BIOAG PROJECT FINAL REPORT

TITLE: *Quantifying the Weed Seedbank Using Quantitative PCR and Soil Community Analysis*

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ABSTRACT:

Quantification of weed seedbanks is essential for the evaluation and improvement of integrated weed management systems by farmers and integral in the Sustaining Resources Grand Challenge. Currently, weed scientists filter seed from the soil and count them – a hopelessly time consuming process that cannot be scaled or used by farmers. If farmers could quantify their seedbanks as they quantify soil nutrients, they could adjust their weed management strategies, improving control of those weeds and improving farm sustainability. Our overarching objective is to develop methods to quantify weed seed using techniques borrowed from soil microbial community analysis.

PROJECT DESCRIPTION:

Weed management is the most important constraint in cropping systems, especially in low-input and organic systems (Cavigelli et al 2008, Bernstein et al 2014). Other than in a very general sense, farmers and scientists do not know how many weed seeds are in soils. Worse, farmers and scientists do not have a way to quantify if control tactics impacted the number of seeds in the soil either positively or negatively from year to year. Developing a truly sustainable integrated weed management system needs to have a rapid and reliable measure that assesses management input effectiveness on the weed seedbank.

Under many current weed management paradigms, growers are typically reactive, responding to what they observe after planting a crop. A common complaint of farmers using preemergence herbicides or pre-plant tillage is that they never know if they needed to spend the money and time on it. The uncertainty of what the weed pressure could be (what species and how many of each) with the complication of what techniques to use and when to use them based on the weed species present often make for less than optimal weed control strategies, and also sets up barriers for adoption of more integrated approaches. Therefore, it is no wonder herbicide resistant crops have had such widespread adoption despite the lack of enthusiasm of the consumer population for this technology (Wunderlich and Gatto 2015). For example, a recent report indicates over 80% of soybeans, cotton, and corn in the United States is genetically engineered to be resistant to an herbicide (USDA Economic Research Service, 2017).

The uncertainty of what the weed pressure could be in a given year could be ameliorated if the numbers of weed seeds in the soil were known (Derksen, et al 2002 and Gallandt 2006). Increasing clarity about weeds in the seedbank will also empower growers and scientists with more information about how to establish targeted management approaches that best suit their individual operational needs. Currently, weed seedbanks can be estimated using grow out methods in a greenhouse, or by sieving the seed out of the soil and counting them. However, these methods are slow and often do not provide useful information in an acceptable time frame, i.e. before the start of the next growing season. Greenhouse based approaches are also very expensive (Wiles et al 1996). There has been research conducted that uses models to estimate the seed input into the soil based on weeds that are not controlled. Although modeling provides a relative estimate of the potential for infestation based on weed emergence, models must be adjusted in hindsight to reflect variation from a previous year and are not reliable for estimating what is already in the soil, or for accounting for variation in future growing seasons (Borgy et al 2015).

Advances in high through-put molecular sequencing techniques in microbes, humans, and model plants and crops have facilitated discovery of predictive genetic markers to characterize biological

identity and response to biotic and abiotic environmental inputs. Therefore, using molecular biology approaches it may be possible to rapidly identify and quantify weed species from a soil sample.

This is, however, where the project hit some insurmountable obstacles. At the same time that the pandemic shut the world down, the PhD and Postdocs who were working on the project had been unable to find a primer set that would successfully amplify weed seed DNA. Without the necessary primers developed and thoroughly tested first by the weed science experts, the sequencing technology within the Sullivan lab could not even be attempted. The first part was crucial (primer development) and the second part (sequencing) could not proceed without that target.

We attempted to use several different weedy species, after it was clear that amplification of downy brome (Bromus tectorum) was not being successful. The recent discoveries of several seed-specific dormancy genes and housekeeping genes (Hauvermale and Burke; unpublished), proved to be elusive targets. Therefore we were not able to achieve our objectives.

OUTPUTS

- Overview of Work Completed and in Progress:
  - Methods have been developed to extract DNA from all seed types by utilizing a standard soil DNA extraction kit.
  - High quality DNA has been extracted from all seed types.
  - Methods for PCR amplification were developed but unsuccessful.

Methods, Results, and Discussion (discussion for final reports only):

DNA extraction from a variety of seeds was achieved through trial and error. Downy brome seeds have been particularly challenging to extract high quality and concentration DNA. For instance, initially downy brome seeds were ground with a mortar and pestle, but it wasn't possible to obtain a fine powder using this method.

Further attempts to grind the seeds with a mortar and pestle were made using a variety of pre-treatments, including imbibing the seeds overnight and then attempting to powderize them in the absence and presence of liquid nitrogen. An industrial strength grinder was then used to powderize downy brome seeds, but the unit was incapable of producing fine powder (in the absence or presence of liquid nitrogen), and this unit required an unreasonably high volume of seeds in order to operate. A smaller industrial grinder was then used to attempt grinding of liquid nitrogen pre-treated seeds, but this did not generate appropriate materials. More attempts were made to grind the seeds in the smaller industrial grinder where dry ice was ground with the seeds. While this last method provided a fairly homogenous powder, the sample absorbed nearly all of the reagents used in DNA extraction.

Eventually, chaff was removed from dry downy brome seeds and the seeds were ground in a consumer grade coffee grinder and rapidly frozen after grinding. The resulting powder still absorbed most of the DNA extraction reagent, so the mass/volume of seed powder was reduced and additional molecular grade water was added to the reagent mix to partially hydrate the sample prior to extraction. This method resulted in relatively low concentrations of DNA of moderate quality that should sufficient for PCR amplification of the 16S gene. Further optimization of downy brome DNA extraction will include imbibing the seeds overnight and freezing in liquid nitrogen prior to homogenization in the commercial-grade coffee grinder. DNA concentrations of successful extractions are shown in the table below.

Species	Replicate	DNA (ng/µl)	260/280	260/230
C. album	1	193.1	1.82	2.05
	2	187.7	1.84	2.15
	3	183.6	1.83	2.05
L. multiflorum	1	69	1.84	1.79
	2	96	1.83	1.37
	3	88.6	1.84	1.8
V. myuros	1	131.3	1.9	1.75
	2	145	1.85	1.97
	3	108.5	1.86	1.87
A. cotula	1	149.5	1.84	2.01
	2	196.8	1.82	2.11
	3	140.5	1.82	1.94
S. tragus	1	183.5	1.81	2.41
	2	139.4	1.86	2.47
	3	154.1	1.84	2.36
B. tectorum	1	17.7	2.2	2.29
	2	14.4	2.37	2.85
	3	30.4	2	2.21

Protocols have been developed for the PCR amplification of the 16S gene, which includes a PCR run method and PCR primer selection.

a. PCR primers to be used for whole 16S gene amplification
    a. GTGCCAGCMGCCGCGGTAA (forward primer)
    b. GGACTACHVGGGTWTCTAAT (reverse primer)
b. PCR master mix:

Component	Concentration
Template DNA	0.1 ng/25 µL rxn
Forward Primer	200 nM
Reverse Primer	200 nM
$MgCl_2$	2 mM
dNTPs	0.2 mM
Platinum *Taq* DNA polymerase	0.25 units/25 µL rxn

c. PCR run method:
    a. Incubate at 94°C for 4 minutes
    b. 23 Cycles
    c. 1 min denature at 94 °C
    d. 30 s anneal at 48 °C
    e. 2 min extension at 72 °C
    f. Hold at 4 °C

- Publications, Handouts, Other Text & Web Products:

- Outreach & Education Activities:
  NA

IMPACTS

- Short-Term/ Intermediate/Long-Term:

Unfortunately the impacts of the project will remain unrealized until we can develop a successful set of primers and PCR conditions.

ADDITIONAL FUNDING APPLIED FOR / SECURED: BioAg funding has been leveraged against the NIFA funded Landscapes in Transition project, www.pnwlit.org.

GRADUATE STUDENTS FUNDED: Pragya Asthana

RECOMMENDATIONS FOR FUTURE RESEARCH: Implementation of the methodology in field trials to determine the real-world applicability of the technique.