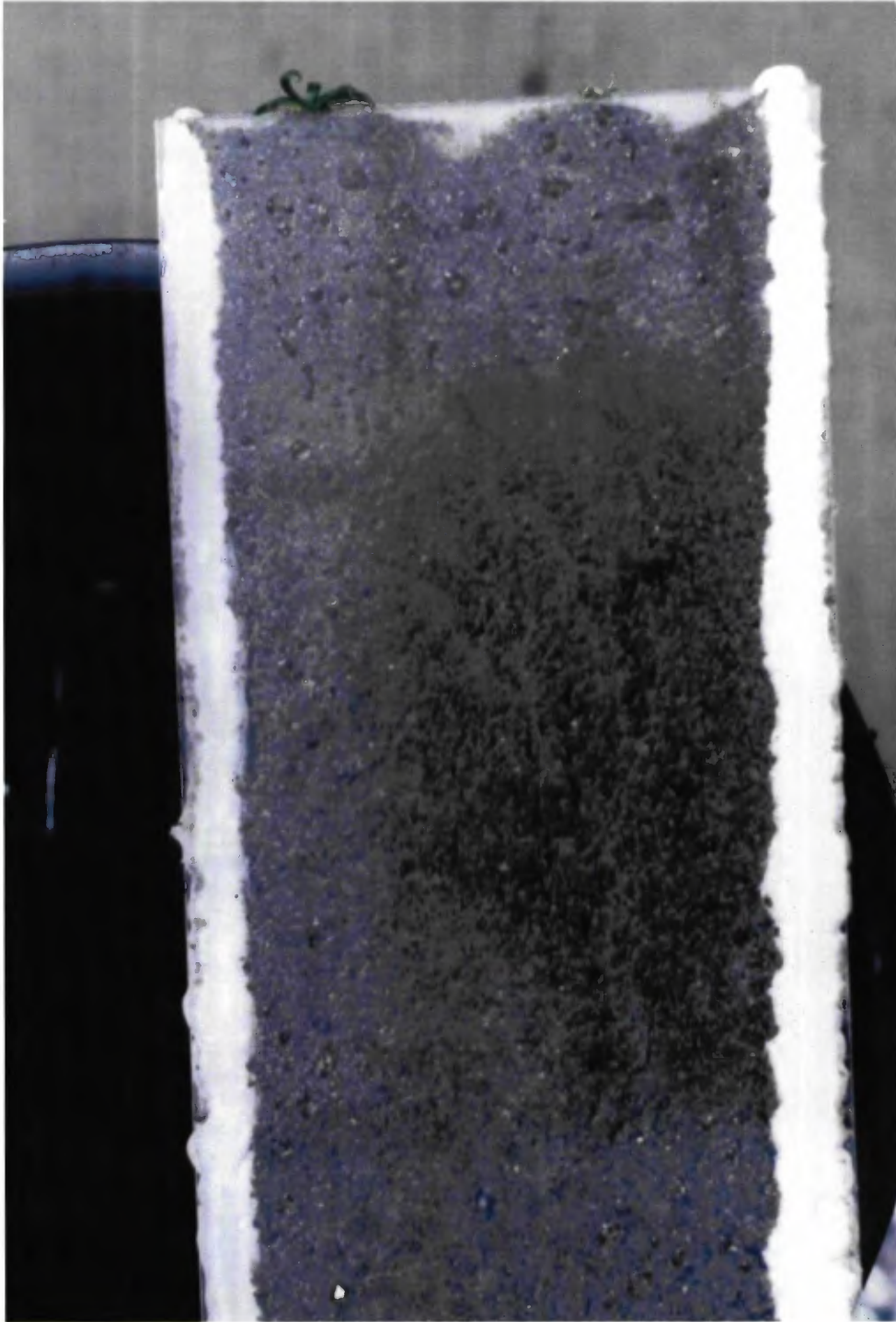


Figure 13. Spineflower roots growing against a glass growth chamber constructed for pot cultures.

Percent Moisture August 1998

Sample	Sample #	Tin	Tin + Wet	Tin + Dry	Moisture	% Moisture	H ₂ O Potential**
SAR 12/2	#1	1.573	6.6066	6.5935	0.0026093	0.26093019	-16.30807709
SAR 12/2	#2	1.673	6.8497	6.8386	0.00214883	0.21488307	-19.52607542
SAR 12/2	#3	1.6826	6.8767	6.8666	0.0019483	0.19483025	-21.11909008
SAR 12/2	#4	1.5948	6.6326	6.625	0.00151087	0.15108743	-25.05956332
SAR 12/2	#5	1.6993	6.7105	6.7035	0.00139882	0.1398825	-26.18215359
SJ 11/25	#1	1.5919	6.7636	6.5392	0.04535807	4.53580741	-0.017136588
SJ 11/25	#2	1.0626	6.262	6.142	0.02362484	2.36248376	-0.503072406
SJ 11/25	#3	1.7826	6.8732	6.7847	0.01769257	1.76925691	-1.265473772
SJ 11/25	#4	1.5873	6.6066	6.5237	0.01679361	1.67936148	-1.455331886
SJ 11/25	#5	1.6002	6.6556	6.6024	0.01063532	1.06353205	-3.791829962
Bau 11/25	#1	1.5829	6.6452	6.576	0.01385913	1.38591256	-2.398006034
Bau 11/25	#2	1.5975	6.8197	6.6374	0.03617135	3.61713526	-0.328446323
Bau 11/25	#3	1.5892	6.7816	6.6719	0.02158302	2.15830169	-1.204950946
Bau 11/25	#4	1.685	6.7885	6.7671	0.00421086	0.42108577	-5.664950975
Bau 11/25	#5	1.5959	6.8563	6.8038	0.01008084	1.00808387	-3.357789708

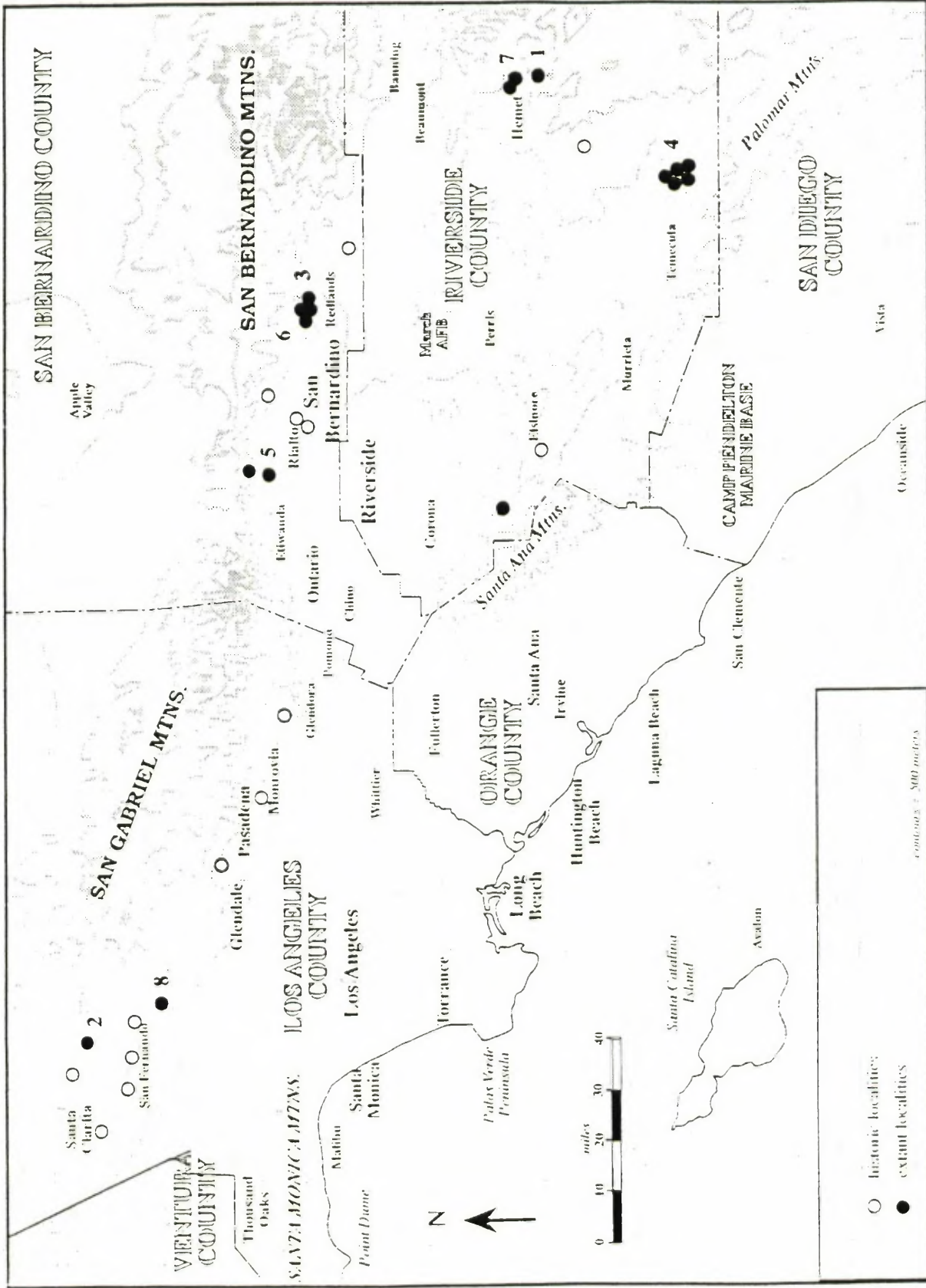
* Bee Canyon not selected as study site until 15 September 1998.

Percent Moisture October 1998

Sample	Sample #	Tin	Tin + Wet	Tin + Dry	Moisture	% Moisture	H ₂ O Potential**
BC 1/5	#1	1.07	6.13	6.09	0.00796813	0.79681275	-69.48239333
BC 1/5	#2	1.56	6.78	6.74	0.00772201	0.77220077	-70.24756999
BC 1/5	#3	1.56	6.69	6.65	0.00785855	0.78585462	-69.82204256
BC 1/5	#4	1.57	6.58	6.52	0.01212121	1.21212121	-57.75795686
BC 1/5	#5	1.75	6.93	6.84	0.01768173	1.76817289	-45.09710822
SAR 1/5	#1	1.58	6.6	6.58	0.004	0.4	-9.466492612
SAR 1/5	#2	0.93	6.07	6.06	0.00194932	0.19493177	-21.11070595
SAR 1/5	#3	1.6	6.15	6.14	0.00220264	0.22026432	-19.11942293
SAR 1/5	#4	1.5	6.71	6.7	0.00192308	0.19230769	-21.32847609
SAR 1/5	#5	1.55	6.18	6.17	0.0021645	0.21645022	-19.40676417
SJ 1/5	#1	1.48	6.82	6.6	0.04296875	4.296875	-0.024847465
SJ 1/5	#2	1.54	6.68	6.57	0.02186879	2.18687873	-0.661030467
SJ 1/5	#3	1.48	6.8	6.72	0.01526718	1.52671756	-1.845214022
SJ 1/5	#4	1.58	6.64	6.58	0.012	1.2	-3.06681963
SJ 1/5	#5	1.55	6.64	6.59	0.00992063	0.99206349	-4.23753759
Bau 1/5	#1	1.56	6.66	6.61	0.00990099	0.99009901	-3.412030079
Bau 1/5	#2	1.53	6.93	6.82	0.02079395	2.07939509	-1.29271498
Bau 1/5	#3	1.39	6.57	6.48	0.01768173	1.76817289	-1.705816053
Bau 1/5	#4	1.47	6.62	6.6	0.00389864	0.38986355	-5.824756768
Bau 1/5	#5	1.4	7.01	6.96	0.00899281	0.89928058	-3.699607268

** H₂O Potential was determined from applying percent moisture to soil moisture curve equations seen in

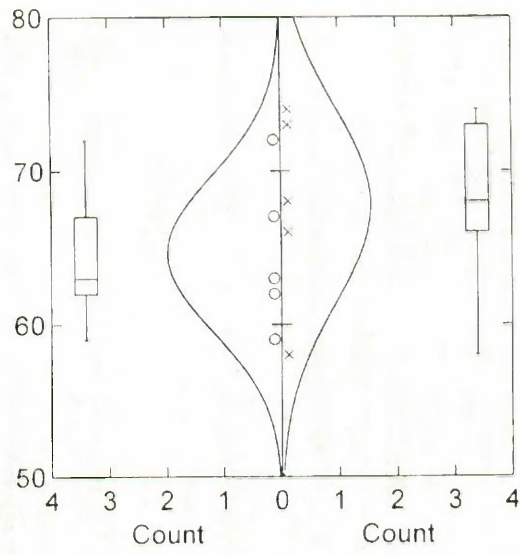
Figure 13.



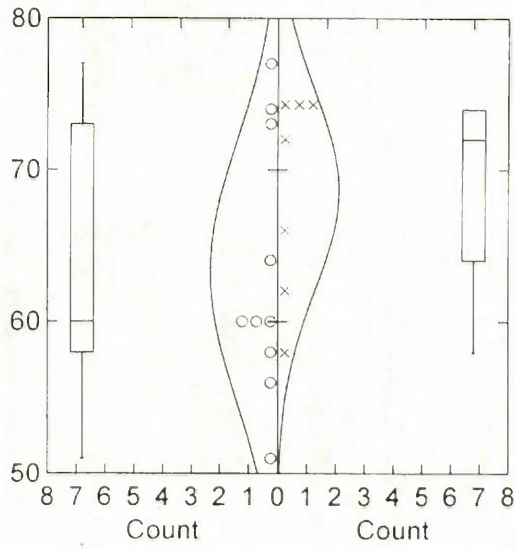
- historic localities
- extent localities

continues on 500 meters

Percent Infection



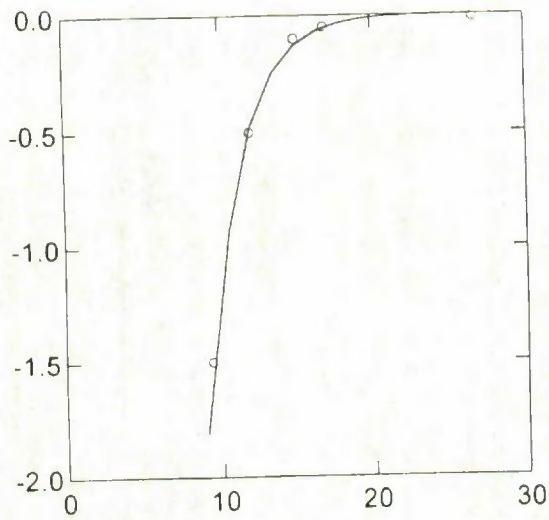
○ Blue corn
× Spfl



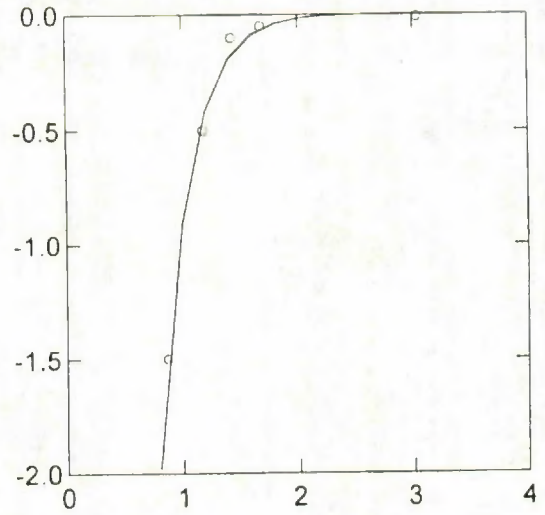
○ Blue corn
× Spfl

Number of Samples Per Y-Value

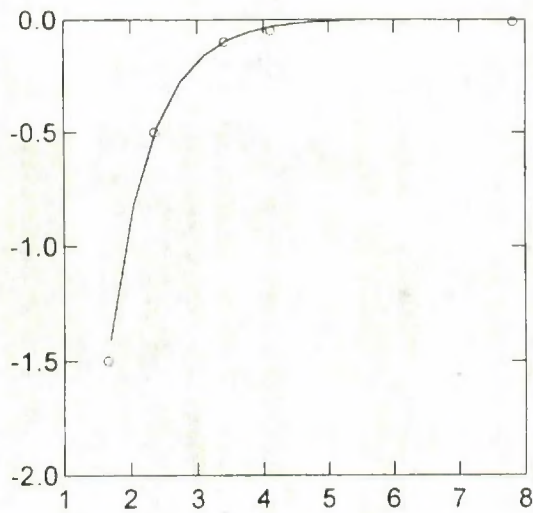
ψ_m (MPa)



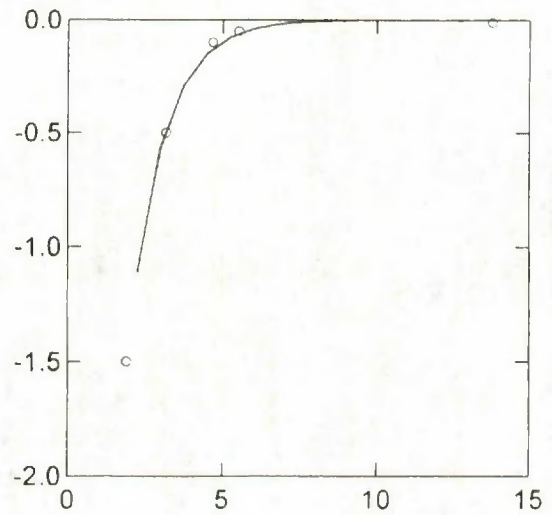
$$y = -99.053 * e^{-0.445x} \quad (R^2 = 0.999)$$



$$y = -45.248 * e^{-3.911x} \quad (R^2 = 0.997)$$



$$y = -19.819 * e^{-1.555x} \quad (R^2 = 1.0)$$



$$y = -8.244 * e^{-0.891x} \quad (R^2 = 0.999)$$

Percent Moisture