BIOAG PROJECT FINAL REPORT

TITLE: Management of seedling blights in organic vegetable production in the Pacific Northwest

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KEY WORDS: vegetables, pea sweet corn, organic, *Pythium*, seedling blight, damping-off, seed treatments, drench treatments, Pacific Northwest

ABSTRACT:

Vegetable production is an important sector of the organic food industry in the Pacific Northwest USA. Significant acres of organic vegetable crops, particularly processing green pea and sweet corn, are grown in the semi-arid Columbia Basin of central Washington and north central Oregon, where Pythium damping-off can cause losses in early spring when cool and wet soil conditions are conducive to the disease. Ana Vida Alcala's PhD project, funded by this grant, addressed management of damping-off in organic vegetables using organic pea production in central Washington as an early season, model crop. In fall 2009, soil samples were collected from 37 certified organic fields in the Columbia Basin to survey for *Pythium* species, from which 305 isolates were baited and identified to 19 species using DNA sequencing. Pathogenicity tests were completed in a growth chamber for as many as 9 isolates of each species on pea in cool and wet soil conditions, in soil sampled from a growers' organic field in the Columbia Basin. Isolates of 9 species were pathogenic, with differences in virulence among species and among isolates within species. *Pythium ultimum* (24.6% of the 305 isolates), *P. irregulare* group 1 (15.1%), and *P. abappressorium* (4.9%) were the most prevalent pathogenic species. Real-time PCR assays detected *P. ultimum* in soil samples from 100% of the 37 fields, compared to 78% for *P. abappressorium* and 57% for *P. irregulare* group 1. Given the lack of highly effective seed treatments for damping-off control in organic production, organic seed and drench treatments were evaluated in 2011 in four field trials: 15 treatments in each of 2 trials in grower-cooperator, certified organic pea crops in the Basin; and 20 treatments in inoculated pea and sweet corn trials at the WSU Mount Vernon NWREC. Nordox seed treatment and seed priming demonstrated the greatest potential for damping-off control. Pea seed priming with 16 h of seed-soaking and 10 h of air-drying optimized priming for rapid emergence. The more promising treatments were evaluated again in 2012 applied to both primed and non-primed seed in each of two field trials, one in Ephrata and one in Mount Vernon. Priming did not improve the efficacy of any of the seed treatments, and even reduced efficacy of some treatments. In two additional grower-cooperator, organic pea trials near Royal City in 2012, the use of primed seed that was dried with biochar instead of air, and then treated with Nordox (organic copper), showed potential for improving pea emergence, height, and yield. Seed exudates produced during germination are measured using electrical conductivity (EC). The EC levels of 17 pea seed lots (representing six cultivars)

significantly affected emergence as well as susceptibility to Pythium damping-off. A negative linear relationship was demonstrated between EC level and emergence, with a stronger regression at higher inoculum levels of *P. ultimum*. The results illustrated that measuring the EC of pea seed lots, combined with quantifying inoculum in soil using real-time PCR assays for each of the prevalent and pathogenic *Pythium* spp. identified in the survey (*P. ultimum*, *P. abappressorium*, and *P. violae*) may enable organic pea growers in central Washington to assess accurately the risk of damping-off. This could result in more effective implementation of management practices for damping-off, such as selection of appropriate fields and specific seed lots to reduce the impact of this disease. Alcala completed her PhD in May 2013.

PROJECT DESCRIPTION:

The overall objective is to enhance organic vegetable production in the Pacific Northwest by providing research-based information that helps growers improve their ability to manage damping-off in organic vegetable crops. Interviews of organic vegetable growers in Washington were completed in 2009 to identify primary concerns regarding damping-off. Based on the responses (detailed in the 2009 CSANR progress report), the focus of the project was revised to address damping-off in pea crops in the Columbia Basin under cold, moist, spring conditions, because growers reported greatest losses to damping-off under these conditions in pea crops. Pea served as a model vegetable for the project. Specific objectives of the project were to:

a) Identify the main *Pythium* species causing damping-off in certified organic fields under low soil temperatures and high soil moisture during early spring planting of pea crops in central Washington.
b) Evaluate microbial and non-microbial products that meet organic standards for controlling damping-off by *Pythium* spp. in order to provide research-based, objective efficacy data to stakeholders on the potential of such treatments for managing damping-off in organic pea crops in early spring.
c) Evaluate seed exudation (electrolyte leakage) of pea cultivars commonly planted in central Washington, and the potential for quantifying the use of electrolyte leakage as a predictive measure of the risk of damping-off under cool, spring planting conditions.

OUTPUTS:

- Work Completed:
    a. A survey of soil sampled from 37 certified organic fields across the Columbia Basin of Washington for *Pythium* isolates, using baiting methods, resulted in a collection of 305 isolates. Of these, 269 isolates were identified to species in 2010 by partial sequencing of the internal transcribed spacer (ITS) region of rDNA. A total of 19 species was identified, with the most predominant being *P. ultimum* (75/269), *P. torulosum* (56/269), *P. irregulare* (46/269), *P. abappresorium* (15/269), *P. adhaerens* (13/269), *P. middletonii* (13/269, a species not previously reported in WA), *P. dissotocum* (10/269), and *P. violae* (10/269). Results of pathogenicity trials on pea completed in a growth chamber in 2011 for as many as 9 isolates of each species in cool and wet conditions using soil from a growers' certified organic field in the Columbia Basin, demonstrated that isolates of 9 of the 19 species were pathogenic, with the most virulent isolates belonging to *P. ultimum*, *P. irregular* group 1, and *P. abappresorium*. In 2012, the pathogenicity tests were repeated for all isolates that were pathogenic in the first test, as well as a subset of isolates of each of the species that were not pathogenic on pea. Significant differences in virulence were observed among the species and among isolates within species.

In 2012, Avi Alcala worked with Kurt Schroeder (USDA ARS plant pathologist in Pullman) to test soil sampled from the 37 fields in the Columbia Basin for each of the three most prevalent and pathogenic species of *Pythium* (*P. ultimum*, *P. irregulare* group 1, and *P. abappressorium*), using a separate real-time PCR assay for each species. Standard curves were first developed using a range in DNA concentrations of each species, and by infesting each of three soil samples from the Basin with a range of inoculum concentrations of each species. A significant linear regression relationship was detected between the CFU/g recovered from the infested soil samples and DNA concentration detected by real-time PCR assay for each *Pythium* species. The real-time PCR assays were then used to determine the amount of inoculum of each of the three *Pythium* spp. detected in each of the 37 certified organic fields surveyed. *P. ultimum* was detected by real-time PCR assay in soil from 100% of the 37 fields, compared to 78% for *P. abappressorium* and 57% for *P. irregulare* group 1. Furthermore, DNA of *P. ultimum* was detected at greater concentrations than that of the other two species, indicating that *P. ultimum* might be the predominant damping-off pathogen of pea in early spring planting conditions in the Columbia Basin.

b. Certified organic seed and drench treatments, or experimental seed and drench treatments with potential for organic certification, were evaluated over two growing seasons (2011 and 2012) in seven organic pea trials and one organic sweet corn trial, in search of effective treatments that growers of organic vegetables can use for managing damping-off in early spring planting conditions. The trials were located at five sites in certified organic grower-cooperator fields in the semi-arid, irrigated, Columbia Basin of central Washington and Oregon, and one research site managed organically in Skagit Co. in the maritime northwestern Washington. The differences in weather, soil properties, cropping systems, and production practices at these sites provided a range of environments for evaluating the treatments against damping-off.

   In 2011, four field trials were completed: 15 treatments were evaluated in each of two trials in grower-cooperator, certified organic pea crops in the Basin (Boardman, OR and Soap Lake, WA); and 20 treatments were evaluated in inoculated pea and sweet corn trials at the WSU Mount Vernon NWREC. Of these treatments, Nordox (organic copper) and seed priming demonstrated the greatest potential for damping-off control. The other treatments lacked efficacy, were inconsistent in performance, or even had negative effects on emergence and growth of pea plants and/or exacerbated damping-off. In companion growth chamber trials, pea seed priming with 16 h of seed-soaking and 10 h of air-drying optimized priming for rapid emergence.

   The seven most promising treatments in the 2011 trials, including Nordox, were evaluated again in 2012, applied to both primed and non-primed seed in each of two field trials, one in a grower-cooperator's pea field near Ephrata, and one in at the WSU Mount Vernon NWREC. The objective was to determine if the potential benefits of these treatments could be enhanced by using primed seed. Contrary to expectations, application of the seed treatments to primed seed significantly reduced emergence compared to applying the treatments to dry seed. Although most of the dry seed treatment combinations improved emergence in both trials compared to control plots planted with primed seed alone, the treatments only improved emergence compared to control plots with dry seed in the

Ephrata trial, and did not improve pea yield in either trial. The results indicated limited potential benefits of these treatments, even when applied to dry pea seed, corroborating the 2011 trial results as well as some stakeholder observations. In fact, two of the treatments (Heads Up Plant Protectant and Natural II) adversely affected emergence and yield when applied to primed seed in the Ephrata trial. Nordox applied to dry pea seed significantly increased stand count 28 days after planting at the Ephrata trial, but did not affect pea yield significantly in either trial.

One of the limitations with priming large-seeded vegetables such as pea is the need to dry the imbibed seed adequately and immediately after priming to facilitate planting, and to facilitate storing the primed seed if planting is delayed. This study attempted to address this limitation by using biochar, a highly porous, charcoal material that absorbs water readily, to treat imbibed seed instead of using forced air. Combinations of priming, using biochar as a desiccant after priming, and Nordox seed treatment were evaluated in each of two grower-cooperator field trials near Royal City (identical trial at each site). Overall, in both trials the plots planted with primed seed had significantly greater pea emergence and taller pea plants compared to plots planted with dry seed. In one trial, plots with primed seed + biochar + Nordox had significantly greater yields than plots with dry seed alone; and pea yields from the former plots were significantly greater than yields from plots with dry seed + Nordox. In the second trial, no significant differences in yield were observed among treatments. In summary, the trials demonstrated the potential for faster emergence and reduced severity of damping-off in pea crops as a result of combining seed priming with biochar as a desiccant, followed by Nordox seed treatment, which could translate to greater pea yields. However, there are limitations to using seed priming because of practical aspects of handling (soaking and drying) the large volumes of seed planted in commercial pea crops in the Columbia Basin.

c. Seed exudates produced during germination are measured using electrical conductivity (EC). The EC levels of 17 pea seed lots (representing six cultivars commonly grown in the Columbia Basin) were obtained from Brotherton Seeds. The seed lots ranged from low to high levels of electrolyte leakage, as measured by EC. Alcala tested the EC of replicate samples of each lot, and then evaluated the severity of damping-off caused by *P. ultimum* for each of the seed lots planted into soil infested at a range of low to high levels of the pathogen (50, 100, and 500 CFU/g soil) compared to non-inoculated soil. Correlation and regression analyses were completed for electrolyte leakage vs. severity of damping-off at each inoculum level. The EC levels of the 17 pea seed lots significantly affected emergence as well as susceptibility to Pythium damping-off. A negative linear relationship was demonstrated between EC level and emergence, with a stronger regression at higher inoculum levels of *P. ultimum*. The results illustrated that measuring the EC of pea seed lots, combined with quantifying inoculum in soil using real-time PCR assays for each of the prevalent and pathogenic *Pythium* spp. identified in the survey (*P. ultimum*, *P. abappressorium*, and *P. violae*) may enable organic pea growers in central Washington and northcentral Oregon to assess accurately the risk of damping-off. This could result in more effective implementation of management practices for damping-off, such as selection of appropriate fields and specific seed lots to reduce the impact of this disease.

- Publications, Handouts, Other Text & Web Products:

  Alcala, A.C., du Toit, L.J., Derie, M.L., Holmes, B., Coffman, G., Gatch, E.W., and Porter, L.D. 2013. Evaluation of priming and Nordox seed treatment for controlling damping-off in organic pea crops in central Washington, 2012. Plant Disease Management Reports 7:ST002.

  Alcala, A.C., Porter, L.D., Derie, M.L., Holmes, B., Coffman, G., and du Toit, L.J. 2013. Evaluation of seed treatments and priming for controlling damping-off in organic pea crops in the semi-arid Columbia Basin and maritime Skagit Valley of Washington, 2012. Plant Disease Management Reports 7:ST001.

  Alcala, A.C., Derie, M.L., Holmes, B., Gatch, E.W., Porter, L.D., Coffman, G., and du Toit, L.J. 2012. Evaluation of organic seed and drench treatments for controlling damping-off in pea and sweet corn in Mount Vernon, WA, 2011. Plant Disease Management Reports 6:ST011.

  Alcala, A.C., Derie, M.L., Holmes, B., Gatch, E.W., Porter, L.D., Coffman, G., and du Toit, L.J. 2012. Evaluation of organic seed and drench treatments for controlling damping-off in organic pea crops in the semi-arid Columbia Basin of Oregon and Washington, 2011. Plant Disease Management Reports 6:ST012.

  Alcala, A. C., Paulitz, T. C., Porter, L. D., and du Toit, L. J. 2011. Profile of *Pythium* spp. in certified organic fields for vegetable production in central Washington. American Phytopathological Society Annual Meeting, Honolulu, Hawaii, 6-10 August 2011. Phytopathology 101:S4. (Poster abstract)

  Alcala, A.C., and du Toit, L.J. 2009. Management of damping-off in organic vegetable crops in the Pacific Northwest. Sustaining the Pacific Northwest 7(4):5-7. http://csanr.wsu.edu/publications/SPNW/SPNW-v7-n4.pdf

  Lindsey du Toit's Vegetable Seed Pathology team website, with a section on Avi Alcala's project: http://www.mountvernon.wsu.edu/VSP/VSP_team.htm#avi

- Outreach & Education Activities:

  Alcala spoke about this project at the WSU Mount Vernon NWREC Field Day in July each year from 2009 through 2012. This event is attended by 150-200 people each year, including growers, extension educators, field representatives, consultants, researchers, seed industry personnel, regulatory agents, etc. In addition, Alcala regularly was asked to give short, impromptu presentations on her project to agricultural-related groups visiting the WSU Mount Vernon NWREC during the 4 years of her PhD program.

  Alcala, A.C., Porter, L.D., and du Toit, L.J. Evaluating methods for management of damping-off in organic pea production. Presentation at the Pacific Northwest Vegetable Association 26th Annual Conference & Trade Show, 14-15 November 2012, Kennewick, WA.

  Alcala, A.C., Porter, L.D., and du Toit, L.J. 2011. Evaluation of organic seed and drench treatments for damping-off in organic pea crop. Presentation at the Pacific Northwest

Vegetable Association 25th Annual Conference & Trade Show, 16-17 November 2011, Kennewick, WA.

Alcala, A.C., and Gatch E.W. 2010. Wilts, blights, rots, damping-off: How to avoid the calling cards of soilborne pathogens. Presentation at the Washington Tilth Producers' Annual Conference, 11-13 November 2010, Port Townsend, WA.

IMPACTS:

- Short-Term: This project has resulted in a greater understanding of the primary *Pythium* spp. that appear to cause damping-off in cool, early spring conditions in certified organic fields in the Columbia Basin in which pea crops are grown. This research revealed the diversity of *Pythium* spp., pathogenic and non-pathogenic, in certified organic fields, and the prevalence of the pathogenic species in relation to non-pathogenic species. In addition, the field trials demonstrated that almost all of the products currently registered for use in certified organic pea crops for damping-off control are either not effective at reducing damping-off in cool, spring conditions in central and western Washington, or are inconsistent. Therefore, the use of these seed treatments by growers is not warranted, which corroborates comments received during an informal survey of organic growers in 2009 regarding their use of organic seed treatments. The 2012 field trials demonstrated that combining organic seed treatments with primed seed did not enhance efficacy of these treatments. In fact, this had negative effects on pea emergence, growth, and yield. However, the use of biochar instead of forced air to dry primed seed, particularly when combined with Nordox, an organic cuprous oxide, improved pea emergence, height, and yield in one of two grower-cooperator trials in the Columbia Basin in 2012, and warrants further investigation (potential intermediate-term impact).

- Intermediate-Term: Results of the pea seed lot EC testing and the soil baiting for *Pythium* spp. pathogenic on pea in cool, moist soil conditions appear promising for helping growers determine the potential risk of damping-off in their fields by measuring: i) the EC value of the seed lots they plant, and ii) inoculum levels of the three primary pathogenic *Pythium* spp. in fields they are considering planting. The real-time PCR assays investigated for the three main pathogenic *Pythium* species provide a means of determining inoculum levels in growers' fields. However, a major limitation of using real-time PCR assays for testing soils for target pathogens is the very limited amount of soil that can be tested (up to 5 g/sample, with potentially no more than 10 samples tested/field in order for the test to be viable economically), which may need to represent fields as large as 150 acres. However, even if the use of real-time PCR assays does not prove viable for testing grower's fields, this research demonstrated increasing growers' awareness of the specific EC measurement of seed lots they will plant will enable the growers to make appropriate decisions based on the relative risk of damping-off for their seed lots. Seed lots with higher EC values can potentially be reserved for planting in fields with a history of no or low risk of damping-off, or for later planting when warmer soil temperatures mean a reduced risk of damping-off. However, there is a limit to the latter choice as most growers double-crop organic pea and sweet corn crops, which means pea crops cannot be planted too late; and early planting of pea crops reduces the difficulty of organic control of nightshade, a weed that produces toxic berries that are very difficult to separate from peas in processing plants.

- Long-Term: The potential practical and economic aspects of priming and drying (with air or biochar) the large volumes of pea seed that are planted in fields in the Columbia Basin each

spring needs to be assessed. Through education and outreach, this research project should result in increased awareness among organic pea growers of the value of knowing the quality of their pea seed lots, measured using EC values, and how to use this information for spring planting decisions (within the boundaries of processor contracts and other practical aspects of relatively large-scale, organic production of pea crops). Real-time PCR assays for the three primary pathogenic *Pythium* species causing damping-off in early spring planting conditions in the Columbia could potentially be offered as a diagnostic service by private labs (e.g., Western Labs in Idaho), to use in combination with pea seed lot EC measurement, to determine which fields to plant with which pea seed lots.

ADDITIONAL FUNDING APPLIED FOR / SECURED: None. Ana Vida Alcala defended her PhD dissertation successfully on 22 May 2013. She accepted a position with Valent USA in Mississippi, working on seed treatments for management of damping-off in field crops.

GRADUATE STUDENTS FUNDED: 1 (PhD student, Avi Alcala, in the Dept. of Plant Pathology, based at the WSU Mount Vernon NWREC in Lindsey du Toit's program).

RECOMMENDATIONS FOR FUTURE RESEARCH:

a. The field trials demonstrated the potential for faster emergence and reduced severity of damping-off in pea crops as a result of combining seed priming with biochar as a desiccant, followed by Nordox seed treatment, which could translate to greater pea yields. However, the logistical and economical aspects of priming and drying (with air or biochar) large volumes of pea seed planted in fields in the Columbia Basin needs to be assessed in detail in cooperation with seed companies, growers, and perhaps engineers.
b. The potential use of EC measurements of pea seed lots to predict the risk of damping-off, combined with testing soils for inoculum levels of the primary *Pythium* spp. pathogenic on pea in cool, spring planting conditions in the Columbia Basin, needs to be evaluated in grower-cooperator fields. The EC research in this project was limited to growth chamber trials, but the results warrant field evaluations. The research could also be extended to a greater diversity of pea cultivars. Apparently, most pea seed companies routinely complete EC tests of their seed lots (Lyndon Porter, USDA ARS legume pathologist, *personal communication*). However, results of these EC tests are not made available to growers, i.e., pea growers typically are not aware of which seed lots might have greater susceptibility to damping-off. An effort should be made to promote awareness of pea seed lot EC readings among pea growers and seed companies as well as pea processors, to utilize EC tests more effectively for management of damping-off in pea production. Ultimately, this should be combined with testing the real-time PCR assays for accuracy in determining levels of inoculum of *Pythium* spp. that cause damping-off in pea crops, particularly in the presence of other soil microflora and multiple *Pythium* spp., pathogenic and non-pathogenic.
c. This research used pea as a model vegetable crop susceptible to damping-off. Almost all vegetables are susceptible to damping-off, and measurement of EC values of seed lots is not an effective method of measuring seed quality and susceptibility to damping-off for small-seeded vegetables as it is for large-seeded vegetables. Research completed in 2005-2007 in Lindsey du Toit's project by an MS student, Jaime Cummings, on management of damping-off in organic vegetable crops, utilized spinach as a model small-seeded vegetable grown in the mild, maritime climate of western Washington. That study demonstrated similar results for the myriad of organic seed and drench treatments evaluated, i.e., very few certified organic seed treatment were effective against damping-off, both in highly controlled greenhouse trials and in grower-

cooperator field trials in western Washington (for three different damping-off pathogens), and the few treatments that demonstrated some efficacy in greenhouse trials were inconsistent in performance when evaluated at multiple field locations. That study, combined with results of this project on pea, a large-seeded vegetable grown in a semi-arid area of irrigated agriculture, highlight the significant limitations to using organic seed treatments to manage damping-off. Therefore, alternative management options, such as developing soil assays for growers to identify fields with higher risk for damping-off, and evaluation of cultural practices for suppressing damping-off (e.g., compost amendments), should be pursued. Management practices that enhance soil health, e.g., biological amendments such as composts and green manure crops; alternative (reduced?) tillage practices; avoiding soil compaction; implementing diverse crop rotations that improve soil health and microbial diversity; etc. are more likely to reduce losses to damping-off effectively in the long term for certified organic (and also conventional) vegetable production than organic seed treatments.