

Narrative:

Project Title: Establishment and maintenance of disease-suppressive soils with green manures in the Pacific Northwest

Relevance to Sustainable Agriculture:

Soil fumigation is required for profitable production of many important agricultural crops and is therefore essential for the livelihoods of the growers, farm workers, and downstream stakeholders and consumers. Prolonged use of fumigants, however, can lead to contamination of the natural resources on which agriculture depends and degradation of the environment. Recognition of this problem has stimulated the regulation and discontinuation of some fumigants and the search for alternatives for pest and pathogen management. Green manures can provide disease control comparable to soil fumigants and contribute to soil quality but variability in disease-suppression has limited grower adoption and thereby prolonged the dependence on fumigants such as metam sodium and chloropicrin. Identification of the practices that consistently establish and maintain disease-suppressive soils is therefore required for sustainable potato cropping systems. Specifically, development of disease-suppressive soils with green manures will (i) eliminate the negative impacts of fumigants and synthetic fertilizers on natural resources and the environment (e.g. water, soil, and biodiversity), (ii) reduce on-farm expenditures on fumigants and fertilizers and inflate profits as farms might qualify for organic certification and produce potatoes that demand higher prices, and (iii) enhance the quality of life by empowering small holder farmers who do not have access to fumigation equipment to grow potatoes, eliminating the risk of fumigation exposure, contamination of the environment on which we all depend.

The importance of alternatives for *Verticillium* wilt management have been recognized by SARE, the National Agricultural Library, and the USDA-NIFA-CRIS:

National SARE database:

Suppression of *Verticillium* wilt of strawberry by different composts was evaluated in the graduate student project “Compost-induced disease-suppressive soils for control of *Verticillium* wilt of strawberry”. Grafting with tomato rootstocks resistant to *V. dahliae* was investigated in the farmer/rancher project “Grafting tomatoes in multi-bay high tunnels as a way to overcome soilborne diseases”. The effect of crop rotation and incorporation of plant residues were determined in the research and education project “Rotations with broccoli- a sustainable alternative to soil chemical fumigants”.

National Agricultural Library:

Alternatives to soil fumigants and the impacts of crop rotation on populations of *V. dahliae* were assessed in the project entitled “Control of pathogens in strawberry and vegetable production systems”.

USDA-NIFA-CRIS Research:

Anaerobic soil disinfestation was tested as an alternative for fumigation for strawberry production systems where *Verticillium* wilt is problematic in “Refining anaerobic soil disinfestation for disease management in strawberry and apple productions”. The impact of rotation crops of potato on soil populations of *V. dahliae* is being investigated in the project “Endophyte dynamics: a new consideration for designing crop rotations for disease management”. Soil amendments are also being investigated as potential alternatives to fumigants in “Optimization/implementation of biologically active soil amendments as a fumigation alternative for soil-borne disease control in California strawberry”.

Relevant literature:

Soils infested with pathogens that do not cause disease when susceptible hosts are grown are disease-suppressive (1). Suppressive soils can be effective alternatives to fumigants for controlling plant diseases caused by soilborne pathogens (2) and are generally established by continuous monoculturing of susceptible crops (3) or, for *Verticillium* wilt of potato, by incorporation of green manure crops for 2-3 successive years (4-7). Remission of wilt is generally observed after two years without green manure incorporation (7) and restoration of suppression can be achieved with incorporation of one additional green manure crop (7). Despite the ability of green manures to establish suppressive soil, variability in the efficacy suppression has prevented widespread grower adoption (8). Asymptomatic infections of green manure crops (9-10) represents a major source of this variability and warrants further investigation to best inform management decisions and accelerate grower adoption.

Citations:

(1) Larkin, R.P. *Annu. Rev. Phytopathol.* 53:199–221, 2015. (2) Larkin, R.P. et al. *Plant Dis.* 95:568-576, 2011. (3) Raaijmakers, J.M and Mazzola. *Science.* 352:1391-1392, 2016. (4) Davis, J. et al. *Am. J. of Pot. Res.* 82:64, 1997. (5) Davis, J. et al. *Phytopathol.* 86:444-453, 1996. (6) McGuire, A.M. *Crop Management.* doi:10.1094/CM-2003-0822-01-RS, 2003. (7) Davis, J. et al. *Am. J. of Pot. Res.* 87:315–326, 2010. (8) Larkin, R.P. *CAB Reviews.* doi: 10.1079/PAVSNR20138037, 2013. (9) Malcolm, G.M et al. *Phytopathol.* 538-544, 2013. (10) Wheeler, D.L. and Johnson, D.A. *Phytopathol.* 106:602-613, 2016.

Innovativeness:

The discovery of asymptomatic infections of green manure crops has precipitated the need to determine the role of these infections in *Verticillium* wilts. The occurrence of these infections, the corresponding rise in populations of *V. dahliae* in soils, and the general absence of disease in potato after green manure incorporation provides promise for green manures in building soils that are fertile and suppress diseases without fumigation. Before widespread grower adoption we need to identify the role of asymptomatic infections in disease-suppression and determine the practices that establish and maintain disease-suppression. We propose to evaluate the two possible scenarios under which asymptomatic infections contribute to suppression: (i) green manure crops select for strains of *V. dahliae*

that are not pathogenic to potato and (ii) green manure crops reduce the aggressiveness of strains of *V. dahliae* that are pathogenic to potato. Both scenarios have precedence in cropping systems but both are novel to Verticillium wilt of potato and underutilized. The former will require growers to plant green manure species or specific genotypes within species that select for pathogen populations that do not cause wilt in potato. The latter will require growers to successively plant green manure species or genotypes that select for pathogen populations that do cause disease on potato but exhibit reduced aggressiveness after infection of green manures. Both scenarios could permit potato cultivation without soil fumigation by diminishing *V. dahliae* populations pathogenic to potato. Finally, identification of the role of asymptomatic infections will likely reduce the variability associated with green manures and increase grower adoption.

Impacts & Outcomes:

Identification of the practices necessary to establish and maintain disease-suppression, for example by planting specific green manure species, will:

1. Reduce grower reliance on fumigants and thus contamination of natural resources.
2. Save growers an estimated \$ 66.00/acre compared to growers who use the fumigant metam sodium (6).
3. Reduce grower reliance on synthetic fertilizers.
4. Improve soil organic matter thereby decreasing erosion, increasing water holding capacity and infiltration rates (6).
5. Allow growers to shorten crop rotations, plant potatoes more often, and thereby inflate their incomes.
6. Allow growers to plant susceptible potato varieties that demand higher prices.
7. Empower growers of other crops in other regions to use green manures to build disease-suppressive soils.
8. Empower small farmers to grow potatoes when fumigation equipment is prohibitively costly.
9. Change the conceptual paradigm that pathogens must be eliminated completely to grow a crop and achieve profits.

Producer involvement:

Producers will be involved at every step of this project:

- Before the experiments begin we will ask growers to (i) complete a survey to determine their perception and current use of green manure crops, and (ii) recommend green manures species and genotypes which we will use in our experiments.
- During the experiments we will collect samples of green manure stems from grower's fields to determine if specific green manures are infected under field conditions.

- After the experiments are completed, we will present the results to growers and request completion of a survey to determine their perception and use of green manure crops in their fields after learning from our results.

Objectives:

1. Determine if green manure crops select for and promote strains of *V. dahliae* that are not pathogenic to potato. This objective will be completed with greenhouse and field experiments:
 - a. Green manure crops will be inoculated with mixtures of *V. dahliae* strains and the pathogen will be recovered from the stems of each crop and the strain identification will be determined.
 - b. Green manure crops will be collected from fields with a history of Verticillium wilt, the pathogen will be recovered from the stems, and the strain identification will be determined and compared with strains from potato.
2. Determine the number of infection cycles necessary on green manure crops to reduce the aggressiveness of strains of *V. dahliae* pathogenic to potato.
 - a. Green manures crops will be repeatedly inoculated with strains of *V. dahliae* pathogenic to potato and the aggressive of each strain will subsequently be determined by inoculating and assessing symptoms in potato after every passage through the green manure crops.

Materials and Methods:

Surveys: Surveys will be administered before and after completion of the proposed experiments to gauge the perception, use, and adoption of green manures. Preexisting listservs from the Northwest Potato Research Consortium will be used to issue the surveys to growers and stakeholders in the PNW. Surveys will also be administered at grower field days and conferences. Examples of survey questions are below. Names and contact information of respondents will be recorded and kept confidential to ensure the same growers are surveyed before and after completion of the experiment.

- Do you currently plant green manure crops in your rotations (Yes or No)?
 - If yes, what species of green manures do you plant (open response)?
 - If yes, for what reason(s) do you plant green manure crops (to enhance soil quality, disease-suppression, or other)?
 - If no, for what reason(s) do you not plant green manure crops (open response)?
- On a scale of 1-10 (1 being not informative and 10 being very informative) rank the value of the information gained from extension bulletins, workshops, social media, and or peer-reviewed journal articles.
- On a scale of 1-10 (1 being not likely and 10 being very likely) rank the likelihood that you will use green manures after learning from the information presented through extension bulletins, workshops, social media, and or peer-reviewed journal articles.

Objective 1:

Greenhouse experiment: This experiment will require a 2-way factorial treatment structure with green manure crops and pathogen strains representing each factor. A randomized completed block design structure (RCBD) will be used and each treatment will be replicated 5 times. The experiment will be repeated once. Potting soil will be infested with mixtures of three strains of *V. dahliae* (one from potato and two from other crops), each pairwise combination and each strain alone. Green manure crops selected based on grower interest will be planted in the infested soil. Plant biomass will be measured and pathogen populations will be quantified from each crop. The pathogen strain will be identified with by comparing segments of DNA from the recovered strain with the three known strains used to inoculate each crop. Assuming the parametric assumptions are satisfied analysis of variance (ANOVA) will be used to detect differences in plant biomass and the percentage of plants infected with each isolate. We hypothesize that green manure crops will select for specific strains in which case we expect to recovery those strains at a higher frequency than other strains.

Field experiment: Fields with a history of Verticillium wilt of potato will be identified by contacting growers directly and through the Northwest Potato Research Consortium listserv. Fields will be divided into strata based on center-pivot wheel tracks and stratified random samples of stems will be collected from each strata. Stems will be surface disinfested and plated onto semi-selected media (NPX), incubated at room temperature, and inspected for *V. dahliae* colonies. *V. dahliae* will be subcultured, DNA will be extracted and molecular markers called microsatellites will be used to compare the strains from green manure crops with the strains from potato. Standard population genetic analyses will be implemented to compare populations from green manure crops vs. potato. We hypothesize that populations from green manures crops will be different from populations from potato.

Objective 2:

This experiment will also require a 2-way factorial treatment structure with green manure crops and pathogen strains representing each factor. A RCBD will be used and each treatment will be replicated ten times. The experiment will be repeated once. Green manure crops will once again be selected based on grower interest gauged from the first survey. Strains of *V. dahliae* pathogenic to potato will be used to inoculate several green manure crops, for example a mustard like brown mustard and a cereal like sudangrass. After green manure crops senesce the pathogen will be recovered, duplicated, and one biological 'copy' will be used to inoculate potato while the other 'copy' will be used to inoculate a new plant of the same species from which the strain was recovered (see figure 1). Disease, plant biomass (yield for potato) will be scored on all crops and *V. dahliae* will be quantified from stems of each plant with culture based techniques. Disease is expected to manifest within 4-5 infection cycles based on experiments by Fordyce and Green in 1960 and Alkher et al in 2009 and is expected to coincide with reduction in aggressiveness of the same strains on potato. ANOVA will be used to detect differences in biomass, disease, and pathogen populations from stems of potatoes inoculated with

strains recovered from potato vs. green manure crops. We hypothesize that potato plants inoculated with strains recovered from green manure crops will display more biomass, less disease, and less pathogen populations than potato plants inoculated with strains recovered from potato.

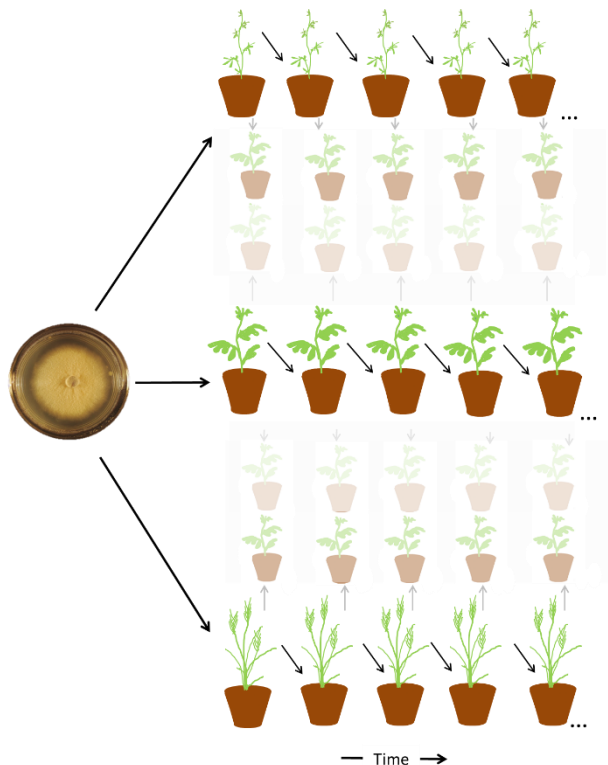


Figure 1. A strain of the potato pathogen *Verticillium dahliae* will be inoculated to a mustard (top row), potato (middle row), and a cereal (bottom row). The strain will be recovered from each crop and used to inoculate the same species of crop and potato. Biomass, disease, and the populations of the pathogen will be measured on potato after each infection cycle for five total cycles. We expect disease and pathogen populations to decrease over time while potato tuber yields increase.

Educational Outreach Plan:

2018

- **Conferences:** Information from the project proposed herein will be presented at the Washington and Oregon Potato Conference in January 2018, the Idaho Potato Conference and Ag Expo, and the American Society of Phytopathology during the summer of 2018. The first two conferences are attended primarily by growers and industry professionals while the APS conference is attended primarily by scientists and industry professionals.
- **Field days:** Information from the proposed project will be presented at potato field days in Othello, Washington and Hermiston, Oregon. Both field days are attended by growers, stakeholders, researchers, and industry professionals from the PNW. Surveys will also be administered at the field days to capitalize on attendance.
- **Social Media:** A facebook page will be established in 2017-2018 to (i) provide immediate research and survey updates (pictures, anecdotes and formal results) to

growers and (ii) receive feedback from growers. The page will be maintained indefinitely and will serve as a forum to discuss alternative disease management practices.

- **Materials:** We will produce presentations for conferences, handouts and extension bulletins for field days, digital posts for the facebook page, and published peer-reviewed manuscripts (minimum of 2, one for each objective). These materials will be disseminated both physically, during field days and conferences, and digitally, via social media and journal articles. The information obtained from the proposed project will be helpful to other producers because (i) *Verticillium* wilts affect hundreds of different crops and green manures can be used in all of the annual cropping systems and, perhaps with less efficacy, in the perennial cropping systems, and (ii) green manures are also effective in reducing other soilborne plant pathogens and pests and the information gleaned herein may therefore be applicable beyond the scope of this proposal.

Scholarly Publications and Educational Materials:

- **Extension bulletins:** At least one extension bulletin will be published through Washington State University. The bulletin will report survey results from before and after project completion and a summary of the results from each objective. Extension bulletins will be handed to growers, researchers, and industry professionals at the field days listed above. A summary of the research will also be submitted to the Potato Progress Report. The regional director, Dr. Andy Jenson, will email the Potato Progress Report to all growers, extensions staff, industry professionals, and scientists on the Northwest Potato Research Consortium's listserv.
- **Refereed scientific journals:** At least two manuscripts, one for each objective, will be submitted for publication in *Phytopathology* or *Plant Disease*.
- **Data:** Data from all experiments will be publicly available upon request if approved by the USDA SARE. DNA sequence data will be published on an online repository entitled Genbank.

Evaluation and Producer Adoption:

A pre-post survey will be administered as detailed above to gauge the perception, use, and adoption of green manures for disease-suppression. The surveys will be issued via an established listserv for the Northwest Potato Research Consortium and by hand at field days and conferences. Respondent names and contact information will be recorded but kept confidential to enable us to survey the same growers before and after they learned from the information disseminated by extension bulletins, field days, conferences, social media and peer-reviewed publications.

Timeline:

2017:

Fall-winter:

- Pre-surveys will be administered to growers via the listserv and advertised on facebook.
- Both greenhouse experiments will commence in greenhouses in Pullman, WA. Greenhouse experiments will be repeated in different greenhouses to expedite completion, permit sufficient time to process samples, analyze data, complete written reports, and repeat experiment(s) if data from trials conflict.
- The field collection for objective 1 will also be completed during this time since growers are actively planting green manure crops in the late summer to fall. The collection of green manure stems from fields will be repeated in 2018 as well.

2018:

Winter-spring:

- Pre-surveys will be administered to growers during conferences and advertised on facebook.
- Reports for both greenhouse trials will be completed.
- Strains of *V. dahliae* will be recovered from the green manure crop stems collected in the fall.
- DNA will be extracted from collected *V. dahliae* strains and DNA will be sequenced.

Spring-Summer:

- Pre-surveys will be administered to growers during field days and advertised on facebook.
- Results from both greenhouse trials and one field collection will be presented at field days, conferences, and via facebook.

Fall-winter

- The second field collection for objective 1 will be completed.
- Strains of *V. dahliae* will be recovered from green manure crop stems, DNA will be extracted and sequenced.
- DNA sequence data will be analyzed to compare strains from green manure crops to strains from potato.
- Extension bulletins and peer-reviewed publications will be prepared and submitted.
- Post-survey will be administered to growers via the listserv and contact information obtained from the pre-surveys.

