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DOE-PER Final Report

"Genetic Analysis of Interacting Trophic Levels in a Stressed Pinyon-Juniper Community: A Model for examining Community Responses to a Rapid and Recent Environmental Changes"

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The goals of this project were to examine the genetic component of a pinyon-juniper woodland that had recently experienced a dramatic environmental change. The environmental change was increased temperature and decreased water associated with the volcanic cinder field at Sunset Crater National Monument. In all of these experiments we have used adjacent soil sites as controls for the effects of the stressed locations. We have examined mycorrhizal colonization and diversity in order to understand this important component in community "adaptation" to climate change. We have examined genetic diversity in the pinyon pine populations to determine what level of genetic differentiation has occurred between stressed and nonstressed locations. In addition, we have recently expanded our environmental parameters to include elevated CO₂ on mycorrhizal performance and diversity.

Mycorrhizal differences due Climate Change

A major question asked in this proposal was: **Do the mycorrhizal symbionts change in a qualitative or quantitative fashion with changes in climate?** We have approached this question in both the field and in greenhouse studies. The climatic conditions that we are most interested in are water, temperature, soil and atmospheric CO₂ concentrations. The first three environmental parameters were tested in field studies while the last was explored in greenhouse trials. We have used quantitative estimations of mycorrhizal colonization, morphological identification techniques and molecular genetic analysis to characterize the mycorrhizae. The combination of these approaches is unequaled by any other research group at this time.

Morphological and Molecular Characterization of Mycorrhizae

As a rapid field screening tool, morphological discrimination among major mycorrhizal types was established. Examination of several thousand ectomycorrhizal root tips from pinyon pine revealed four major morphological categories. The morphological discriminators included color, texture and shape. DNA sequencing of 25 representative isolates in the rDNA ITS region, revealed phylogenetically coherent morphological groups. Three morphological groups were found in the Basidiomycotina while one was

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found in the Ascomycotina. As a crude level, species diversity can be evaluated using morphological examination, though a combination of morphology and molecular analysis is best.

Mycorrhizal Community Dynamics Related to Environmental Contrasts

We have used RFLP analysis of the ITS regions of ectomycorrhizal fungi to quantify diversity in this community and how it relates to the two environmental types. We analyzed six location - three cinder and three soil. These sites were pair such that the geographic distance between a soil-cinder pair was ca. 1 mile but the distance separating pairs was > 10 miles. This allows us to detect differences due to spatial differences as opposed to difference based upon the environment. We found the soil types were dominated by one or a few abundant ectomycorrhizal types. These were different between soil types, but not between replications within a soil type. Interestingly, the differences were strongly biased towards basidiomycotina (85%) at the soil locations and towards the ascomycotina on the cinder sites (57%). Cluster analysis of the locations based upon ectomycorrhizae revealed the strong correlation between like soil type communities. This study is one of the most comprehensive studies to document a community shift due to abiotic forces (Gehring et al. 1997).

Pinyon Pine Genetic Analysis

As part of our research to understand the effects of altered climate on the Pinyon-Juniper woodland community, we asked the question: **Are there genetic difference between pinyon populations on cinder sites and adjacent soil sites?** Our logic is that pinyon pine must have undergone genetic changes in order to adapt to the hotter and drier cinder sites.

Our approach was to use a multi-locus DNA fingerprinting technology called Amplified Fragment Length Polymorphism (AFLP) to estimate genetic similarity between the locations and to estimate genetic differentiation. As a part of this strategy, we wanted to establish AFLP markers are Mendelian genetic characters and estimated the genomic arrangement of these marker loci. As an approach to this goal, we constructed a genetic map using 40 haploid megagametophyte tissues from a single individual. These are essentially the female meiotic product (like and egg or ovule) and a collection of these represent a "Mendelian family" that can be used for chromosome/genetic mapping. The genetic map contains 25 genetic linkage groups, covering 2,012 centimorgans, and has linked together over 338 AFLP markers (attached figure; Travis et al. 1997). This work establishes the validity of using AFLP markers for population differentiation estimates.

Population differentiation between the soil and cinder was estimated using 137 AFLP markers in order to accurately estimate the level of differentiation. Two statistical analyses were performed on these data; Analysis of Molecular Variance (AMOVA) and theta calculation. Statistical significance was established in both cases through permutations tests on the data themselves (i.e. bootstrapping). The multilocus theta values was 0.013 with statistical significance of $p < 0.00005$. This level of differentiation was low,

but a control study using 231 AFLP markers between two sets of individuals on the cinder site had a $\theta = 0.000$ with $p=0.309$. Thus, these studies indicate low level but significant genetic differentiation between soil and cinder locations. Consistent with the idea that pinyon pines have genetically adapted to the cinder locations.

Increased Carbon Dioxide affect on Mycorrhizae

This work was initiated in the last year to the proposal in collaboration with Dr. George Koch and Mr. Jean-Marc Patnoe. The basic experimental design was to test mycorrhizal colonization on pinyon seedlings in a greenhouse treatments where the CO₂ is either ambient or elevated to twice ambient. The pinyon seeds were blocked for source effects and sampling was carried out over a 6 month experimental time frame. We evaluated the effect of CO₂ by examining mycorrhizal colonization, seedling growth, morphology of mycorrhizal colonist and the molecular genetic characterization of the symbionts. These experiments are still in progress, but quantitative estimates of colonization indicate a marked effect of CO₂ treatment. We are currently using molecular analysis to determine if the quantitative effect is reflected the diversity of fungal types observed.

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