SCRI Project (2010-51181-21140) Progress Report – 2013

Integrated Management of Zoosporic Pathogens and Irrigation Water Quality for a Sustainable Green Industry

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TARGET AUDIENCE

Nursery growers and greenhouse producers
Garden centers, retailers, and landscapers
Agricultural industry scientists and consultants
Extension specialists and agents
Plant disease diagnosticians, regulatory personnel
Undergraduate and graduate students, postdoctoral associates
Educators, horticulturists, mycologists, and researchers
Conservation biologists
Government policy makers

PRODUCTS

Publications

Book Chapters

Copes, W. E., Barbeau, B., and Chastagner, G. A. Chapter 21. Chlorine Dioxide for Irrigation Water Treatment. Pages ##-## in: Biology, Detection and Management of Pathogens in Irrigation Water. C. Hong, G. Moorman, W. Wohanka, and C. Büttner (eds.) APS Press, St. Paul, MN. Accepted.

Elmer, W. H. Buck, J., Ahonsi, M. O., and Copes, W. E. Chapter 24. Emerging Technologies for Irrigation Water Treatments. Pages ##-##. In: Biology, Detection and Management of Pathogens in Irrigation Water. C. Hong, G. Moorman, W. Wohanka, and C. Büttner (eds.) APS Press, St. Paul, MN. Accepted.

Kong, P. and Lea-Cox, J. D. Chapter 28. Water Quality Dynamics: Implications for Managing Waterborne Pathogens. Pages ##-##. In: Biology, Detection and Management of Plant Pathogens in Irrigation Water. C. H. Hong, G. W. Moorman, W. Wohanka, and C. Büttner (eds.) APS Press, St. Paul, MN. Accepted

Lea-Cox J. L., and Ross, D. S. Chapter 32. Water Management to Minimize Pathogen Movement in Containerized Production Systems. Pages ##-##. In: Biology, Detection and Management of Plant Pathogens in Irrigation Water. C. H. Hong, G. W. Moorman, W. Wohanka, and C. Büttner (eds.) APS Press, St. Paul, MN. Accepted

Ristvey, A. G., and Moorman, G. W. Chapter 29. An Integrated Approach to Minimizing Plant Pathogens in Runoff Water from Containerized Production Systems. Pages ##-##. In: Biology, Detection and Management of Plant Pathogens in Irrigation Water. C. H. Hong, G. W. Moorman, W. Wohanka, and C. Büttner (eds.) APS Press, St. Paul, MN. Accepted

Books

Hong, C. X., Moorman, G. W., Wohanka, W., and Büttner, C. 2014. Biology, Detection and Management of Plant Pathogens in Irrigation Water. American Pathological Society, St. Paul, MN (in press)

Conference Papers and Presentations

Dart, N., and Hong, C. X. 2013. Significance of soil inoculum in the epidemiology of boxwood blight caused by *Calonectria pseudonaviculata.* Phytopathology 103:S2.33

Hao, W., and Hong, C. X. 2013. Biological control of *Phytophthora nicotianae* by heat-tolerant bacteria in irrigation water. Phytopathology 103:S2.56

Hong, C. X. 2013. Diversity and significance of waterborne pathogens to plant biosecurity under a changing climate. 10th International Congress of Plant Pathology, Beijing, China, August 25-31, 2013

Hong, C. X., and Moorman, G. W. 2013. Diversity and significance of plant pathogens as agricultural water contaminants. 2013 AWRA Spring Specialty Conference on Agricultural Hydrology and Water Quality II, St. Louis, MO, March 25-27, 2013

Kong, P., and Hong, C. X. 2013. Aquatic biology of oomycete plant pathogens. 10th International Congress of Plant Pathology, Beijing, China, August 25-31, 2013

Lea-Cox, J. D., Belayneh, B. E., and Ristvey, A.G. 2013. Daily and seasonal changes in the water quality of irrigation containment ponds. In: Workshop - The challenges of using alternative and recycled water sources for horticultural use. American Society for Horticultural Science Annual Meeting, 24 July, 2013, Palm Desert, CA. HortScience 48:S106.

Richardson, P. A., Kong, P., Cafà, G., and Hong, C. X. 2013. Tracking water quality dynamics in a multi-basin agricultural water recycling system, 2013 AWRA Spring Specialty Conference on Agricultural Hydrology and Water Quality II, St. Louis, MO, March 25-27, 2013

Richardson, P. A., Kong, P., Cafà, G., Lea-Cox, J. D., Belayneh, B. E., Ristvey, A. G., and Hong, C. X. 2013. Dramatic fluctuations of water quality in agricultural runoff containment basins. 2013 AWRA Spring Specialty Conference on Agricultural Hydrology and Water Quality II, St. Louis, MO, March 25-27, 2013

Yang, X., Copes, W. E., and Hong, C. X. 2013. Three novel *Phytophthora* species from irrigation water in Mississippi. Phytopathology 103:S2.164

Journal Articles

Burgos-Garay, M. L., Hong, C. X., and Moorman, G. W. 2013. Effects of heterotrophic bacteria from recycled irrigation water on *Pythium in vitro* and *in vivo*. Plant Disease. Accepted pending revision.

Majsztrik, J.C., and Lea-Cox, J.D. 2013. Water quality regulations in the Chesapeake Bay: Working to more precisely estimate nutrient loading rates and incentivize best management practices in the nursery and greenhouse industry. HortScience 48: 1097-1102

Pistininzi, M., Weiss, E., Achtemeier, L., and Hong, C. X. 2013. Zoospore production biology of pythiaceous plant pathogens. Journal of Phytopathology doi: 10.1111/jph.12154

Yang, X., Copes, W. E., and Hong, C. X. 2013. *Phytophthora mississippiae* sp. nov., a new species recovered from irrigation reservoirs at a plant nursery in Mississippi. Journal of Plant Pathology and Microbiology http://dx.doi.org/10.4172/2157-7471.1000180

Yang, X., Copes, W. E., and Hong, C. X. 2013. Two novel species representing a new clade and cluster of *Phytophthora*. Fungal Biology (Accepted)

Yang, X., Gallegly, M. E., and Hong, C. X. 2013. A new species in the subclade 9a of the genus *Phytophthora*: *P. hydrogena* sp. nov. Mycologia (in press)

Yang, X., and Hong, C. X. 2013. *Phytophthora virginiae* sp. nov., a high-temperature tolerant species from irrigation water in Virginia. Mycotaxon (Accepted)

Yang, X., Kong, P., and Hong, C. X. 2013. Evaluation of fungicide drenches for control of Phytophthora root rot of petunia, 2012. Plant Disease Management Reports 7:OT002

Yang, X., Richardson, P. A., and Hong, C. X. 2013. Evaluation of nonregistered fungicide for control of rose downy mildew, 2012. Plant Disease Management Reports 7:OT001

Yang, X., Richardson, P. A., Kong, P., and Hong, C. X. 2013. Fungicidal control of Phytophthora aerial blight on annual vinca in Virginia, 2012. Plant Disease Management Reports 7:OT005

Yang, X., Richardson, P. A., Olson, H. A., and Hong, C. X. 2013. Root and stem rot of begonia caused by *Phytopythium helicoides* in Virginia. Plant Disease 97:1385

Theses/Dissertation

Burgos-Garay, Maria L. 2013. Effect of heterotrophic bacterial communities on *Pythium spp.* in recycled irrigation water. Ph. D. Dissertation. The Pennsylvania State University, University Park, PA

Cultice, Alyssa. 2013. Horticultural producers' willingness to adopt water recirculation technology in the Mid-Atlantic region. M.S. Thesis, Virginia Tech, Blacksburg, VA

Hao, Wei. 2012. An energy-saving heat treatment for re-circulated irrigation water and the potential biological mechanisms. Ph. D. Dissertation. Virginia Tech, Blacksburg, VA

Other Products

Physical Collections

Numerous isolates of *Pythium* from the continuous baiting of recycling irrigations systems in two commercial greenhouses in Pennsylvania were accumulated and some are part of a working collection of over 100 isolates from this research. DNA from each isolate was extracted and is stored at -80C as part of this collection in the Moorman Lab at the Pennsylvania State University.

Many isolates of *Phytophthora*, including the type cultures of three new species, were recovered from one commercial nursery in Mississippi. The type cultures of these three and other new species along with over 2000 bacterial strains from pond surveys in Virginia are part of a working collection maintained in the Hong Lab at Virginia Tech. More than 1000 DNA sequences of *Phytophthora* species and bacteria have been deposited at the Genbank for public access.

Survey Instruments

A producer survey was developed to assess growers' disease and irrigation management practices and to quantify their willingness to capture and recycle irrigation water.

ACCOMPLISHMENTS

What was accomplished under the proposed goals?

Seven phylogenetically distinct groups of nursery water isolates that do not belong to any known species of *Phytophthora* were named following detailed morphological examination, physiological and molecular analyses. Three groups isolated from Mississippi in 2011 and 2012 were named *Phytophthora mississippiae*, *P. stricta*, and *P. macilentosa*. The other four groups were from Virginia with two formally described as *P. hydrogena*, and *P. virginiae*, and the rest provisionally named *P. stagnum* and *Halophytophthora fluviatilis*. To assess the potential pathogenicity of these new species, isolates of *P. mississippiae*, *P. stricta* and *P. macilentosa* were inoculated on wounded roots, non-wounded roots, and wounded collars of six plant hosts: *Rhododendron* 'Scintallation' x white form of *R. hyperythrum* Southgate™ 'Brandi™', *Pieris japonica, Hydrangea quercifolia* 'Snowflake', *Ilex* 'Liberty', *Gardenia jasminoides* 'August Beauty', and *Catharanthus roseus*. The experiment is being repeated, but to date results indicate none of the three new Mississippi species is pathogenic on any of these six plants under

the test conditions in the Gulf Coast region. Descriptions of these new species will help diagnosticians, regulatory personnel and researchers in correctly identifying Phytophthora pathogens, and avoid the risk of misidentifying high-impact species such as *P. ramorum*, *P. kernoviae*, and *P. alni*. This information along with those in the literature are being developed into fact sheets to help farmers better understand, assess and manage crop health risks posed by *Phytophthora* species in irrigation systems.

Several new *Pythium* species were isolated from greenhouse water in Pennsylvania and their crop health risk is being assessed. DNA sequences from the internal transcribed spacer region (ITS) of isolates of *Pythium* very frequently obtained by continuously baiting recycling irrigation water tanks indicates that these isolates may be species new to science. They can be put into groups that are similar to but not identical to known isolates as follows. Group 1 (similar to *P. dissotocum, P. diclinum, P. lutarium, P. coloratum*), group 2 (similar to *P. adhaerens, P. chondricola, P. porphyrae*), group 3 (similar to *P. flevoense, P. capillosum, P. pectinolyticum*) and group 4 (similar to *P. apleroticum, P. aquatile, P. pachycaule, P. oopapillum*). The cox II region is being sequenced to further characterize these isolates. Three known species frequently found in the water are *Pythium helicoides*, *P. chamaehypon*, and *P. middletoni*. Some of the new species are pathogenic on geranium seedlings. None of these species is associated with significant crop losses in commercial greenhouses. Co-inoculations of geranium seedlings indicate that the new species do not inhibit the pathogenic activity of *P. aphanidermatum*, a highly pathogenic species often associated with crop losses.

The effect of three selected bacteria isolated from recycled irrigation water on Pythium root rot of geranium (*Pelargonium* cv. 'White Orbit') in greenhouse ebb and flow experiments was tested. Individual bacteria that inhibited or enhanced *Pythium* sporangium and zoospore development in culture did not inhibit *P. aphanidermatum* or *P. cryptoirregulare* on geraniums in the greenhouse. Studies using q-PCR to quantify changes in bacterial populations when *P. aphanidermatum, P. cryptoirregulare,* and *P irregulare* were added to recycled irrigation water samples indicate that γ-proteobacteria populations increase in the presence of all three *Pythium* species.

To better understand the pathogen dynamics along water path in single-basin irrigation recycling systems and at every step in multi-basin systems, pathogen aquatic studies have been expanded from zoospores to include chlamydospores and oospores in the lab and microbial characterization to multiple basins at three production facilities in Virginia. Specifically, chlamydospores produced by six isolates of *P. nicotianae*, *P. palmivora* and *P. tropicalis* and oospores by three isolates of *P. hedraiandra* and *P. pini* were assessed against five water pH levels from 3.0 to 11.0. Both chlamydospores and oospores survived much longer in this range of water pH than zoospores. These new data are an integral part of the pathogen aquatic biology. Bacterial diversity was surveyed in six basins at two production nurseries each with a unique runoff water recycling system. At least eighteen phyla of bacteria were identified from these basins with proteobacteria, bacterioidetes, actinobacteria, verrucomicrobia and firmicutes being most abundant. These studies offer numerous clues to revealing naturally-occurring pathogen-suppressing agents in irrigation runoff containment basins. A small subset of over 2000 bacterial strains was evaluated individually and in combinations of two, three and four against zoospores of *P. nicotianae in vitro* and *in vivo*. These studies indicate that some bacterial strains suppressed zoospore survival and disease development on lupine seedlings while others promoted both. These new data are foundational for developing guidelines on water recycling system designs for pathogen mitigation and, consequently, the profitability and sustainability of the ornamental horticulture industry.

Nine nursery ponds (six in Virginia, two in Maryland, and one in Mississippi) have been monitored continuously for surface water temperature, electrical conductivity (EC), pH, dissolved oxygen (DO), oxidation-reduction potential (ORP), and chlorophyll a. Results of this expanded monitoring in Mid-Atlantic and Gulf Coast regions supports the hypotheses that dramatic seasonal and diurnal fluctuations

of water quality are common in runoff containment basins and that these fluctuations are tied to the photosynthetic activities which, in turn, are affected by physical and environmental factors including light, temperature and nutrient load into the basins. Other water quality parameters, pathogen survival, crop health, productivity and horticultural product quality data are being collected and analyzed. Water samples were taken from selected nurseries in three states for N, P, and micronutrient analyses and the results will be used to better understand water quality dynamics. Microclimatic weather data also are being continuously monitored at each of the selected Maryland and Virginia nurseries. The impacts of seasonal and diurnal water pH fluctuations on the performance of fungicides, insecticides, herbicides, and plant growth regulators, as well as on chlorine treatment used in the horticultural industry have been assessed. Recommendations to help farmers maximize their agricultural chemical performance have been developed and disseminated through invited lectures at universities, newsletters and presentations at various extension venues. Information and the recommendations developed through this project are being delivered via a monthly webinar series launched in October 2013 (www.irrigation-pathogens.info) and incorporated into an online knowledge center.

A producer survey was developed and distributed to approximately 2,000 growers in Pennsylvania, Maryland, and Virginia. It was found that (i) a majority of the respondents considered any threat to water resources is important but they are unaware of growing global water scarcity; (ii) 60-70% of small and medium nurseries and 20% of large facilities do not capture runoff because of cost and disease concerns; (iii) Both Phytophthora and Pythium diseases are considered as major limiting factors in large production facilities, and (iv) chlorination is the most widely used water treatment for pathogen risk mitigation. Some of these data have been delivered to the industry via the webinar on November 6, 2013.

What opportunities for training and professional development has the project provided?

Nine Graduate Research Assistantships (GRAs): four at doctoral level (Burgos-Garay, Dart, Hao, Yang) and five at masters level (Cultice, D'Alession, Lanze, Ree, Xu)
Four Postdoctoral Research Fellowships (Burgos-Garay, Cafà, Hao, Nikrad)
One visiting scientist scholarship (Ben)
Two internships (Hu, Pistininzi), and
One nationwide webinar series: Irrigation Pathogens and Water Quality, for the industry

How have the results been disseminated to communities of interest?

Grower Meetings

Dart, N., and Hong, C. X. 2013. Microsclerotia formation and soil biology of the box blight pathogen, *Calonectria pseudonaviculata*, Boxwood Blight Webinar (Organized by the American Nursery and Landscape Association). 2/25/2013

Hong, C. X. 2013. Making the most out of the agricultural chemical dollars. Eastern Shore Agricultural Conference, Belle Haven, VA. 1/4/2013

Hong, C. X. 2013. Getting down to the basics with *Phytophthora.* Beech Summit, Longwood Garden, Kennett Square, PA. 4/5/2013

Hong, C. X., 2013. Water treatment updates. VNLA Field Day, Gloucester, VA. 8/8/2013

Kong, P., Cafa, G., Hao, W., Yang, X., Ghimire, S., Richardson, P. A., and Hong, C. X. 2013. Irrigation systems for crop health, water security and environmental sustainability. VNLA Field Day, Gloucester, VA. 8/8/2013

Moorman, G. W. Plant pathogens in irrigation water. Northwestern Pennsylvania Greenhouse Seminar. Erie, PA. 2/21/13

Newsletter Articles

Yang, X., Richardson, P. A., Kong, P., Olson, H. A., and Hong, C. X. 2013. New pathogens causing root and stem rot of Begonia. Virginia Nursery and Landscape Association (VNLA) Newsletter 83(2):46-48.

Hong, C. X. 2013. SCRI Project Update Series I – Recycled Water Quality: Recycled water quality and management implications. VNLA Newsletter 83(3):55-56

Project Meetings & Field Visits

Research results were shared with collaborating growers and advisory panel members via regular web conferences and annual project meeting in Virginia Beach, VA. They also were disseminated to other growers via field visits, etc.

Lab Tours at Virginia Tech's Hampton Roads AREC

Shared the project results with two groups:

Fifteen members of the Dean's Council in the College of Agriculture and life Science (CALS) of Virginia Tech visited the Hong lab on May 2, 2013

Fourteen people including new CALS faculty members and two international visiting scholars visited the lab on May 29, 2013

Invited Lectures and Presentations

Hong, C. X. 2013. Plant health management in a thirsty world. Department of Molecular Biology and Chemistry, Christopher Newport University, Newport News, VA. 2/22/2013

Hong, C. X. 2013. Recycled water quality dynamics and implications for ornamental horticultural crop health and production. Horticulture Department, Virginia Tech, Blacksburg, VA. 5/6/2013

Hong, C. X. 2013. Treasure hunt in agricultural runoff water containment basins, Recent Advancements in Biological Control session of APS Potomac Division Meeting, Shepherdstown, WV. 4/4/2013

Hong, C. X. 2013. Water dispersal of *Phytophthora* species. Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY. 6/26/2013

Hong, C. X. 2013. Recycling irrigation system – a focal point of plant biosecurity and agricultural water sustainability in the 21st century. EITC: Precision Agriculture – Future Challenges and Directions. Ithaca, NY, 6/27/2013

What do you plan to do during the next reporting period to accomplish the goals?

In addition to planned activities, we are striving to expand the research horizon and expedite the technology delivery.

New research horizons

Understanding the interactions between the isolates of *Pythium* very frequently found in recirculating irrigation water but not associated with crop losses and those species of *Pythium* that are known to cause significant crop losses in commercial greenhouses. This research is being conducted by a graduate student as part of her Master of Science degree research project at Penn State University.

Expanding the hunt in runoff containment basins for naturally occurring biological control agents. This work has begun with investigations into the ammonium-oxidizing bacteria and archaea in this aquatic ecosystem.

Modelling the seasonal and diurnal water quality fluctuations in runoff containment basins. This research integrates the initial three years of environmental and water quality data for better understanding the water quality dynamics. It aims to provide a tool for water quality manipulation in

order to suppress pathogens, improve crop productivity and quality as well as a tool for assessing water resource conservation and protection benefits of capturing and recycling runoff water.

Expedited technology delivery

We have launched a monthly webinar series on plant pathogens found in irrigation water and irrigation water quality. These webinars aim to expedite the delivery of new knowledge and technologies resulting from this project and put our research data into practice as quickly as possible.

Information gathered to date on pathogen diversity in irrigation water, their aquatic biology and recycled water quality will be summarized and made available in the online Knowledge Center. A user-friendly interactive form allowing Knowledge Center users to assess the threat of plant pathogens in irrigation water in their production facility will be developed and tested in the Knowledge Center.

We have initiated a field demonstration plot at one of the collaborating nurseries in Virginia in 2013 and will expand this effort in 2014 to demonstrate (i) how locating the pump house away from the runoff entrance will mitigate pathogen risk in a single-basin water recycling system, and (ii) how a multi-basin water recycling system with a stepwise water flow will further reduce pathogen dissemination via irrigation water.