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Evaluating Fungal Pathogen Inoculum Loads in Field Seed Banks

Taryn Lori Williamson
Brigham Young University

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Taryn Lori Williamson

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Craig Eliot Coleman, Chair
John M. Chaston
Susan E. Meyer

Department of Plant and Wildlife Sciences

Brigham Young University

ABSTRACT

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Taryn Lori Williamson
Department of Plant and Wildlife Sciences, BYU
Master of Science

Quantification of soilborne pathogen inoculum loads is important in both agricultural and wildland settings. Quantitative Polymerase Chain Reaction (qPCR) methods using SYBR Green chemistry have been shown to be useful for quantifying fungal inoculum loads in environmental samples. The purpose of this study was to develop a method to quantify fungal pathogen inoculum loads in soil seed banks using a qPCR method with SYBR Green chemistry. The invasive annual grass *Bromus tectorum* was chosen for this seed bank study. There were three objectives: 1) to design target-specific primers for three fungal pathogens known to be important in *Bromus tectorum* seed banks, 2) to develop a procedure for measuring inoculum loads in field samples, including optimization of qPCR standard curves and protocols, for these pathogens, and 3) to perform qPCR using this methodology on a representative set of field samples to quantify pathogen DNA in seed bank soil and surface litter. The three pathogens were chosen for quantification based on their hypothesized roles in *Bromus tectorum* stand failure: the seed pathogen *Pyrenophora semeniperda*, an undescribed species of *Fusarium* seed rot pathogen belonging to the *F. tricinctum* species group (FTSG), and the newly-described causal agent of bleach blonde syndrome (*Clarireedia capillus-albis*). Primers designed for each pathogen were shown to be target-specific in tests against each other and 12 other fungal species cultured from *B. tectorum* seed banks. Subsequently developed standard curves for each pathogen had R^2 values > 0.98, efficiencies between 90 and 110 percent, and generally optimal dissociation curves. Inoculum loads were expressed for each pathogen as picograms of DNA per microliter of extracted soil or surface litter. Significant differences in measured inoculum loads were found between the targeted pathogens and between soil and litter samples for each pathogen. The data provided reinforces that the SYBR Green qPCR method provides a potentially useful tool for the study of field seed and seedling diseases across a wide spectrum of both wildland and agronomic applications.

Keywords: qPCR, cheatgrass, *Bromus tectorum,* stand failure

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INTRODUCTION

Quantifying fungal pathogen inoculum loads in soil is important, challenging, furthers research on their biology, and can help control and monitor prevalence of the disease.

Many methods have been developed to identify and quantify soilborne fungal pathogens leading up to current methods of quantitative polymerase chain reaction (qPCR). Conventional methods are centered on culture and isolation of fungi, for example, from the soil, and when combined with dilution plate methods on selective media, quantification of pathogens is attainable (1, 2). Limitations of this approach include: Not all fungi can be detected under standard laboratory conditions, this method relies on the accuracy and expertise of the individual giving the diagnosis, and this method can be time consuming when results are needed promptly (3, 4).

An immunological technique, enzyme-linked immunosorbent assay (ELISA), has been used for quantification of pathogens in soil through the recognition of antigens present by antibodies, and through monitoring of population changes of inoculum over time and space, ultimately the behavior of the fungal pathogen in the soil can be quantified (5, 6, 7). One limitation of these approaches is that they are expensive and require highly optimized, specific assay kits (4).

Molecular methods have been developed to improve specificity and sensitivity when identifying and quantifying pathogens in soil. One method is PCR combined with denaturing gradient gel electrophoresis (DGGE). van Elsas et al. (8) and Ma et al. (9) both used this method targeting the 18s ribosomal RNA genes for detection and analysis of the pathogens in soils. This method is not flawless, in that not all fungal groups can be determined through this technique (8).

Metagenomics is another molecular approach to quantify pathogens in soil. Metagenomics allows for a direct analysis of genomes within an environmental sample through high-throughput sequencing (10). Shotgun metagenomics examines all DNA and the collective microbial genome in the sample, whereas marker gene metagenomics targets only certain genes through PCR (10). Moussa et al. (11) looked at the microbiome of fungi in soil in Saudi Arabia. The ITS1/2 and ITS3/4 regions were amplified and pyrosequencing was used for sequencing. Panelli et al. (12) also used metagenomics to analyze fungal communities in Italian soils. The ITS1 region was amplified and Illumina sequencing was carried out in this metagenomics method. Metagenomics can be a powerful tool when looking at fungal communities, however, variations, including PCR amplification biases, sequencing and software errors, can lead to inaccurate results (10).

Increased accuracy and sensitivity for detecting and quantifying pathogens has been demonstrated with using qPCR coupled with SYBR Green chemistry relative to previously stated methods. For example, this particular method was used to detect and quantify pathogens by (13) targeting the ITS 1 and ITS 2 regions of *Exophiala jeanselmei* in air-conditioners water reservoir samples. Tests were conducted on the limit of detection, selectivity of primers, efficiency, dynamic range, and repeatability. Al-gabr et al. (14) studied fungal pathogens in multiple water sources in Xiamen, China. Fungi were identified phenotypically and by sequencing the ITS region, while quantification was carried out by targeting the ITS and 18s regions through qPCR.

SYBR Green qPCR has also been used to quantify fungal pathogens in soil. Filion et al. (15) targeted the *EF1α* gene of *Fusarium solani* f. sp. *phaseoli* and the SSU rRNA gene region of *Glomus intraradices*. Quantitative PCR was run on DNA from sterile and non-sterile soil, and the products were run on an agarose gel. Lievens et al. (16) targeted the ITS 1 and ITS 2 regions of oomycete tomato pathogens in soil. The relationship between inoculum density and quantified

DNA was examined in artificial and naturally infested samples. Kernaghan (17) targeted ITS 1 and 2 regions to quantify *Pythium* in ginseng soils. In naturally occurring samples each crude DNA extraction was split into 3 replicate extractions to increase precision in samples that had lower concentrations of DNA. The results of qPCR were analyzed on agarose gels. This qPCR method was also combined with dilution plating to quantify the pathogens. Kurth et al. (18) used SYBR Green qPCR to quantify and study the relationship between a basidiomycete fungus, *Piloderma croceum,* and a helper bacterium. Primers were designed in both the ITS region and an intergenic region of *P. croceum*. Both primer pairs were found to work effectively in quantifying the specific fungus.

The qPCR methods reviewed above have been adapted here for quantifying seed bank pathogens. Utilizing SYBR Green with qPCR to detect and quantify seed bank pathogens could have utility in both agricultural and natural ecosystems. The work reported here on methods development is part of a larger study focused on *Bromus tectorum* (cheatgrass) and how fungal pathogen abundance in the soil changes over time. The pathogens chosen for this study were chosen based on their putative relationship with cheatgrass stand failure, but the methods described are not specific to cheatgrass or the pathogens discussed.

Cheatgrass stand failure occurs when the in situ seed bank fails to produce cheatgrass plants even under favorable conditions (19). It is not known for certain what causes cheatgrass stand failure, but there are three fungal seed pathogens believed to play a role: an undescribed *Fusarium* species belonging to the *F. tricinctum* (FTSG) group, *Pyrenophora semeniperda*, and *Clarireedia capillus-albis* sp.n. (20, 21). The majority of *Fusarium* isolates identified in stand failure sites were primarily found to be in the *tricinctum* group, but other isolates were related to

other strains, allowing for the *Fusarium* species to not be positively identified at the species level (22). The *Fusarium* species will be referred to as the *Fusarium* sp. throughout this paper.

The *Fusarium* sp. identified is widely found in stand failure sites. *Fusarium* has been found to be the most abundant genus of fungi isolated from stand failure soils, with *Fusarium* from the FTSG most frequently found (22). This pathogen is able to cause seed death and targets non-dormant seeds (23, 24). *Pyrenophora semeniperda* is not a primary player in directly causing cheatgrass stand failure, but it could have a major impact on the post-stand failure seed bank and therefore on stand recovery (24). Furthermore, *P. semeniperda* can also negatively affect the seeds of native grasses. The pathogen uses cheatgrass as a reservoir from which it may spill over and impact native grasses (25). *Clarireedia capillus-albis* causes bleaching, sterility, and premature death in cheatgrass (20). This pathogen is closely related to the disease dollar spot (*Clarireedia homoeocarpa*), where individual leaves of the grass are bleached within circular areas about the size of a silver dollar (26, 27).

This study developed a method to quantify fungal pathogen inoculum loads in soil seed banks using SYBR Green qPCR. Methods were developed as part of a larger study focused on quantifying seed bank fungal pathogens to determine how inoculum load for each pathogen varies as a function of time since stand failure. However, the method developed here is broadly applicable to quantification of any fungal seed bank pathogen and also to other soilborne fungal pathogens and soil fungi in general. Study objectives were: 1) to design target-specific primers for three fungal pathogens known to be important in *Bromus tectorum* seed banks, 2) to develop a procedure for measuring inoculum loads in field samples, including optimization of qPCR standard curves and protocols, for these pathogens, and 3) to perform qPCR on a representative set of field samples to quantify pathogen DNA in seed bank soil and surface litter.

MATERIAL AND METHODS

The process of developing and executing the methodology to obtain quantitative data on inoculum loads for specific pathogens in field seed bank soil and litter samples using qPCR with SYBR Green chemistry is described as a series of six steps (Fig. 1).

Step 1. Primer Design

Primers were designed using the RealTime qPCR Assay Entry on-line tool (Integrated DNA Technologies, Coralville, Iowa). Primers for *P. semeniperda* were designed from the ITS region. Because ITS is not adequate for species identification in *Fusarium* and because specificity problems were encountered with the use of ITS-based primers for *C. capillus-albis*, primers for these two pathogens were designed to amplify a portion of the *EF1α* gene. Primers for *Fusarium* were designed to be specific to the *Fusarium tricinctum* species group (FTSG). Primer parameters included length between 18-30 base pairs and T_m of 59-62 °C. Additional design parameters included an amplicon length range of 70-150 bp and a primer GC content between 35-65% (Table 1).

Step 2. Primer Specificity Testing

Isolates from target and non-target species were used to determine primer specificity. One isolate for each target pathogen was selected from an existing collection of previously identified strains (Table 2). Non-target fungal species were cultured from soil samples obtained from stand failure sites (Table 3). Isolates were grown on potato dextrose agar in pure culture, transferred to liquid culture for five days, then centrifuged and dried as described in Boose et al. (28). DNA

was extracted from the dried fungal mycelium using the ZR Fungal/Bacterial DNA Mini Prep kit and protocol (Zymo Research, Irvine, California). The DNA was quantified using the Qubit dsDNA BR Assay kit and protocol and a Qubit Fluorometer (Invitrogen, Eugene, Oregon).

Non-target isolate DNA was amplified with the ITS4 and ITS5 primers (29) using Mytaq Red Mix, 2x Master Mix (Bioline, London, United Kingdom). Cycling conditions and protocol followed manufacturer's recommendations. The reaction was then run on a 1 percent agarose gel to determine amplification. The DNA was cleaned using a DNA Clean & Concentrator -5 kit (Zymo Research, Irvine, California) and sent to the BYU DNA Sequencing Center for Sanger Sequencing. The GenBank nonredundant database was searched using BLAST within the Geneious software package (Biomatters, Auckland, New Zealand) to identify the non-target isolates (Table 3).

Primers were tested for amplification with their respective target species as well as with each other target and non-target species, to ensure that target species amplified but that cross-amplification did not occur. All PCRs were performed using HotStarTaq Master Mix (Qiagen, Germantown, Maryland), and run on Master Cycler Pro thermocyclers (Eppendorf, Hauppauge, New York). Reaction and thermocycler parameters were set according to manufacturer's recommendations. Amplicons were visualized on 3% MetaPhor Agarose gels (Lonza, Rockland, Maine) run in 1X TBE for 60 minutes at 90 V.

Step 3. Quantitative PCR Standard Curve Development

Unlike conventional PCR, where DNA analysis is conducted at the end, qPCR monitors the reaction in real-time, quantifying after each cycle. This is accomplished with the use of the

fluorescent dye SYBR Green. SYBR Green binds to any double stranded DNA and releases a fluorescence signal that reflects the amount of product formed. A C_t (cycle threshold) value is defined as the number of amplification cycles required to reach a threshold where the fluorescence signal is statistically significant from the background noise (30). A smaller C_t value means that fewer cycles are required to detect the signal. This in turn means that the initial concentration of the target DNA was higher. Thus, the number of cycles required to reach the detection threshold is a direct function of initial concentration.

Standard curves for the target amplicon of each pathogen were generated using SYBR Green PCR Master Mix and the 7300 Real Time PCR System machine (Applied Biosystems, Foster City, California). Standard curves were initially generated with 25 µL solutions. Each solution contained 12.5 µL of SYBR Green PCR master mix, 7 µL of double deionized water, 1.5 µL (15 pmol) of forward primer, 1.5 µL(15 pmol) of reverse primer, and 2.5 µL of template DNA extracted from fungal cultures grown in the lab. The template DNA was prepared at 5 dilutions that varied in 10-fold intervals from 1ng to 100 fg. To generate standard curves each dilution, including a no template control, were run in duplicate. Thermocycling conditions were as follow: 10 minutes at 95 °C, 15 seconds at 95 °C for 40 cycles and one minute at 60 °C, followed by a dissociation stage of 15 seconds at 95 °C, 30 seconds at 60 °C, and 15 seconds at 95 °C. The Applied Biosystems 7300/7500 SDS software package (Version 1.2.3) was used to obtain C_t values and dissociation curves for each template dilution, along with an R^2 value for the plot of template dilution x C_t value (standard curve). The R^2 value is a measure of the reliability of the relationship expressed in the standard curve. The dissociation curve is a measure of the homogeneity of the PCR product, which in turn is a check on the formation of nonspecific product such as primer-dimers during the reaction. This is important because SYBR Green is not sequence-specific, but instead causes fluorescence of any product that is formed. The efficiency

of the run was determined by using an online qPCR Efficiency Calculator from Thermo Fisher Scientific. Efficiency is a measure of the ability of the PCR product to double in quantity with each cycle as predicted by theory.

Step 4. Sample Collection and Processing

Samples for methods development were collected from one site in Skull Valley, south of Dugway, Utah (*P. semeniperda*: 40.13880 -112.71130) and two sites in Buena Vista Valley (Dun Glen Valley) west of Winnemucca, Nevada (*Fusarium* sp.: 40.69839 -118.04401, *C. capillus-albis*: 40.69445 -117.93802) as part of a larger study of *B. tectorum* stand failure history. At each site, seedbank surface litter and soil samples were collected with tin sampling cans and placed in paper bags. Along four 10-meter transects, nine random positions were sampled. Surface litter and soil samples from each transect were randomly bulked in groups of three for a total of 12 surface litter and 12 soil samples per site.

Samples were dried at room temperature and ambient humidity (ca, 35%) for at least three days. Rocks were removed by screening and samples were homogenized in a coffee grinder. A subsample was taken from each homogenized sample (0.22 g of soil or 0.08 g of litter). DNA was extracted from these subsamples using the Quick-DNA Fecal/Soil Microbe Microprep (Zymo Research, Irvine, California) and quantified using the Qubit fluorometer.

Step 5. Quantitative PCR with Field Samples

Quantitative PCR was performed using an optically clear 96-well plate (Thermo Fisher Scientific, Waltham, Massachusetts). Each plate consisted of two replicates of the serial dilutions

and the no template control for the standard curve, for a total of twelve wells. The remaining wells accommodated the 24 samples for one site (12 soil samples and 12 surface litter samples) run in triplicate (three technical replicates per sample) for measurement of the initial concentration of DNA for the target pathogen in each sample.

The 7300 Real Time PCR System machine was used to carry out the quantification of the targeted fungal pathogen. Each reaction consisted of 20 µL solutions. Each solution contained 1X SYBR Green PCR master mix, 1 µL deionized water, 1.5 µL (15 pmol) of forward primer, 1.5 µL (15 pmol) of reverse primer, and 6µL of DNA. The DNA from each sample was standardized to 30 ng/µL to minimize variation among technical replicates. The thermocycling conditions were the same as those used for standard curve development described earlier. The SDS software package was used to obtain a C_t value and a dissociation curve for each sample, along with an R^2 value for the standard curve. The efficiency of each run was determined by using the online qPCR Efficiency Calculator from Thermo Fisher Scientific. The SDS software package was also used to determine the quantity of target DNA for each sample by interpolation from the standard curve.

Step 6. Converting qPCR Results to a Relative Abundance

A standard quantity of DNA (180 ng) was used for each reaction. To calculate the target DNA content in relative abundance, the quantity of target DNA from the qPCR expressed in picograms per sample was divided by 180 ng to determine the fraction of total DNA that was target DNA. The resulting number represents the concentration of target DNA in pg-μL^{-1} of soil or surface litter dry weight.

The data was analyzed using analysis of variance with RStudio (31), with pathogen (*Pyrenophora, Fusarium*, or *Clarireedia*) and sample type (soil or litter) as the independent main effects and log transformed inoculum concentration in pg/µL as the dependent variable. Technical replicates were averaged prior to analysis, so each pathogen by sample type combination was represented by the twelve mean values obtained from the field replicates, for a total of 72 experimental units.

RESULTS

Primer Design and Specificity

To design *Fusarium* primers for qPCR from the *EF1α* gene that would be specific for species in the FTSG, an alignment of *EF1α* sequences from various *Fusariam* species was used to identify regions conserved in the FTSG but not in other species. The primers were tested for specificity with eight *Fusarium* isolates collected from stand failure sites (Table 1). Six of the isolates were known to belong to the FTSG, while two isolates did not belong to the group. To test specificity, DNA extracted from these representative *Fusarium* species collected in stand failure areas was amplified using the *EF1α* primers. There was amplification from isolates in the FTSG, but no amplification from non-FTSG isolates (Fig. 2). This confirmed that the primers could differentiate between the FTSG and at least two non-FTSG isolates.

The primers for *Fusarium* sp., *P. semeniperda*, and *C. capillus-albis* were then tested for amplification with their respective pathogen, for cross amplification against the other pathogens, and against a no-template control. Each of the primers amplified DNA from their own respective fungal pathogen. No cross amplification of DNA from the other pathogens or the no-template control was detected (Fig. 3).

The primers from the three target pathogens were tested against DNA from isolates representing 12 unique species (Table 3) for cross amplification. Primers developed for amplification of DNA from the target pathogens showed no cross amplification with DNA from any of the 12 non-target species (Fig. 4). These results showed that any DNA amplified from field samples using the primers for a target pathogen would represent DNA from that specific pathogen.

Development of Standard Curves

Standard curves were generated for each of the three pathogens to use as basis to determine the initial quantity of target DNA in field samples. The standard curve for each of the pathogens (Fig. 5) was successfully optimized to have an R^2 value and efficiency (Table 4) that fell within the acceptable range (32). Dissociation curves also showed little or no amplification of non-target DNA (Fig. 6).

Quantitative PCR on Field Samples

For each pathogen, samples collected from a single representative site were processed for DNA quantification as described earlier and a mean value for the 12 samples collected for each pathogen and sample type was calculated (Fig. 7). Analysis of variance demonstrated that the main effect differences among pathogens and also between sample types (soil vs. litter) were highly significant (pathogen main effect df= 2, 66, F=57.61, P<0.0000; sample type main effect df =1, 66, F=51.67, P<0.0000). *Clarireedia capillus-albis* samples contained significantly more inoculum than *P. semeniperda* samples, and *Fusarium* sp. samples contained significantly less inoculum than either *P. semeniperda* or *C. capillus-albis* samples. Litter samples contained

significantly more inoculum than soil samples. The interaction between pathogen and sample type was also highly significant (pathogen x sample type interaction, df =2, 66, F=42.49, P<0.0000). For *Fusarium* sp., inoculum concentrations were low in both litter and soil, which did not differ significantly. Levels of *P. semeniperda* inoculum were intermediate, while *C. capillus-albis* levels were high. In both the latter cases, there was significantly more inoculum in litter than in soil samples. As samples for each pathogen were obtained from different sites, these comparisons are not meant to be interpreted directly, but are only intended to show that meaningful differences in changes in relative abundance of DNA inoculum load can be detected using this methodology.

DISCUSSION

In this study, a method using SYBR Green qPCR was used to quantify field inoculum loads for three seed bank soil pathogens. We demonstrated the utility of this method for detection and quantification of fungal pathogens in soil seed banks. It is a relatively simple procedure that uses high-throughput techniques to process large numbers of samples. This enables the collection of data sets that capture in truly quantitative terms both pathogen abundance and the variability in pathogen inoculum load commonly encountered in heterogeneous field soils. These advantages make it useful in both agricultural and natural ecosystems. However, one drawback is that the pathogen of interest must be determined prior to quantification, as this method yields only information on targeted, known pathogens.

This method includes quality controls to assure accuracy and sensitivity. A standard curve is generated by a serial dilution of a standard, or known template. The threshold cycle is plotted against the dilution factor to create a straight line. The correlation coefficient of this line should

be 0.98 or higher (32). This curve is then used as a standard to interpolate the DNA content of unknown samples. By including a replicated standard curve on every run, there is assurance that run-to-run variability is not introduced as error in interpolation on the curve. The efficiency of amplification is another quality control. The number of products should ideally double after each cycle, indicating 100% efficiency. An efficiency of 90%-110% is needed to assure that there is proper primer function and appropriate reaction conditions are present (32). In addition, dissociation curves are examined at the end of qPCR to insure specificity. The end product for each sample is denatured at 95 °C, brought down to the annealing temperature, and then brought back to 95 °C. The fluorescence is monitored through this process, and when the derivative of the data is analyzed, a single peak should result. Non-single peak formation could indicate primer dimers or a non-homogenous product (32). Lastly, technical replicates are used, i.e., the amplification of a single sample is repeated multiple times, typically in duplicate or triplicate. This allows for the monitoring of precision. It also increases the likelihood of obtaining usable information even in the event of an occasional failed amplification.

The primers designed showed optimal specificity in this study. No cross amplification occurred, indicating that these primers would only amplify the targeted pathogens. In previous studies, the ITS and EF1α regions have been deemed very effective barcode markers and regions to identify fungi (33, 34).

Dissociation curves were run for each of the samples. The curves for *P. semeniperda* and *C. capillus-albis* showed a single peak, while *Fusarium* sp. exhibited a double peak or shoulder for some samples (Figure 7). Double peaks and shoulders typically indicate that there is non-homogeneous amplification occurring and the primers are not specific (32). However, this is not always the case. The GC content of the product can have an effect on the melting rate resulting

in multiple peaks, but only one product (35). More testing, including gel-electrophoresis, needs to be performed to determine if the amount of end product and GC content could be contributing factors, or if non-homogeneous amplification is taking place.

qPCR technical replicates were analyzed to determine the precision and accuracy of the results. There are some reports that technical replicates from the same sample need to have a C_t standard deviation of ≤0.3 and/or or an R^2 value ≥0.99, while others deem standard deviations of <0.5 cycles acceptable (36, 32). High standard deviations were detected during trial runs of qPCR in this study, probably due to the increased variability associated with low and variable DNA concentrations. By standardizing each reaction to 30ng/μL of DNA, the technical replicate standard deviation was reduced to an acceptable level. This improvement, along with R^2 values of ≥0.99, indicates that the reported results can be regarded as accurate.

The ability of qPCR to detect significant differences in relative pathogen load is partly a function of the accuracy and repeatability of the method and partly a function of adequate sample replication. A sample number of 12 replicates per treatment level (pathogen species, seed bank soil vs. surface litter) was more than adequate to detect differences, especially for pathogens at a higher level of abundance. In this study, the site chosen for quantifying *Fusarium* sp. did not have a high relative inoculum load, so a difference between soil and surface litter was harder to detect. However, qPCR could clearly be useful in furthering our understanding of how the abundance of these pathogens in the seed bank changes as a function of time since *B. tectorum* stand failure.

The method presented here for quantifying fungal inoculum loads in soil seed banks can readily detect meaningful differences in the field. Pathogens on *B. tectorum* were used for

methods development, but the method itself is universal and could be used advantageously for many other seed bank pathogens and also in the study of soilborne fungal pathogens in general.

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FIGURES

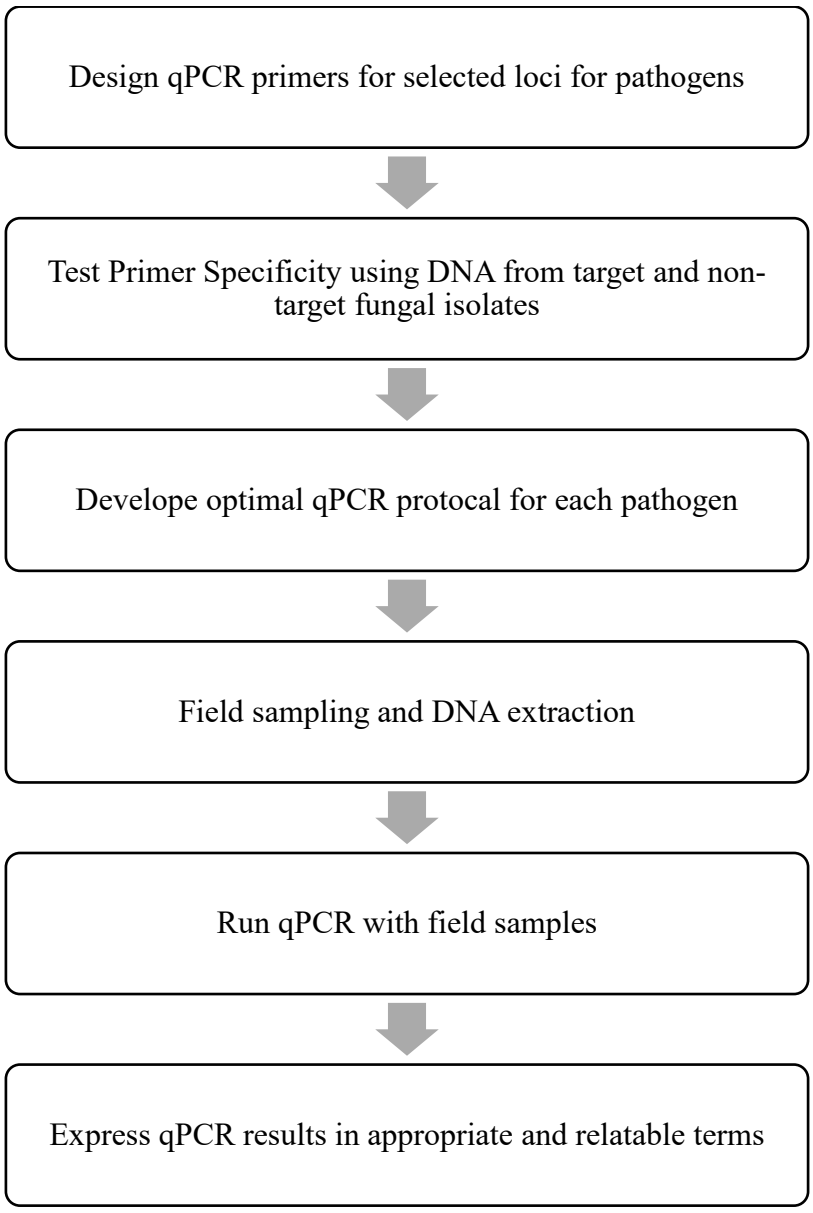


Figure 1. Flow chart. Steps followed to conduct the method for quantifying fungal pathogens in soil.

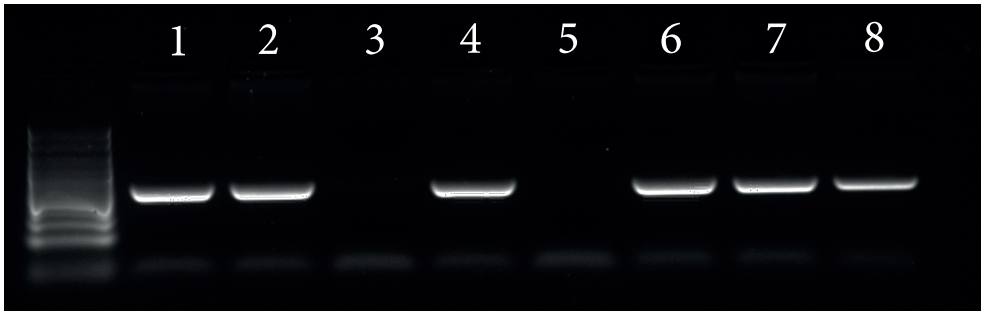


Figure 2. *Fusarium* sp. primers found in Table 1 tested against *Fusarium* species isolates in the FTSG and non-*Tricinctum* groups. The samples used for template DNA were collected in Skull Valley, UT and Eden Valley, NV (21). Lanes 1, 2, 4, and 6-8 are isolates from the *Tricinctum* group. Lane 3 and lane 5 are both isolates from non-*Tricinctum* groups.

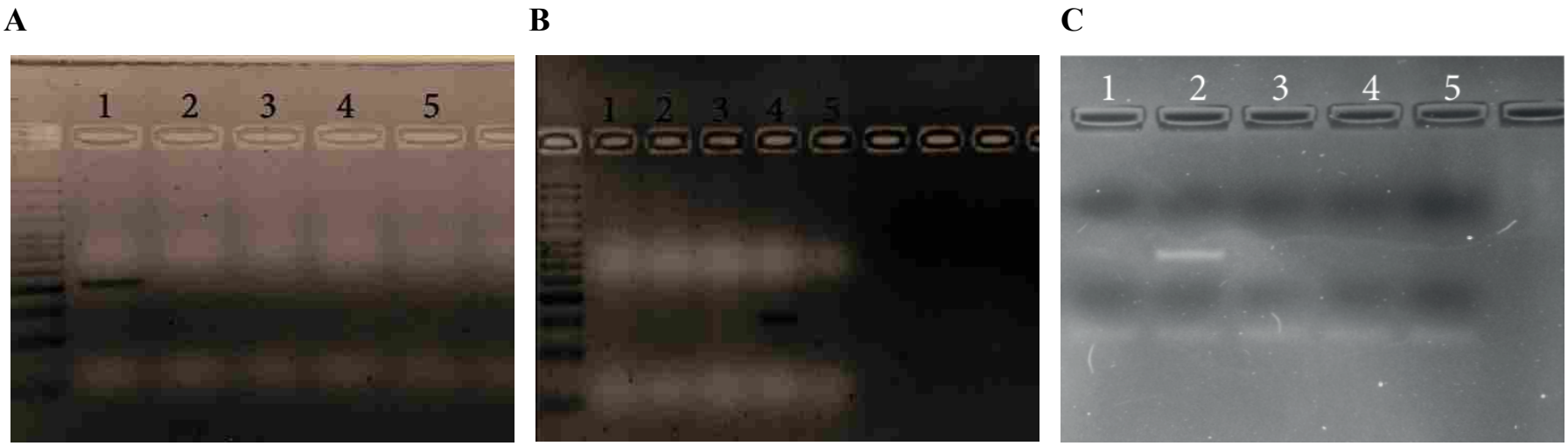
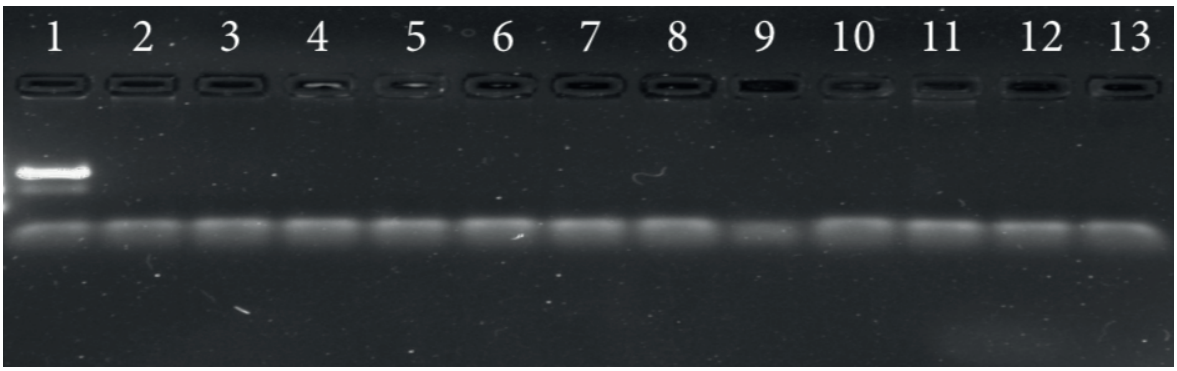


Figure 3. The primer sets for each of the pathogens was tested for amplification against all three pathogens, another pathogen *Epicoccum nigrum*, and a no template control: (A) *Fusarium* sp. (B) *Pyrenophora semeniperda* (C) *Clarireedia capillus-albis*. For all three, each lane shows the amplification products using DNA from each of the isolates as template; lane 1- *Fusarium* sp. DNA; lane 2 - *C. capillus-albis* DNA; lane 3 - *E. nigrum* DNA; lane 4 - *P. semeniperda* DNA; lane 5 - no template control.

A

B



C

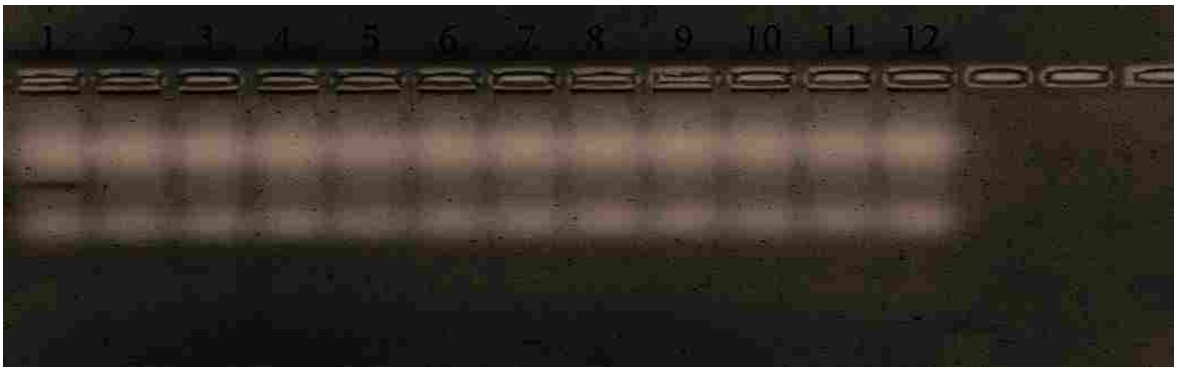


Figure 4. The primer sets for each of the pathogens was tested for amplification against 11 or 12 unique isolates from stand-failure sites. (A) *Fusarium* sp. Lane 1 is amplification from *Fusarium* sp. template DNA. (B) *Pyrenophora semeniperda*. Lane 1 is amplification from *P. semeniperda* template DNA. (C) *Clarireedia capillus-albis*. Lane 1 is amplification from *C. capillus-albis* template DNA. For A and B, lanes 2- 13 correlate with Table 3 samples 1-12. For C, lanes 2-10 correlate with Table 3 samples 1-9; lane 11 is *Alternaria alternata*, and lane 12 is *Alternaria tellustris*.

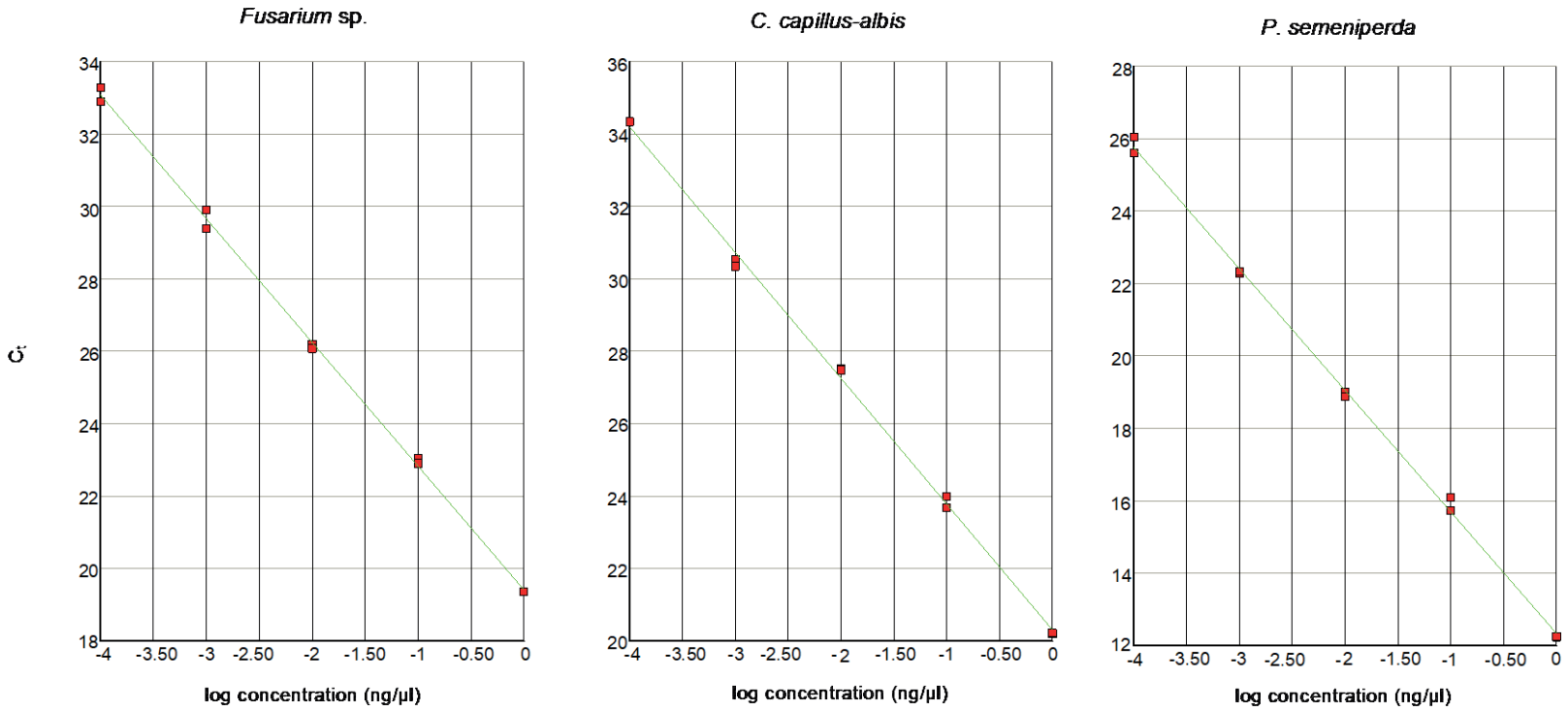


Figure 5. Standard curves generated for each fungal pathogen. The threshold value (C_t) was plotted against the Log of the concentration of the pathogen.

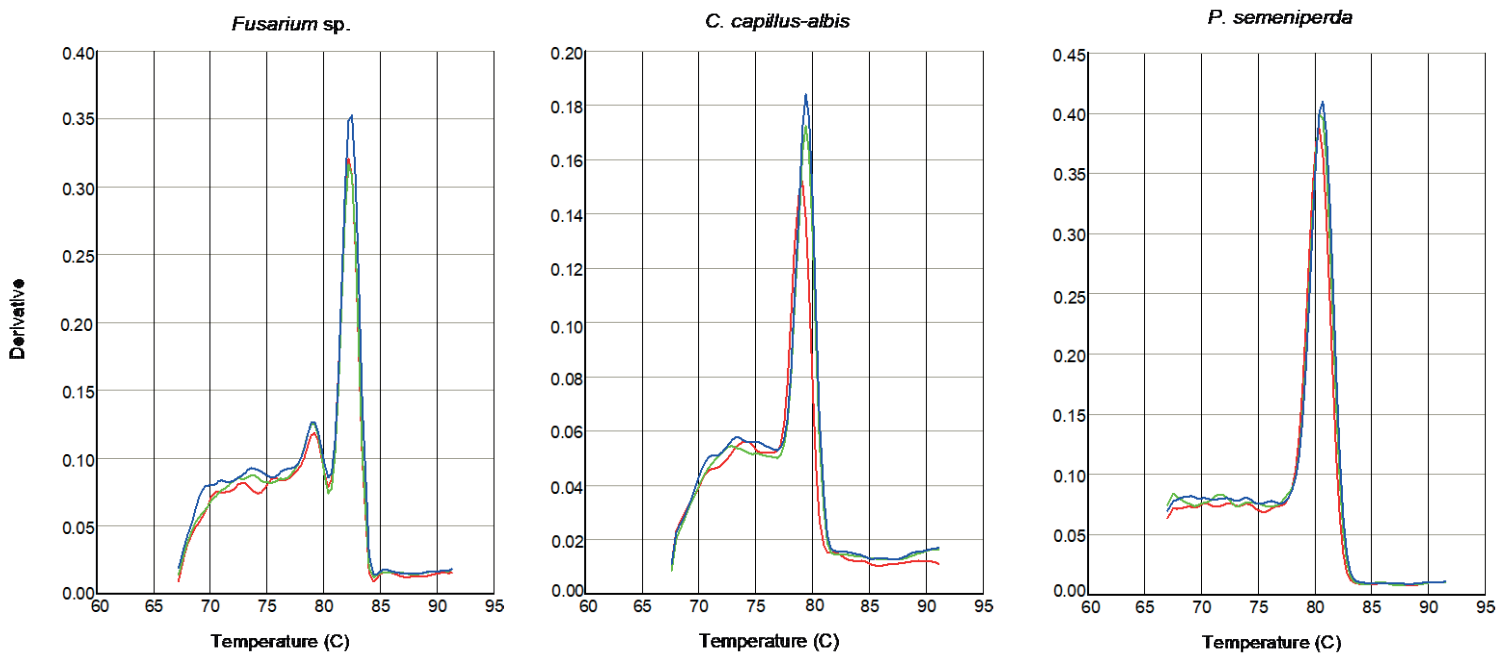


Figure 6. Dissociation curves from soil field samples. The y-axis is the derivative of the change in fluorescence intensity as a function of temperature. The temperature on the x-axis represents the temperature throughout the melt curve analysis. The different colors represent the individual samples from a set of technical replicates from one sample. The dissociation curve for *Fusarium* sp. is from a 1990 stand-failure. The dissociation curve for *P. semeniperda* is from a 2013 stand-failure. The dissociation curve for *C. capillus-albis* is from a 2010 stand-failure.

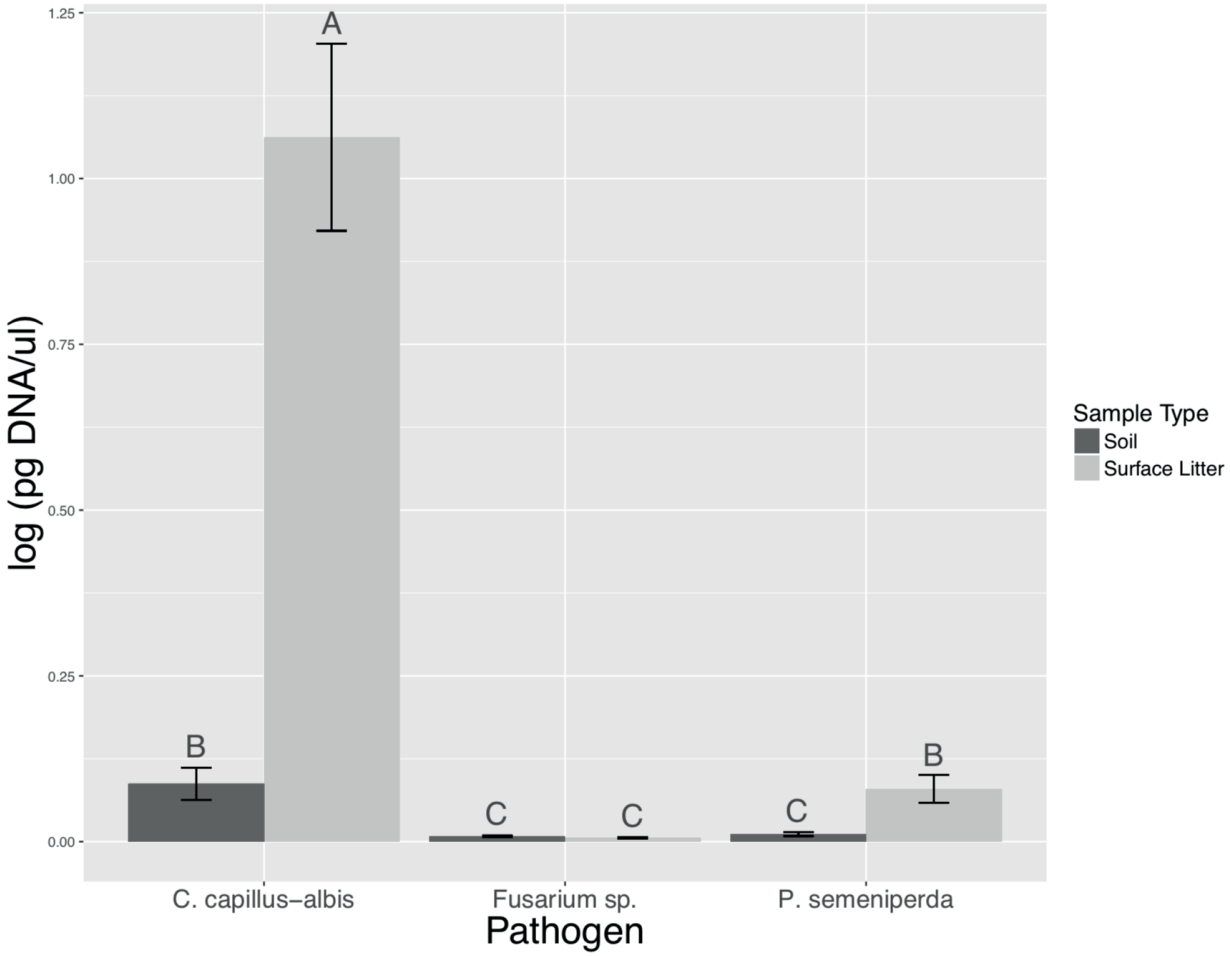


Figure 7. The relative abundance of DNA for each pathogen expressed as the weight of DNA per unit of sample extracted in representative field samples from *Bromus tectorum* monocultures. Error bars represent the standard error of the mean.

TABLES

Table 1. Primer design for the three fungal pathogens being targeted.

Primer	**Sequence**	**Amplicon Length (bp)**
***Fusarium*_EF1F**	GTATCTTACCCCGCCACTC	
***Fusarium*_EF1R**	AGTGGTTAGTGACTGCAAGAC	103
***Pyrenophora*_ITSF**	CCACGACTCGCCTTAAAATC	
***Pyrenophora*_ITSR**	GGACTGAGCGCAAAAATGT	73
***Clarireedia*_EF1F**	TTACGCACCAGTTCTTGAC	
***Clarireedia*_EF1R**	TCAATCTTTTGAAGGAGCTCAG	71

Table 2. Origin of strains used in primer design and testing for targeted pathogens.

Pathogen	Name of Location	GPS Coordinates
***Fusarium* sp. (FTSG)**	Eden Valley, NV	41.17083 -117.41361
Pyrenophora semeniperda	Ten Mile Creek, UT	41.86488 -114.13594
Clarireedia capillus-albis	Whiterocks, UT	40.32938 -112.77861

Table 3. Isolates of non-target species used to test for specificity of primers.

Sample	Identification	Location
1	*Mucor plumbeus*	Dun Glen Study Site, NV
2	*Ascochyta hordei*	Thistle, UT
3	*Alternaria infectoria*	Thistle, UT
4	*Chaetopyrena penicillata*	Thistle, UT
5	*Podospora tetraspora*	Thistle, UT
6	*Fusarium proliferatum*	Thistle, UT
7	*Penicillium cremeogriseum*	Thistle, UT
8	*Comoclathris spartii*	Davis Mountain Site, Skull Valley, UT
9	*Phoma cladoniicola*	Davis Mountain Site, Skull Valley, UT
10	*Peyronellaea glomerata*	Stansbury Island, UT
11	*Alternaria alternata*	Stansbury Island, UT
12	*Alternaria tellustris*	Stansbury Island, UT

Table 4. Values tested for the optimization of qPCR standard curves.

Pathogen	R-squared Value	Efficiency
Fusarium sp.	0.998759	96.30
Pyrenophora semeniperda	0.998496	98.64
Clarireedia capillus-albis	0.997852	94.10

APPENDIX A

Site locations where samples were collected for the larger study on *B. tectorum* stand failure history. Appendix A is included to aid in interpretation of data in Appendix B. Appendix A is not part of the manuscript or used in the manuscript, but is additional information.

Stand Failure Year	Location	GPS Coordinates
1990	Dun Glen, NV	40.69205 -117.93819
2003	Dun Glen, NV	40.68962 -117.96403
2009	Dun Glen, NV	40.69183 -117.95852
2009	Dun Glen, NV	40.69305 -117.92267
2010	Dun Glen, NV	40.69839 -118.04401
2013	Dun Glen, NV	40.69445 -117.93802
2014	Dun Glen, NV	40.68664 -117.98334
2015	Dun Glen, NV	40.68791 -117.96607
No Stand Failure	Dun Glen, NV	40.69066 -117.89386
No Stand Failure	Dun Glen, NV	40.69890 -117.89944
1990	Skull Valley, UT	40.13880 -112.71130
2008	Skull Valley, UT	40.17711 -112.72815
2009	Skull Valley, UT	40.39453 -112.94844
2010	Skull Valley, UT	40.27520 -112.63137
2013	Skull Valley, UT	40.32838 -112.77701
2014	Skull Valley, UT	40.34031 -112.68634
2015	Skull Valley, UT	40.29299 -112.77019
No Stand Failure	Skull Valley, UT	40.14190 -112.66871
No Stand Failure	Skull Valley, UT	40.13996 -112.64128

APPENDIX B

Data set from the qPCR analysis targeting the *Fusarium tricinctum* species group, including all samples taken for the larger study on *B. tectorum* stand failure. These data were not included in the manuscript because of issues with reaction efficiency, but are included here for possible use in comparison with a metagenomics study on the same sample set. No Stand Failure is abbreviated as NSF.

Location	Year	Sample	Weight of Sample in Extraction (g)	Final Solution (µL)	Qubit (ng/µL)	Total DNA in Reaction	qPCR mean Quantity	Total DNA (ng/g)
Dun Glen, NV	1990	1990_soil_1_wm	0.2257	95	50	180	0.0273	3.19E-09
Dun Glen, NV	1990	1990_soil_2_wm	0.2228	91	79.6	180	65801.93	0.01188517
Dun Glen, NV	1990	1990_soil_3_wm	0.2225	90	54.6	180	0	0
Dun Glen, NV	1990	1990_soil_4_wm	0.2269	88	60	180	0	0
Dun Glen, NV	1990	1990_soil_5_wm	0.2284	96	31	180	0.00271	1.96E-10
Dun Glen, NV	1990	1990_soil_6_wm	0.229	97	61.2	180	0.0026	3.74E-10
Dun Glen, NV	1990	1990_soil_7_wm	0.2199	96	74.6	180	0.00558	1.01E-09
Dun Glen, NV	1990	1990_soil_8_wm	0.2267	95	75.2	180	0.00151	2.64E-10
Dun Glen, NV	1990	1990_soil_9_wm	0.2258	96	58.6	180	0.00442	6.12E-10
Dun Glen, NV	1990	1990_soil_10_wm	0.2257	91	58.8	180	0.0158	2.08E-09
Dun Glen, NV	1990	1990_soil_11_wm	0.2235	92	56.6	180	0.0073	9.45E-10
Dun Glen, NV	1990	1990_soil_12_wm	0.2203	92	30.8	180	0.00315	2.25E-10
Dun Glen, NV	1990	1990_sl_1_wm	0.1006	98	61.8	180	0.132	4.41E-08
Dun Glen, NV	1990	1990_sl_2_wm	0.101	99	73.4	180	0.0289	1.16E-08
Dun Glen, NV	1990	1990_sl_3_wm	0.1041	97	97.2	180	0.017	8.55E-09
Dun Glen, NV	1990	1990_sl_4_wm	0.1024	98	128	180	0.000881	6.00E-10
Dun Glen, NV	1990	1990_sl_5_wm	0.105	98	72.2	180	0.0135	5.05E-09
Dun Glen, NV	1990	1990_sl_6_wm	0.1048	99	101	180	0.0272	1.44E-08
Dun Glen, NV	1990	1990_sl_7_wm	0.1002	99	136	180	0.0142	1.06E-08
Dun Glen, NV	1990	1990_sl_8_wm	0.1019	99	76.6	180	0.018	7.44E-09
Dun Glen, NV	1990	1990_sl_9_wm	0.1033	98	66.6	180	0.0438	1.54E-08

Dun Glen, NV	1990	1990_sl_10_wm	0.1075	99	83.4	180	0.0109	4.65E-09
Dun Glen, NV	1990	1990_sl_11_wm	0.1056	100	122	180	0.0617	3.96E-08
Dun Glen, NV	1990	1990_sl_12_wm	0.0996	98	101	180	0.00943	5.21E-09
Dun Glen, NV	2003	2003_soil_1_wm	0.22	99	47.2	180	0.00267	3.15E-10
Dun Glen, NV	2003	2003_soil_2_wm	0.2228	74	61.2	180	0.00634	7.16E-10
Dun Glen, NV	2003	2003_soil_3_wm	0.2204	98	39.4	180	0.00423	4.12E-10
Dun Glen, NV	2003	2003_soil_4_wm	0.2301	99	49.2	180	0.00487	5.73E-10
Dun Glen, NV	2003	2003_soil_5_wm	0.2219	97	54.8	180	0.00793	1.06E-09
Dun Glen, NV	2003	2003_soil_6_wm	0.2228	98	41.4	180	0.00287	2.90E-10
Dun Glen, NV	2003	2003_soil_7_wm	0.2239	100	38.8	180	0.00365	3.51E-10
Dun Glen, NV	2003	2003_soil_8_wm	0.2292	99	51.4	180	0.0029	3.58E-10
Dun Glen, NV	2003	2003_soil_9_wm	0.227	99	46.6	180	0.00375	4.23E-10
Dun Glen, NV	2003	2003_soil_10_wm	0.2223	100	50	180	0.0044	5.50E-10
Dun Glen, NV	2003	2003_soil_11_wm	0.2283	97	49.4	180	0.00324	3.78E-10
Dun Glen, NV	2003	2003_soil_12_wm	0.2251	99	81.4	180	0.00167	3.32E-10
Dun Glen, NV	2003	2003_sl_1_wm	0.0806	98	53.4	180	0.00795	2.87E-09
Dun Glen, NV	2003	2003_sl_2_wm	0.072	97	48.4	180	0.0177	6.41E-09
Dun Glen, NV	2003	2003_sl_3_wm	0.0742	96	43.8	180	0.0108	3.40E-09
Dun Glen, NV	2003	2003_sl_4_wm	0.073	96	49	180	0.00407	1.46E-09
Dun Glen, NV	2003	2003_sl_5_wm	0.072	97	55.4	180	0.00826	3.42E-09
Dun Glen, NV	2003	2003_sl_6_wm	0.0721	97	49.2	180	0.00807	2.97E-09
Dun Glen, NV	2003	2003_sl_7_wm	0.0775	97	37.8	180	0.0163	4.28E-09
Dun Glen, NV	2003	2003_sl_8_wm	0.0717	95	65.8	180	0.00613	2.97E-09
Dun Glen, NV	2003	2003_sl_9_wm	0.0576	96	29.8	180	0.00426	1.18E-09
Dun Glen, NV	2003	2003_sl_10_wm	0.0742	98	53.2	180	0.0051	1.99E-09
Dun Glen, NV	2003	2003_sl_11_wm	0.077	96	50.8	180	0.00784	2.76E-09
Dun Glen, NV	2003	2003_sl_12_wm	0.0766	97	47.4	180	0.00175	5.84E-10
Dun Glen, NV	2009	2009_soil_1_wm	0.237	83	78.4	180	0.00611	9.32E-10
Dun Glen, NV	2009	2009_soil_2_wm	0.2278	72	177	180	0.0189	5.87E-09
Dun Glen, NV	2009	2009_soil_3_wm	0.2314	85	120	180	0.0183	4.48E-09
Dun Glen, NV	2009	2009_soil_4_wm	0.2408	87	100	180	0.0139	2.79E-09

Dun Glen, NV	2009	2009_soil_5_wm	0.2379	88	131	180	0.029	7.81E-09
Dun Glen, NV	2009	2009_soil_6_wm	0.2312	93	96	180	0.0199	4.27E-09
Dun Glen, NV	2009	2009_soil_7_wm	0.2293	82	107	180	21622.36	0.004596467
Dun Glen, NV	2009	2009_soil_8_wm	0.2298	81	102	180	0.0102	2.04E-09
Dun Glen, NV	2009	2009_soil_9_wm	0.2189	76	138	180	0.0103	2.74E-09
Dun Glen, NV	2009	2009_soil_10_wm	0.2281	85	64.4	180	0.0091	1.21E-09
Dun Glen, NV	2009	2009_soil_11_wm	0.2106	89	141	180	0.0102	3.38E-09
Dun Glen, NV	2009	2009_soil_12_wm	0.2169	87	64.4	180	0.0052	7.46E-10
Dun Glen, NV	2009	2009_sl_1_wm	0.0883	100	68.4	180	0.00861	3.71E-09
Dun Glen, NV	2009	2009_sl_2_wm	0.0852	99	62.4	180	0.0143	5.76E-09
Dun Glen, NV	2009	2009_sl_3_wm	0.0916	98	68	180	0.00465	1.88E-09
Dun Glen, NV	2009	2009_sl_4_wm	0.0896	99	48.8	180	0.0139	4.16E-09
Dun Glen, NV	2009	2009_sl_5_wm	0.0905	99	62.6	180	0	0
Dun Glen, NV	2009	2009_sl_6_wm	0.0835	97	62	180	0.0216	8.64E-09
Dun Glen, NV	2009	2009_sl_7_wm	0.0879	99	73.2	180	0.0217	9.94E-09
Dun Glen, NV	2009	2009_sl_8_wm	0.0872	99	65.8	180	0.0261	1.08E-08
Dun Glen, NV	2009	2009_sl_9_wm	0.08	99	44	180	0.108	3.27E-08
Dun Glen, NV	2009	2009_sl_10_wm	0.0844	96	61	180	0.00867	3.34E-09
Dun Glen, NV	2009	2009_sl_11_wm	0.0821	99	45.4	180	0.0453	1.38E-08
Dun Glen, NV	2009	2009_sl_12_wm	0.08	99	42.2	180	0.00406	1.18E-09
Dun Glen, NV	2009	2009(2)_soil_1_wm	0.2275	97	57.8	180	0.0147	2.01E-09
Dun Glen, NV	2009	2009(2)_soil_2_wm	0.2299	82	52.8	180	0.017	1.78E-09
Dun Glen, NV	2009	2009(2)_soil_3_wm	0.2293	97	64.8	180	0.00503	7.66E-10
Dun Glen, NV	2009	2009(2)_soil_4_wm	0.2251	99	50.6	180	0.0154	1.90E-09
Dun Glen, NV	2009	2009(2)_soil_5_wm	0.2228	97	54.2	180	0.0126	1.65E-09
Dun Glen, NV	2009	2009(2)_soil_6_wm	0.2216	99	54	180	0.0023	3.08E-10
Dun Glen, NV	2009	2009(2)_soil_7_wm	0.2161	97	53.8	180	0.043	5.77E-09
Dun Glen, NV	2009	2009(2)_soil_10_wm	0.2198	98	69.4	180	0.0088	1.51E-09
Dun Glen, NV	2009	2009(2)_soil_11_wm	0.2275	90	58	180	0.000237	3.02E-11
Dun Glen, NV	2009	2009(2)_soil_12_wm	0.2227	97	63.2	180	0.173	2.65E-08
Dun Glen, NV	2009	2009(2)_sl_1_wm	0.0779	95	60.6	180	0.0377	1.55E-08

Dun Glen, NV	2009	2009(2)_sl_2_wm	0.0805	96	52.4	180	0.0233	8.09E-09
Dun Glen, NV	2009	2009(2)_sl_3_wm	0.0711	94	49.8	180	0.00765	2.80E-09
Dun Glen, NV	2009	2009(2)_sl_4_wm	0.0799	96	42.2	180	0.0267	7.52E-09
Dun Glen, NV	2009	2009(2)_sl_5_wm	0.1015	99	54.2	180	0.0291	8.55E-09
Dun Glen, NV	2009	2009(2)_sl_6_wm	0.0806	96	56	180	0.0826	3.06E-08
Dun Glen, NV	2009	2009(2)_sl7_wm	0.0946	97	46.8	180	0.0086	2.29E-09
Dun Glen, NV	2009	2009(2)_sl10_wm	0.0741	94	53.8	180	0.0213	8.08E-09
Dun Glen, NV	2009	2009(2)_sl11_wm	0.0722	96	45.4	180	0.0229	7.68E-09
Dun Glen, NV	2009	2009(2)_sl12_wm	0.0804	93	44	180	0.0408	1.15E-08
Dun Glen, NV	2010	2010_soil_1_wm	0.2294	93	68	180	0.00834	1.28E-09
Dun Glen, NV	2010	2010_soil_2_wm	0.2277	89	83.8	180	29557.6	0.00537858
Dun Glen, NV	2010	2010_soil_3_wm	0.226	90	109	180	0.0115	2.77E-09
Dun Glen, NV	2010	2010_soil_4_wm	0.2205	96	148	180	0.0167	5.98E-09
Dun Glen, NV	2010	2010_soil_5_wm	0.2233	89	78.4	180	27465.12	0.004767891
Dun Glen, NV	2010	2010_soil_6_wm	0.2279	91	92	180	0.0424	8.65E-09
Dun Glen, NV	2010	2010_soil_7_wm	0.2236	93	120	180	0.011	3.05E-09
Dun Glen, NV	2010	2010_soil_8_wm	0.2261	91	83.4	180	25772.31	0.004806044
Dun Glen, NV	2010	2010_soil_9_wm	0.227	88	70.4	180	29548.25	0.004480111
Dun Glen, NV	2010	2010_soil_10_wm	0.2207	92	95.6	180	0.0106	2.35E-09
Dun Glen, NV	2010	2010_soil_11_wm	0.223	95	95	180	0.0331	7.44E-09
Dun Glen, NV	2010	2010_soil_12_wm	0.2278	97	72.4	180	0.00808	1.38E-09
Dun Glen, NV	2010	2010_sl_1_wm	0.0806	99	55.6	180	0.00823	3.12E-09
Dun Glen, NV	2010	2010_sl_2_wm	0.0736	97	57.4	180	0.0385	1.62E-08
Dun Glen, NV	2010	2010_sl_3_wm	0.0705	96	54.4	180	0.0114	4.69E-09
Dun Glen, NV	2010	2010_sl_4_wm	0.0736	99	58.4	180	0.0482	2.10E-08
Dun Glen, NV	2010	2010_sl_5_wm	0.0751	99	40	180	0.0238	6.97E-09
Dun Glen, NV	2010	2010_sl_6_wm	0.0781	98	54.8	180	0.0189	7.22E-09
Dun Glen, NV	2010	2010_sl_7_wm	0.0767	99	49.8	180	0.00479	1.71E-09
Dun Glen, NV	2010	2010_sl_8_wm	0.0782	98	58.4	180	0.0216	8.78E-09
Dun Glen, NV	2010	2010_sl_9_wm	0.0768	98	45.2	180	0.0198	6.34E-09
Dun Glen, NV	2010	2010_sl_10_wm	0.0786	98	56.6	180	0.012	4.70E-09

Dun Glen, NV	2010	2010_sl_11_wm	0.0762	98	45	180	0.0639	2.05E-08
Dun Glen, NV	2010	2010_sl_12_wm	0.073	97	34.6	180	0.00285	7.28E-10
Dun Glen, NV	2013	2013_soil_1_wm	0.2235	97	52.6	180	0.00259	3.28E-10
Dun Glen, NV	2013	2013_soil_2_wm	0.2233	98	51	180	0.00618	7.68E-10
Dun Glen, NV	2013	2013_soil_3_wm	0.2264	97	72.2	180	0.0035	6.01E-10
Dun Glen, NV	2013	2013_soil_4_wm	0.2196	98	51.8	180	0.00521	6.69E-10
Dun Glen, NV	2013	2013_soil_5_wm	0.2257	98	56.6	180	0.00869	1.19E-09
Dun Glen, NV	2013	2013_soil_6_wm	0.2254	99	65.8	180	0.0279	4.48E-09
Dun Glen, NV	2013	2013_soil_7_wm	0.229	100	51.6	180	0.00429	5.37E-10
Dun Glen, NV	2013	2013_soil_8_wm	0.2225	97	50.6	180	0.00691	8.47E-10
Dun Glen, NV	2013	2013_soil_9_wm	0.2238	98	60	180	0.00337	4.92E-10
Dun Glen, NV	2013	2013_soil_10_wm	0.2367	98	31.6	180	0	0
Dun Glen, NV	2013	2013_soil_11_wm	0.2207	99	45	180	0.00531	5.95E-10
Dun Glen, NV	2013	2013_soil_12_wm	0.2252	94	71.6	180	0.00792	1.31E-09
Dun Glen, NV	2013	2013_sl_1_wm	0.0817	98	44.2	180	0.00236	6.95E-10
Dun Glen, NV	2013	2013_sl_2_wm	0.0913	99	45.6	180	0.00637	1.75E-09
Dun Glen, NV	2013	2013_sl_3_wm	0.084	96	47	180	0.0028	8.36E-10
Dun Glen, NV	2013	2013_sl_4_wm	0.0861	98	57	180	0.00736	2.65E-09
Dun Glen, NV	2013	2013_sl_5_wm	0.0846	97	44	180	0.0379	1.06E-08
Dun Glen, NV	2013	2013_sl_6_wm	0.082	99	50.4	180	0.146	4.94E-08
Dun Glen, NV	2013	2013_sl_7_wm	0.0828	99	43.8	180	0.0912	2.65E-08
Dun Glen, NV	2013	2013_sl_8_wm	0.0851	98	41.6	180	0.00898	2.39E-09
Dun Glen, NV	2013	2013_sl_9_wm	0.0866	98	45	180	0.0504	1.43E-08
Dun Glen, NV	2013	2013_sl_10_wm	0.0854	99	54.2	180	0.00218	7.61E-10
Dun Glen, NV	2013	2013_sl_11_wm	0.0905	100	47.8	180	0.0282	8.27E-09
Dun Glen, NV	2013	2013_sl_12_wm	0.0904	98	37.4	180	0.0375	8.45E-09
Dun Glen, NV	2014	2014_soil_1_wm	0.2464	99	29.4	180	0	0
Dun Glen, NV	2014	2014_soil_2_wm	0.2308	98	32	180	9.46E-05	7.14E-12
Dun Glen, NV	2014	2014_soil_3_wm	0.232	99	50.6	180	0.00133	1.60E-10
Dun Glen, NV	2014	2014soil4_wm	0.2307	99	33.2	180	6.00E-04	4.75E-11
Dun Glen, NV	2014	2014_soil_5_wm	0.2372	87	132	180	0.00988	2.66E-09

Dun Glen, NV	2014	2014_soil_6_wm	0.2298	88	104	180	0.00233	5.16E-10
Dun Glen, NV	2014	2014_soil_7_wm	0.2327	89	98	180	0.00411	8.56E-10
Dun Glen, NV	2014	2014_soil_8_wm	0.2374	86	111	180	0.00294	6.57E-10
Dun Glen, NV	2014	2014_soil_9_wm	0.234	84	109	180	0.00611	1.33E-09
Dun Glen, NV	2014	2014_soil_10_wm	0.2337	84	146	180	0.00429	1.25E-09
Dun Glen, NV	2014	2014_soil_11_wm	0.2329	90	106	180	0.00305	6.94E-10
Dun Glen, NV	2014	2014_soil_12_wm	0.2369	87	93.6	180	0.00319	6.09E-10
Dun Glen, NV	2014	2014_sl_1_wm	0.0808	97	59.4	180	0.0232	9.19E-09
Dun Glen, NV	2014	2014_sl_2_wm	0.0808	97	53	180	0.00399	1.41E-09
Dun Glen, NV	2014	2014_sl_3_wm	0.083	95	56.2	180	0.0258	9.22E-09
Dun Glen, NV	2014	2014_sl_4_wm	0.0813	96	73.6	180	0.00804	3.88E-09
Dun Glen, NV	2014	2014_sl_5_wm	0.0827	93	65.2	180	0.00451	1.84E-09
Dun Glen, NV	2014	2014_sl_6_wm	0.0865	97	45.2	180	0.0135	3.80E-09
Dun Glen, NV	2014	2014_sl_7_wm	0.0845	98	65	180	0.0183	7.66E-09
Dun Glen, NV	2014	2014_sl_8_wm	0.0841	95	40	180	0.00887	2.23E-09
Dun Glen, NV	2014	2014_sl_9_wm	0.0829	96	52.2	180	0.0209	7.02E-09
Dun Glen, NV	2014	2014_sl_10_wm	0.0814	97	26	180	0.0162	2.79E-09
Dun Glen, NV	2014	2014_sl_11_wm	0.08	90	29	180	0.0219	3.97E-09
Dun Glen, NV	2014	2014_sl_12_wm	0.0831	94	49.8	180	0.00162	5.07E-10
Dun Glen, NV	2015	2015_soil_1_wm	0.1923	71	82.2	180	0.00327	5.51E-10
Dun Glen, NV	2015	2015_soil_2_wm	0.2205	99	43	180	0.0099	1.06E-09
Dun Glen, NV	2015	2015_soil_3_wm	0.1943	68	82	180	0.000502	8.00E-11
Dun Glen, NV	2015	2015_soil_4_wm	0.1933	67	85	180	0	0
Dun Glen, NV	2015	2015_soil_5_wm	0.192	72	89.6	180	0.00561	1.05E-09
Dun Glen, NV	2015	2015_soil_6_wm	0.1989	78	77.8	180	0	0
Dun Glen, NV	2015	2015_soil_7_wm	0.1948	71	106	180	0	0
Dun Glen, NV	2015	2015_soil_8_wm	0.1972	94	48.8	180	0.0326	4.21E-09
Dun Glen, NV	2015	2015_soil_9_wm	0.2226	92	117	180	0.00962	2.58E-09
Dun Glen, NV	2015	2015_soil_10_wm	0.2283	92	71.2	180	0	0
Dun Glen, NV	2015	2015_soil_11_wm	0.2305	93	52.6	180	0.00461	5.44E-10
Dun Glen, NV	2015	2015_soil_12_wm	0.2202	95	127	180	0.00488	1.49E-09

Dun Glen, NV	2015	2015_sl_1_wm	0.0735	96	48.6	180	0.0155	5.47E-09
Dun Glen, NV	2015	2015_sl_2_wm	0.0756	95	44.8	180	0.00199	6.22E-10
Dun Glen, NV	2015	2015_sl_3_wm	0.1	96	47.8	180	0.000986	2.51E-10
Dun Glen, NV	2015	2015_sl_4_wm	0.0707	98	44.2	180	0.0197	6.71E-09
Dun Glen, NV	2015	2015_sl_5_wm	0.0738	99	47.2	180	0.00188	6.61E-10
Dun Glen, NV	2015	2015_sl_6_wm	0.0738	98	44.8	180	0.015	4.96E-09
Dun Glen, NV	2015	2015_sl_7_wm	0.0738	100	43.4	180	0.0127	4.15E-09
Dun Glen, NV	2015	2015_sl_8_wm	0.0838	98	41.4	180	0.0782	2.10E-08
Dun Glen, NV	2015	2015_sl_9_wm	0.075	98	32.8	180	0.0152	3.62E-09
Dun Glen, NV	2015	2015_sl_10_wm	0.0725	97	44.2	180	0.00307	1.01E-09
Dun Glen, NV	2015	2015_sl_11_wm	0.0747	98	30.2	180	0.0778	1.71E-08
Dun Glen, NV	2015	2015_sl_12_wm	0.0737	98	52.8	180	0.00165	6.44E-10
Dun Glen, NV	NSF	NDO_8_soil_1_wm	0.2176	88	63.4	180	0.0104	1.48E-09
Dun Glen, NV	NSF	NDO_8_soil_2_wm	0.2256	90	82.6	180	0.00412	7.54E-10
Dun Glen, NV	NSF	NDO_8_soil_3_wm	0.2224	86	79.6	180	0.00328	5.61E-10
Dun Glen, NV	NSF	NDO_8_soil_4_wm	0.2214	85	58.8	180	0.00751	9.42E-10
Dun Glen, NV	NSF	NDO_8_soil_5_wm	0.222	88	72	180	0.00314	4.98E-10
Dun Glen, NV	NSF	NDO_8_soil_6_wm	0.2236	89	42.6	180	0.00234	2.20E-10
Dun Glen, NV	NSF	NDO_8_soil_7_wm	0.2264	80	44.6	180	0.00135	1.18E-10
Dun Glen, NV	NSF	NDO_8_soil_8_wm	0.2232	82	60.4	180	0.00717	8.84E-10
Dun Glen, NV	NSF	NDO_8_soil_9_wm	0.2298	83	42.8	180	0.00199	1.71E-10
Dun Glen, NV	NSF	NDO_8_soil_10_wm	0.222	86	115	180	0.00672	1.66E-09
Dun Glen, NV	NSF	NDO_8_soil_11_wm	0.2214	91	79.4	180	0.00661	1.20E-09
Dun Glen, NV	NSF	NDO_8_soil_12_wm	0.224	85	53.6	180	0.00425	4.80E-10
Dun Glen, NV	NSF	NDO_8_sl_1_wm	0.0767	108	45	180	0.0171	6.02E-09
Dun Glen, NV	NSF	NDO_8_sl_2_wm	0.0882	109	34.6	180	0.0258	6.13E-09
Dun Glen, NV	NSF	NDO_8_sl_3_wm	0.087	109	36	180	0.0252	6.31E-09
Dun Glen, NV	NSF	NDO_8_sl_4_wm	0.1084	108	46.4	180	0.0107	2.75E-09
Dun Glen, NV	NSF	NDO_8_sl_5_wm	0.0857	101	30.2	180	0.0334	6.60E-09
Dun Glen, NV	NSF	NDO_8_sl_6_wm	0.0926	106	36	180	0.00567	1.30E-09
Dun Glen, NV	NSF	NDO_8_sl_7_wm	0.0798	108	37.2	180	0.00862	2.41E-09

Dun Glen, NV	NSF	NDO_8_sl_8_wm	0.0829	106	38.8	180	0.00794	2.19E-09
Dun Glen, NV	NSF	NDO_8_sl_9_wm	0.0963	103	33	180	0.048	9.41E-09
Dun Glen, NV	NSF	NDO_8_sl_10_wm	0.0975	107	51	180	0.0205	6.37E-09
Dun Glen, NV	NSF	NDO_8_sl_11_wm	0.0875	108	40.4	180	0.046	1.27E-08
Dun Glen, NV	NSF	NDO_8_sl_12_wm	0.0966	108	40.6	180	0.0189	4.77E-09
Dun Glen, NV	NSF	NDO_9_soil_1_wm	0.2233	89	38.8	180	0.0243	2.09E-09
Dun Glen, NV	NSF	NDO_9_soil_2_wm	0.2225	90	54	180	0	0
Dun Glen, NV	NSF	NDO_9_soil_3_wm	0.228	89	56.4	180	0.0206	2.52E-09
Dun Glen, NV	NSF	NDO_9_soil_4_wm	0.2254	84	29.6	180	0.044	2.70E-09
Dun Glen, NV	NSF	NDO_9_soil_5_wm	0.2399	100	35.6	180	0.0106	8.74E-10
Dun Glen, NV	NSF	NDO_9_soil_6_wm	0.2238	94	43.2	180	0.0342	3.45E-09
Dun Glen, NV	NSF	NDO_9_soil_7_wm	0.2218	90	37	180	0.0565	4.71E-09
Dun Glen, NV	NSF	NDO_9_soil_8_wm	0.2224	96	84.4	180	23817.41	0.004820598
Dun Glen, NV	NSF	NDO_9_soil_9_wm	0.2304	90	49.6	180	0.077	8.29E-09
Dun Glen, NV	NSF	NDO_9_soil_10_wm	0.223	89	61.2	180	39080.43	0.005303022
Dun Glen, NV	NSF	NDO_9_soil_11_wm	0.222	92	87.8	180	0.0432	8.73E-09
Dun Glen, NV	NSF	NDO_9_soil_12_wm	0.224	91	31.4	180	0.0232	1.64E-09
Dun Glen, NV	NSF	NDO_9_sl_1_wm	0.1058	99	33.2	180	0.0387	6.68E-09
Dun Glen, NV	NSF	NDO_9_sl_2_wm	0.1309	99	27.8	180	0.0855	9.99E-09
Dun Glen, NV	NSF	NDO_9_sl_3_wm	0.0719	99	35.2	180	0.0348	9.37E-09
Dun Glen, NV	NSF	NDO_9_sl_4_wm	0.0653	99	9.68	58.08	0.371	9.37E-08
Dun Glen, NV	NSF	NDO_9_sl_5_wm	0.0604	99	26.4	180	0.0728	1.75E-08
Dun Glen, NV	NSF	NDO_9_sl_6_wm	0.0527	98	16.2	97.2	0.0104	3.22E-09
Dun Glen, NV	NSF	NDO_9_sl_7_wm	0.0647	97	26.4	180	0.508	1.12E-07
Dun Glen, NV	NSF	NDO_9_sl_8_wm	0.0629	96	38.8	180	0.07	2.30E-08
Dun Glen, NV	NSF	NDO_9_sl_9_wm	0.0857	99	31.4	180	0.05	1.01E-08
Dun Glen, NV	NSF	NDO_9_sl_10_wm	0.0674	98	18.4	110.4	0.0294	7.12E-09
Dun Glen, NV	NSF	NDO_9_sl_11_wm	0.0802	99	20.2	121.2	0.14	2.88E-08
Dun Glen, NV	NSF	NDO_9_sl_12_wm	0.0827	99	27.2	180	0.0754	1.36E-08
Skull Valley, UT	1990	1990_soil_1_sv	0.2226	100	53	180	0	0
Skull Valley, UT	1990	1990_soil_2_sv	0.2279	100	50	180	0.00284	3.46E-10

Skull Valley, UT	1990	1990_soil_3_sv	0.2286	100	60	180	0.00352	5.13E-10
Skull Valley, UT	1990	1990_soil_4_sv	0.2204	99	55.2	180	0.00445	6.13E-10
Skull Valley, UT	1990	1990_soil_5_sv	0.2267	99	55.8	180	0.00237	3.21E-10
Skull Valley, UT	1990	1990_soil_6_sv	0.2234	99	58.8	180	0.007	1.01E-09
Skull Valley, UT	1990	1990_soil_7_sv	0.2244	100	58	180	0.00455	6.53E-10
Skull Valley, UT	1990	1990_soil_8_sv	0.2294	99	55.8	180	0.00249	3.33E-10
Skull Valley, UT	1990	1990_soil_9_sv	0.2224	97	50.8	180	0.0027	3.32E-10
Skull Valley, UT	1990	1990_soil_10_sv	0.2238	96	52.4	180	0.00619	7.73E-10
Skull Valley, UT	1990	1990_soil_11_sv	0.2236	89	34.6	180	0.00366	2.80E-10
Skull Valley, UT	1990	1990_soil_12_sv	0.2226	98	56.2	180	0.000387	5.32E-11
Skull Valley, UT	1990	1990_sl_1_sv	0.0759	98	62.2	180	0.00433	1.93E-09
Skull Valley, UT	1990	1990_sl_2_sv	0.0745	97	51.2	180	0.00171	6.33E-10
Skull Valley, UT	1990	1990_sl_3_sv	0.0711	98	57.2	180	0.00589	2.58E-09
Skull Valley, UT	1990	1990_sl_4_sv	0.0788	98	60.6	180	0.00446	1.87E-09
Skull Valley, UT	1990	1990_sl_5_sv	0.0769	97	53	180	0.00348	1.29E-09
Skull Valley, UT	1990	1990_sl_6_sv	0.074	99	61.4	180	0	0
Skull Valley, UT	1990	1990_sl_7_sv	0.0769	98	54.4	180	0.00279	1.07E-09
Skull Valley, UT	1990	1990_sl_8_sv	0.0782	99	58	180	0.000112	4.57E-11
Skull Valley, UT	1990	1990_sl_9_sv	0.0732	100	38.4	180	0.00224	6.53E-10
Skull Valley, UT	1990	1990_sl_10_sv	0.0775	99	55.8	180	0.00251	9.94E-10
Skull Valley, UT	1990	1990_sl_11_sv	0.076	98	39.4	180	0.00121	3.42E-10
Skull Valley, UT	1990	1990_sl_12_sv	0.0777	99	43.6	180	0.000787	2.43E-10
Skull Valley, UT	2008	2008_soil_1_sv	0.2247	85	62	180	0.0031	4.04E-10
Skull Valley, UT	2008	2008_soil_2_sv	0.2251	98	38.2	180	0.00477	4.41E-10
Skull Valley, UT	2008	2008_soil_3_sv	0.2251	97	49.2	180	0.00354	4.17E-10
Skull Valley, UT	2008	2008_soil_4_sv	0.2282	99	52.4	180	0.00511	6.45E-10
Skull Valley, UT	2008	2008_soil_5_sv	0.2242	97	48.4	180	0.00696	8.10E-10
Skull Valley, UT	2008	2008_soil_6_sv	0.2218	98	49.2	180	0.00344	4.15E-10
Skull Valley, UT	2008	2008_soil_7_sv	0.2238	99	48.8	180	0.0042	5.04E-10
Skull Valley, UT	2008	2008_soil_8_sv	0.2251	99	44.8	180	0.00246	2.69E-10
Skull Valley, UT	2008	2008_soil_9_sv	0.2264	98	58.8	180	0.00369	5.22E-10

Skull Valley, UT	2008	2008_soil_10_sv	0.2222	98	50.4	180	0.00631	7.79E-10
Skull Valley, UT	2008	2008_soil_11_sv	0.226	98	49.2	180	0.00417	4.94E-10
Skull Valley, UT	2008	2008_soil_12_sv	0.2218	97	64.2	180	0.00184	2.87E-10
Skull Valley, UT	2008	2008_sl_1_sv	0.0846	91	78.4	180	0.00684	3.20E-09
Skull Valley, UT	2008	2008_sl_2_sv	0.0828	86	58.6	180	0.0228	7.71E-09
Skull Valley, UT	2008	2008_sl_3_sv	0.0878	91	72.8	180	0.00527	2.21E-09
Skull Valley, UT	2008	2008_sl_4_sv	0.09	92	70.6	180	0.00214	8.58E-10
Skull Valley, UT	2008	2008_sl_5_sv	0.0803	93	60.4	180	0.0267	1.04E-08
Skull Valley, UT	2008	2008_sl_6_sv	0.0855	92	52.2	180	0.0152	4.74E-09
Skull Valley, UT	2008	2008_sl_7_sv	0.0851	93	62.8	180	0.116	4.42E-08
Skull Valley, UT	2008	2008_sl_8_sv	0.0808	94	63.8	180	0.0272	1.12E-08
Skull Valley, UT	2008	2008_sl_9_sv	0.0838	93	60.6	180	0.204	7.62E-08
Skull Valley, UT	2008	2008_sl_10_sv	0.085	95	62.6	180	0.00458	1.78E-09
Skull Valley, UT	2008	2008_sl_11_sv	0.0775	94	60.6	180	0.0246	1.00E-08
Skull Valley, UT	2008	2008_sl_12_sv	0.0868	94	52.8	180	0.000861	2.74E-10
Skull Valley, UT	2009	2009_soil_1_sv	0.2208	97	43.8	180	0.00702	7.50E-10
Skull Valley, UT	2009	2009_soil_2_sv	0.2264	99	49	180	0.00647	7.70E-10
Skull Valley, UT	2009	2009_soil_3_sv	0.2238	97	48.6	180	0.00366	4.28E-10
Skull Valley, UT	2009	2009_soil_4_sv	0.2222	98	47.6	180	0.00359	4.19E-10
Skull Valley, UT	2009	2009_soil_5_sv	0.2251	100	63	180	0.00694	1.08E-09
Skull Valley, UT	2009	2009_soil_6_sv	0.2239	99	63.4	180	0.00543	8.46E-10
Skull Valley, UT	2009	2009_soil_7_sv	0.225	99	49.4	180	0.00591	7.14E-10
Skull Valley, UT	2009	2009_soil_8_sv	0.2246	98	61.6	180	0.00374	5.58E-10
Skull Valley, UT	2009	2009_soil_9_sv	0.2243	99	56.2	180	0.00767	1.06E-09
Skull Valley, UT	2009	2009_soil_10_sv	0.2285	98	61.2	180	0.00567	8.27E-10
Skull Valley, UT	2009	2009_soil_11_sv	0.2268	99	50.2	180	0.00469	5.71E-10
Skull Valley, UT	2009	2009_soil_12_sv	0.2245	98	49	180	0.0053	6.30E-10
Skull Valley, UT	2009	2009_sl_1_sv	0.0791	89	66.8	180	0.00802	3.35E-09
Skull Valley, UT	2009	2009_sl_2_sv	0.0824	93	55.4	180	0.0157	5.45E-09
Skull Valley, UT	2009	2009_sl_3_sv	0.0832	90	71	180	0.00562	2.40E-09
Skull Valley, UT	2009	2009_sl_4_sv	0.0805	94	61.6	180	0.00596	2.38E-09

Skull Valley, UT	2009	2009_sl_5_sv	0.0829	93	58	180	0.0122	4.41E-09
Skull Valley, UT	2009	2009_sl_6_sv	0.0839	92	53.8	180	0.00559	1.83E-09
Skull Valley, UT	2009	2009_sl_7_sv	0.0851	91	61.2	180	0.00269	9.78E-10
Skull Valley, UT	2009	2009_sl_8_sv	0.0827	93	54	180	0.00975	3.29E-09
Skull Valley, UT	2009	2009_sl_9_sv	0.0907	91	58.2	180	0.00754	2.45E-09
Skull Valley, UT	2009	2009_sl_10_sv	0.0958	93	81	180	0.00418	1.83E-09
Skull Valley, UT	2009	2009_sl_11_sv	0.0997	94	53.2	180	0.00615	1.71E-09
Skull Valley, UT	2009	2009_sl_12_sv	0.083	91	52.2	180	0.00224	7.12E-10
Skull Valley, UT	2010	2010_soil_1_sv	0.2228	100	51.2	180	0.000709	9.05E-11
Skull Valley, UT	2010	2010_soil_2_sv	0.2221	98	37.8	180	0.00362	3.35E-10
Skull Valley, UT	2010	2010_soil_3_sv	0.2274	98	56.4	180	0.0055	7.43E-10
Skull Valley, UT	2010	2010_soil_4_sv	0.222	99	33.6	180	0.00643	5.35E-10
Skull Valley, UT	2010	2010_soil_5_sv	0.2256	99	37.2	180	0.00341	3.09E-10
Skull Valley, UT	2010	2010_soil_6_sv	0.2268	98	46	180	0.00244	2.69E-10
Skull Valley, UT	2010	2010_soil_7_sv	0.2249	98	44.4	180	0.00271	2.91E-10
Skull Valley, UT	2010	2010_soil_8_sv	0.2231	99	44.8	180	0.00556	6.14E-10
Skull Valley, UT	2010	2010_soil_9_sv	0.2218	100	46.2	180	0.00472	5.46E-10
Skull Valley, UT	2010	2010_soil_10_sv	0.2226	99	41.2	180	0.00517	5.26E-10
Skull Valley, UT	2010	2010_soil_11_sv	0.225	99	40	180	0.00545	5.33E-10
Skull Valley, UT	2010	2010_soil_12_sv	0.2268	99	47.6	180	0.00637	7.35E-10
Skull Valley, UT	2010	2010_sl_1_sv	0.0774	99	26.8	180	0	0
Skull Valley, UT	2010	2010_sl_2_sv	0.0769	98	49.4	180	0.00958	3.35E-09
Skull Valley, UT	2010	2010_sl_3_sv	0.0743	99	54.4	180	0.00477	1.92E-09
Skull Valley, UT	2010	2010_sl_4_sv	0.0773	99	54.8	180	0.00352	1.37E-09
Skull Valley, UT	2010	2010_sl_5_sv	0.0759	99	45.4	180	0.00103	3.39E-10
Skull Valley, UT	2010	2010_sl_6_sv	0.076	99	30	180	0.00402	8.73E-10
Skull Valley, UT	2010	2010_sl_7_sv	0.0779	98	38.6	180	0.01	2.70E-09
Skull Valley, UT	2010	2010_sl_8_sv	0.0777	100	42	180	0.00652	1.96E-09
Skull Valley, UT	2010	2010_sl_9_sv	0.0767	98	34.8	180	0.0519	1.28E-08
Skull Valley, UT	2010	2010_sl_10_sv	0.0775	99	36.6	180	0.00336	8.73E-10
Skull Valley, UT	2010	2010_sl_11_sv	0.0745	99	37	180	0.0127	3.47E-09

Skull Valley, UT	2010	2010_sl_12_sv	0.076	99	33.6	180	0.00224	5.45E-10
Skull Valley, UT	2013	2013_soil_1_sv	0.2204	95	61.8	180	0.00201	2.97E-10
Skull Valley, UT	2013	2013_soil_2_sv	0.2237	96	65.6	180	0.00262	4.10E-10
Skull Valley, UT	2013	2013_soil_3_sv	0.2244	98	64.2	180	0.00427	6.65E-10
Skull Valley, UT	2013	2013_soil_4_sv	0.2252	98	64	180	0.00669	1.04E-09
Skull Valley, UT	2013	2013_soil_5_sv	0.2238	98	43.8	180	0.00485	5.17E-10
Skull Valley, UT	2013	2013_soil_6_sv	0.2246	98	59.4	180	0.0047	6.77E-10
Skull Valley, UT	2013	2013_soil_7_sv	0.225	97	54.4	180	0.00434	5.65E-10
Skull Valley, UT	2013	2013_soil_8_sv	0.2279	98	57.6	180	0.00461	6.34E-10
Skull Valley, UT	2013	2013_soil_9_sv	0.2267	98	70.6	180	0.00415	7.04E-10
Skull Valley, UT	2013	2013_soil_10_sv	0.2251	96	49.8	180	0.00533	6.29E-10
Skull Valley, UT	2013	2013_soil_11_sv	0.2248	98	63.8	180	0.00347	5.36E-10
Skull Valley, UT	2013	2013_soil_12_sv	0.2248	99	54.8	180	0.0017	2.28E-10
Skull Valley, UT	2013	2013_sl_1_sv	0.0838	87	51.6	180	0	0
Skull Valley, UT	2013	2013_sl_2_sv	0.0819	95	47	180	0.000323	9.78E-11
Skull Valley, UT	2013	2013_sl_3_sv	0.0844	97	47	180	0.00202	6.06E-10
Skull Valley, UT	2013	2013_sl_4_sv	0.0829	98	45	180	0.00708	2.09E-09
Skull Valley, UT	2013	2013_sl_5_sv	0.082	98	52.4	180	0.013	4.52E-09
Skull Valley, UT	2013	2013_sl_6_sv	0.0851	98	51	180	0.00451	1.47E-09
Skull Valley, UT	2013	2013_sl_7_sv	0.0793	97	52.2	180	0.00305	1.08E-09
Skull Valley, UT	2013	2013_sl_8_sv	0.0814	97	58.2	180	0.122	4.70E-08
Skull Valley, UT	2013	2013_sl_9_sv	0.0866	98	52.4	180	0.205	6.75E-08
Skull Valley, UT	2013	2013_sl_10_sv	0.0899	97	50	180	0.00298	8.93E-10
Skull Valley, UT	2013	2013_sl_11_sv	0.0917	98	49	180	0.0439	1.28E-08
Skull Valley, UT	2013	2013_sl_12_sv	0.0794	98	49.2	180	0.00293	9.88E-10
Skull Valley, UT	2014	2014_soil_1_sv	0.2201	98	29.4	180	0.00869	6.32E-10
Skull Valley, UT	2014	2014_soil_2_sv	0.2214	96	35.8	180	0.00826	7.12E-10
Skull Valley, UT	2014	2014_soil_3_sv	0.2214	98	31.2	180	0.00971	7.45E-10
Skull Valley, UT	2014	2014_soil_4_sv	0.2292	88	39.2	180	0.0174	1.45E-09
Skull Valley, UT	2014	2014_soil_5_sv	0.2264	96	37.6	180	0.0276	2.44E-09
Skull Valley, UT	2014	2014_soil_6_sv	0.2252	95	42.6	180	0.017	1.70E-09

Skull Valley, UT	2014	2014_soil_7_sv	0.2234	98	39.4	180	0.00837	8.04E-10
Skull Valley, UT	2014	2014_soil_8_sv	0.2206	98	44.6	180	0.00831	9.15E-10
Skull Valley, UT	2014	2014_soil_9_sv	0.2282	87	49.8	180	0.00543	5.73E-10
Skull Valley, UT	2014	2014_soil_10_sv	0.2224	97	45.4	180	0.0282	3.10E-09
Skull Valley, UT	2014	2014_soil_11_sv	0.2281	95	31	180	0.0204	1.46E-09
Skull Valley, UT	2014	2014_soil_12_sv	0.2218	94	30	180	0.00799	5.64E-10
Skull Valley, UT	2014	2014_sl_1_sv	0.1079	104	24.4	146.4	0.0274	4.40E-09
Skull Valley, UT	2014	2014_sl_2_sv	0.0712	101	20.2	121.2	0.0858	2.03E-08
Skull Valley, UT	2014	2014_sl_3_sv	0.0946	100	35.4	180	0.00033	6.86E-11
Skull Valley, UT	2014	2014_sl_4_sv	0.0755	98	32.4	180	0.0102	2.38E-09
Skull Valley, UT	2014	2014_sl_5_sv	0.081	98	32.6	180	0.000508	1.11E-10
Skull Valley, UT	2014	2014_sl_6_sv	0.0787	98	51	180	7.56E-05	2.67E-11
Skull Valley, UT	2014	2014_sl_7_sv	0.0987	99	33.2	180	0	0
Skull Valley, UT	2014	2014_sl_8_sv	0.103	99	32.6	180	0.000584	1.02E-10
Skull Valley, UT	2014	2014_sl_9_sv	0.0767	99	26.2	180	0.0477	8.96E-09
Skull Valley, UT	2014	2014_sl_10_sv	0.0766	99	37.4	180	0.00235	6.31E-10
Skull Valley, UT	2014	2014_sl_11_sv	0.0782	99	40	180	0.0668	1.88E-08
Skull Valley, UT	2014	2014_sl_12_sv	0.1	99	43.4	180	0	0
Skull Valley, UT	2015	2015_soil_1_sv	0.2276	97	52	180	0.00199	2.45E-10
Skull Valley, UT	2015	2015_soil_2_sv	0.2238	100	46	180	0.00495	5.65E-10
Skull Valley, UT	2015	2015_soil_3_sv	0.2234	99	60.2	180	0.00608	9.01E-10
Skull Valley, UT	2015	2015_soil_4_sv	0.2276	100	51.2	180	0.00343	4.29E-10
Skull Valley, UT	2015	2015_soil_5_sv	0.2245	99	57.6	180	0.00689	9.72E-10
Skull Valley, UT	2015	2015_soil_6_sv	0.224	98	54.8	180	0.00489	6.51E-10
Skull Valley, UT	2015	2015_soil_7_sv	0.2216	99	54.8	180	0.00449	6.11E-10
Skull Valley, UT	2015	2015_soil_8_sv	0.2254	99	54.2	180	0.00367	4.85E-10
Skull Valley, UT	2015	2015_soil_9_sv	0.2249	97	38.2	180	0.00177	1.62E-10
Skull Valley, UT	2015	2015_soil_10_sv	0.2222	98	49	180	0.00384	4.61E-10
Skull Valley, UT	2015	2015_soil_11_sv	0.2209	97	51.2	180	0.00613	7.66E-10
Skull Valley, UT	2015	2015_soil_12_sv	0.2231	98	62.6	180	0.0118	1.80E-09
Skull Valley, UT	2015	2015_sl_1_sv	0.0796	98	51.4	180	0.00196	6.89E-10

Skull Valley, UT	2015	2015_sl_2_sv	0.0792	98	55.4	180	0.0157	5.98E-09
Skull Valley, UT	2015	2015_sl_3_sv	0.0759	98	56.4	180	0.0127	5.14E-09
Skull Valley, UT	2015	2015_sl_4_sv	0.0752	89	52.8	180	0.00128	4.44E-10
Skull Valley, UT	2015	2015_sl_5_sv	0.0767	97	44.8	180	0.0169	5.32E-09
Skull Valley, UT	2015	2015_sl_6_sv	0.077	97	61	180	0.0251	1.07E-08
Skull Valley, UT	2015	2015_sl_7_sv	0.0787	98	47.6	180	0.0239	7.87E-09
Skull Valley, UT	2015	2015_sl_8_sv	0.0808	98	43.6	180	0.0279	8.20E-09
Skull Valley, UT	2015	2015_sl_9_sv	0.077	98	65	180	0.0134	6.16E-09
Skull Valley, UT	2015	2015_sl_10_sv	0.0774	99	52.2	180	0.0172	6.38E-09
Skull Valley, UT	2015	2015_sl_11_sv	0.0789	98	50.2	180	0.0607	2.10E-08
Skull Valley, UT	2015	2015_sl_12_sv	0.0779	99	55	180	0.00735	2.85E-09
Skull Valley, UT	NSF	NDO_1_soil_1_sv	0.2287	96	56.2	180	0.0248	3.25E-09
Skull Valley, UT	NSF	NDO_1_soil_2_sv	0.2211	98	49	180	0.013	1.57E-09
Skull Valley, UT	NSF	NDO_1_soil_3_sv	0.2207	97	48.4	180	0.0154	1.82E-09
Skull Valley, UT	NSF	NDO_1_soil_4_sv	0.2294	97	50.4	180	0.00864	1.02E-09
Skull Valley, UT	NSF	NDO_1_soil_5_sv	0.2223	98	50.8	180	0.0812	1.01E-08
Skull Valley, UT	NSF	NDO_1_soil_6_sv	0.2217	98	48.6	180	0.0143	1.71E-09
Skull Valley, UT	NSF	NDO_1_soil_7_sv	0.2216	98	51.4	180	0.015	1.89E-09
Skull Valley, UT	NSF	NDO_1_soil_8_sv	0.224	97	49.6	180	0.0176	2.10E-09
Skull Valley, UT	NSF	NDO_1_soil_9_sv	0.2249	97	59.6	180	0.0094	1.34E-09
Skull Valley, UT	NSF	NDO_1_soil_10_sv	0.2235	98	49.2	180	0.0103	1.23E-09
Skull Valley, UT	NSF	NDO_1_soil_11_sv	0.2211	97	44.8	180	0.00611	6.67E-10
Skull Valley, UT	NSF	NDO_1_soil_12_sv	0.2271	96	52	180	0.0167	2.04E-09
Skull Valley, UT	NSF	NDO_1_sl_1_sv	0.0789	98	51.8	180	0.0242	8.65E-09
Skull Valley, UT	NSF	NDO_1_sl_2_sv	0.0766	98	52.6	180	0.0113	4.22E-09
Skull Valley, UT	NSF	NDO_1_sl_3_sv	0.0757	92	45.2	180	0.0121	3.69E-09
Skull Valley, UT	NSF	NDO_1_sl_4_sv	0.0741	97	41.6	180	0.0115	3.48E-09
Skull Valley, UT	NSF	NDO_1_sl_5_sv	0.0774	83	61.2	180	0.0064	2.33E-09
Skull Valley, UT	NSF	NDO_1_sl_6_sv	0.0797	97	46.4	180	0.0298	9.35E-09
Skull Valley, UT	NSF	NDO_1_sl_7_sv	0.078	98	55	180	0.0284	1.09E-08
Skull Valley, UT	NSF	NDO_1_sl_8_sv	0.078	97	44.4	180	0.0468	1.44E-08

Skull Valley, UT	NSF	NDO_1_sl_9_sv	0.0773	98	38.4	180	0.0439	1.19E-08
Skull Valley, UT	NSF	NDO_1_sl_10_sv	0.0764	94	48.6	180	0.0427	1.42E-08
Skull Valley, UT	NSF	NDO_1_sl_11_sv	0.079	97	42	180	0.0132	3.78E-09
Skull Valley, UT	NSF	NDO_1_sl_12_sv	0.0773	94	51.6	180	0.00378	1.32E-09
Skull Valley, UT	NSF	NDO_2_soil_1_sv	0.224	88	41.4	180	0.00702	6.34E-10
Skull Valley, UT	NSF	NDO_2_soil_2_sv	0.2232	89	36.4	180	0.00413	3.33E-10
Skull Valley, UT	NSF	NDO_2_soil_3_sv	0.2276	88	17	102	0.0033	2.13E-10
Skull Valley, UT	NSF	NDO_2_soil_4_sv	0.2218	88	67.4	180	0.00662	9.83E-10
Skull Valley, UT	NSF	NDO_2_soil_5_sv	0.2261	89	50	180	0.0082	8.97E-10
Skull Valley, UT	NSF	NDO_2_soil_6_sv	0.2243	90	58.2	180	0.00844	1.09E-09
Skull Valley, UT	NSF	NDO_2_soil_7_sv	0.227	89	30	180	0.0126	8.23E-10
Skull Valley, UT	NSF	NDO_2_soil_8_sv	0.2228	87	50.2	180	0.0181	1.97E-09
Skull Valley, UT	NSF	NDO_2_soil_9_sv	0.2222	88	18.9	113.4	0.00946	6.24E-10
Skull Valley, UT	NSF	NDO_2_soil_10_sv	0.2254	88	20.4	122.4	0.0101	6.57E-10
Skull Valley, UT	NSF	NDO_2_soil_11_sv	0.2274	90	22	132	0.0101	6.66E-10
Skull Valley, UT	NSF	NDO_2_soil_12_sv	0.2208	89	4.68	28.08	0.00669	4.49E-10
Skull Valley, UT	NSF	NDO_2_sl_1_sv	0.0774	95	44.6	180	0.0112	3.41E-09
Skull Valley, UT	NSF	NDO_2_sl_2_sv	0.0811	95	54	180	0.029	1.02E-08
Skull Valley, UT	NSF	NDO_2_sl_3_sv	0.0899	96	44.2	180	0.0226	5.93E-09
Skull Valley, UT	NSF	NDO_2_sl_4_sv	0.0841	95	51.2	180	0.00792	2.54E-09
Skull Valley, UT	NSF	NDO_2_sl_5_sv	0.0754	95	49.6	180	0.0111	3.85E-09
Skull Valley, UT	NSF	NDO_2_sl_6_sv	0.0738	87	48.2	180	0.00825	2.60E-09
Skull Valley, UT	NSF	NDO_2_sl_7_sv	0.077	90	53.4	180	0.00664	2.30E-09
Skull Valley, UT	NSF	NDO_2_sl_8_sv	0.081	95	48.4	180	0.0157	4.95E-09
Skull Valley, UT	NSF	NDO_2_sl_9_sv	0.0789	95	48.4	180	0.04	1.30E-08
Skull Valley, UT	NSF	NDO_2_sl_10_sv	0.0799	94	53.6	180	0.0207	7.25E-09
Skull Valley, UT	NSF	NDO_2_sl_11_sv	0.0766	94	50.6	180	0.0255	8.80E-09
Skull Valley, UT	NSF	NDO_2_sl_12_sv	0.0764	94	62	180	0.0369	1.56E-08