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By Paul Fisher, Dustin Meador, Jennifer Parke, Robert Wick and William Argo

What's in your water?

BIOLOGICAL WATER QUALITY

Second in a four-part series on monitoring irrigation water for floriculture crops

If you are recirculating irrigation water or drawing water from a pond, testing the biological water quality can be important. What might you test for? In addition to waterborne pathogens, microbial water issues result in algae on the growing substrate and floors, and clogged equipment from bacteria and biofilm. For growers producing edible crops, human pathogens, including strains of *E. coli*, are regulated.

Working with a testing lab

Most tests of biological water quality require samples to be analyzed by professional laboratory services, which are provided by water treatment companies, university extension plant pathology labs and private microbiology labs. Onsite detection methods for biological water quality are being developed, but tend to be useful as indicators rather than specific diagnostic tools.

For laboratory tests, contact the lab before you submit samples. Things you should discuss with the lab include:

- What to test for. The lab needs to understand whether you are interested in testing for a particular pathogen species or a class of organisms (such as aerobic bacteria) so it is ready to run the appropriate test when the samples arrive.
- How to collect samples. The samples should be representative of the irrigation water. Take the samples after the irrigation water has run for 5 minutes or long enough to ensure that you are not sampling water that has been sitting in the piping. For ponds, the usual procedure is to sample from the intake pipe depth.
- The sample volume and containers. Some laboratories provide sterile sampling containers. Typically 350 milliliters is enough. If sterile containers are

not available, then purchase distilled water containers from a grocery store, empty the container and flush several times with the sample.
- Proper labeling and documenting samples including lab forms.
- Storing samples and packaging (usually in insulated containers with a cool pack).
- When to ship samples (usually early in the week with overnight shipping).

Testing for pathogens

Plant pathology laboratories use either baiting (attracting pathogen spores to a leaf) or filtration to concentrate pathogens in the water sample. The sample is then plated out on agar substrates (Figure 1) that selectively grow different classes of organisms, to identify the presence or absence of pathogens and the number of colonies. This type of test for pathogens is typically to the genus level, with tests for *Pythium* and *Phytophthora* being the most widely available. For example, the University of Massachusetts provides this service for $50 per sample, with a one to two week turnaround to allow time to culture the sample (Table 1).

Pathogen testing could be used to determine if a water treatment technology is controlling *Pythium*. To test treatment efficacy, water samples could be collected from sources before and after the point of water treatment (with activated peroxygens, chlorine, chlorine dioxide, copper, ozone, UV radiation or another technology) and tested for living colonies of *Pythium*. Biological water quality changes very quickly. Therefore, repeat testing over the growing season is an advised monitoring strategy if waterborne pathogens are suspected.

Identification of pathogens to the species level requires a specialist, but many species of *Pythium* and *Phytophthora* can be identified by conventional or molecular techniques. Identification to species level is most important when there is there is a specific pathogen suspected, such as *Phytophthora ramorum*.

The presence of *Pythium* does not necessarily mean there will be root disease but *Phytophthora* is more aggressive and should always be considered a problem. Nevertheless, if a lab reports the presence of *Pythium* or *Phytophthora* at the genus level, it is prudent to assume that a pathogen is present and to treat the water and crops accordingly. Follow up with additional water tests after crop management treatments are carried out.

The University of Guelph can identify the pathogen species based on characteristics of their DNA (Table 1). This test can evaluate a number of species in a single analysis. However, the test will not differentiate between live and dead microbes.

Whether pathogens are viable is important if you are treating water with a sanitizing agent technology and want to know whether the pathogens are killed by the treatment. In this case, culture-plating may be more useful than DNA analysis.

Diagnostic kits

Researchers are developing protocols to use ELISA diagnostic kits as a preliminary screening test for waterborne pathogens. Field diagnostic kits are available for *Pythium* and *Phytophthora*. The kits use monoclonal or polyclonal antibodies to detect the pathogens. This is the same technology used in home pregnancy test kits. Kits are available as dipsticks or as lateral flow devices (see Table 1 for suppliers).

TOP: Figure 1. Eucalyptus leaf discs for baiting *Phytophthora* are placed onto a selective agar culture medium to test for the presence of the pathogen (the thread-like rings around the leaf discs). BOTTOM: Figure 2. High microbial concentrations in the irrigation water can lead to clogging of irrigation equipment.

Many growers already use these ELISA kits to identify pathogens on leaves, with instant results. Trialing at Oregon State University has found that some ELISA tests for *Phytophthora* cross-react with a few *Pythium* species, so that if the test is positive, it is important to follow up with laboratory tests. Lab testing is also advised to rule out the presence of *Phytophthora ramorum* from water that has tested positive in a *Phytophthora* test kit. Test kits detect both live and dead forms of these pathogens.

Total microbial load

Total colony count expressed as colony forming units (CFUs) of either bacteria or fungus is a non-specific measurement, meaning these tests estimate the amount of large groups of diverse organisms. Total CFUs of bacteria or fungi are not a good indicator of plant pathogens, because most microorganisms in water samples are likely to be beneficial or benign, rather than pathogenic.

The goal of treating irrigation water is not to eliminate bacteria or fungus, but to keep the CFUs managed so that they don't degrade water quality or limit control of pathogenic organisms. Greenhouses are not sterile environments, and attempting to completely sterilize water, all surfaces and the growing substrate would require high doses of chemicals that are likely to be phytotoxic to crops. Removing beneficial organisms

Photo by Rob Wick, University of Massachusetts Photo by Paul Fisher, University of Florida

can also increase the likelihood that pathogenic organisms will cause disease. Clogging of irrigation equipment is, however, a common problem resulting from high microbial density in water (Figure 2).

Total colony counts within irrigation systems indicate the biological productivity of the water. If water treatment technology has been installed, you want to know whether waterborne pathogens are likely to be controlled if they arise in the source water. You can test the active ingredient dose of the sanitizing agent compared with published recommendations, and analyze indicator organisms such as total bacteria and fungus CFUs from samples both before and after the point of treatment in the irrigation line. The change in microbial density before and after treatment may be a useful bio-indicator of the general efficacy (or failure) of the treatment system to control microorganisms.

Sampling at points along an irrigation system can also identify where conditions favor growth of microorganisms. Excessive organic matter increases the number of microorganisms and the risk of clogging equipment. Changes in CFUs from the source water to the emitters can also help monitor the growth of microbes within the irrigation lines.

There is currently little standardization across private horticulture laboratories on how samples are processed for total density of bacteria and fungus. A threshold of 10,000 colony forming units (cfu) of aerobic bacteria per milliliter is generally recommended to reduce clogging of drip lines and micro-emitters.

One technology used (see sidebar) at the University of Florida to measure total density of bacteria and fungus is 3M Petrifilms (www.3m.com). Petrifilms are plastic cards coated in a dehydrated nutrient film with microbial indicators. A droplet of water placed on the Petrifilm can be stored for three to five days in the dark at room temperature, and each colony forming units makes a colored dot on the Petrifilm to allow counting. Different types of Petrifilm are available for aerobic bacteria, yeasts and molds (i.e., fungus and fungal-like organisms), or other pathogens of importance to human health. University of Florida researchers have trained growers with the Petrifilms, who have found the method easy to use.

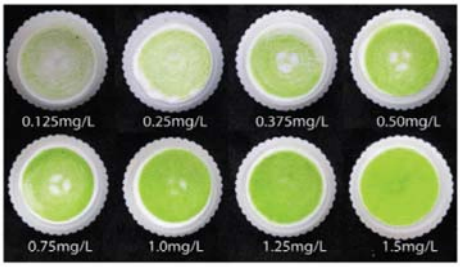
Algae testing

In horticulture, algae are often thought of as the "green stuff" that workers slip on, causes blooms in ponds and coats the growing substrate, benches, floors and irrigation lines. In reality, algae are a complex of many unrelated species and groups (cyanobacteria, green and red algae) that range from unicellular bacteria-like organisms to complex aquatic plants.

The horticulture industry will probably need to become more educated about algal biology as control methods are evaluated. For example, pond chemicals vary in control of different types of algae (www.extension.purdue.edu/extmedia/HO/HO-247-W.pdf).

Samples can be sent to a testing laboratory for identification and quantification of algae and other aquatic weeds, which is most likely to benefit growers drawing water from catchment basins with algal bloom issues.

Growers can quantify algal concentration by sending a sample to a specialist laboratory that will measure total chlorophyll. A grower-friendly kit is available for extracting algae (Table 1, Figure 3), which involves filtration to concentrate the algae on a disc, and extraction of the chlorophyll. However, this testing method is qualitative. The color of the filter disc or extracted solution can be viewed and determined if it is "clear", "green" or "very green". In a laboratory setting, a spectrophotometer is used to quantify chlorophyll concentration in the water sample. Standards have not been defined for "acceptable" or "problem" levels for algae in greenhouse applications, although researchers at the University of Florida are starting to accumulate some data.



TOP: Figure 3. Algae at different total chlorophyll concentrations on white filter discs after filtering pond water with a Lamotte testing kit. BOTTOM: Figure 4. Testing water quality for human pathogens is essential when producing edible crops.

Human pathogen testing

Levels of human pathogens are regulated when using water for drinking supplies, irrigating edible fruits (especially with methods that wet foliage or fruit), and for post-harvest washing of produce. Some states have regulations for coliform levels in water used to irrigate non-food crops.

Outbreaks of human disease caused by contaminated produce emphasize that monitoring to ensure irrigation and wash water are free of human pathogens is a critical food safety issue (Figure 4). More information is available on water testing for human pathogens at http://edis.ifas.ufl.edu/ss482.

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Photo by Paul Fisher, University of Florida Photo by Gavin Mohammad-Pour, University of Florida

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Why you should test

Tests for biological quality can help avoid crop losses from diseases, unsightly algae, clogging of equipment, and health issues from contaminated produce. Most organisms in water are too small to see with the human eye. Consider monitoring to catch biological water problems before they cost you money. **GM**

Paul Fisher is associate professor and extension specialist, Dustin Meador is graduate student, Environmental Horticulture Department, University of Florida, pfisher@ufl.edu. Robert Wick is professor of plant pathology, Department of Plant, Soil and Insect Sciences, rwick@pltpath.umass.edu. Jennifer Parke is associate professor, Department of Crop and Soil Science, Oregon State University, jennifer.parke@oregonstate.edu. William Argo is technical manager, Blackmore Co., bargo@blackmoreco.com.

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Water Education Alliance For Horticulture
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Testing total density of micro-organisms in irrigation water

University of Florida researchers have adapted this method for testing irrigation water using 3M Petrifilms.

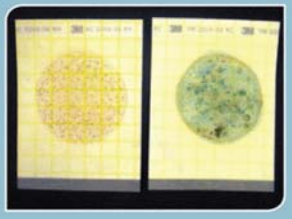
1 Allow the irrigation water to flow for 5 minutes, or long enough to ensure sampled water has been not been sitting in the piping. Collect a sample of irrigation water in a sterile disposable container. Rinse the collection container three to five times in the representative irrigation water. Be careful not to contaminate the sample by touching the rim of the container with the irrigation emitter or dirty hands.

2 Dilute a 1 milliliter (ml) of the water sample by dispensing it with a sterile disposable dropper into 99 ml of distilled water in a sterile vial. Close the top of the vial and mix the diluted sample by inverting the vial three times.

3 With a new sterile dropper, remove 1 ml from the vial and place it onto a Petrifilm card. There are two types of Petrifilm most commonly used (aerobic bacteria or yeasts and molds) to culture different groups of organisms. For testing organisms related to biofilm and equipment clogging, use the aerobic bacteria Petrifilm.

4 A 3M spreader plate is used to spread the 1 ml sample evenly to cover 20 squares.

5 After three to five days of incubation at a controlled room temperature in the dark (i.e., an office drawer), the Petrifilm can be counted to determine if there are more than 100 dots, which represents 10,000 colony forming units per ml (cfu/ml). More than 10,000 cfu/ml indicates high risk for biofilm development. There are no recommended thresholds available for yeasts and molds. Use these Petrifilms as a relative measure for samples taken before and after treatment with a sanitizing agent. The image shows aerobic bacteria (red dots, left) and yeasts and molds (blue dots, right).



For more information on this testing method and to source the supplies, contact Paul Fisher, pfisher@ufl.edu.

TABLE 1. KEY BIOLOGICAL WATER QUALITY MEASUREMENTS FOR HORTICULTURE

Biological measurement	Analysis	Significance	Cost	Example sources	Units and target range
Culture plating of plant pathogens	Laboratory	Identifies living pathogens, usually to the genus level.	$50-$100	Various laboratories. For example, *Pythium* and *Phytophthora* at University of Massachusetts, (413) 545-3209; http://extension.umass.edu/agriculture/index.php/services/plant-problem-diagnostics University of Florida Extension Plant Disease Clinic, http://plantpath.ifas.ufl.edu/pdc	Lab-specific. May be presence/absence or quantitative Some plant pathogens are quarantine issues or under regulatory control
Pathogen DNA analysis	Laboratory	DNA scan. Highly specific, but won't differentiate between live and dead organisms	$125-$195	Various labs. University of Guelph Laboratory Services (519) 767-6227; www.guelphlabservices.com	Presence/absence or semi-quantitative
ELISA-based test kits for Pythium and Phytophthora	Onsite	Not species-specific. Some cross-reactivity between *Phytophthora* species and *Pythium* species. Does not differentiate between live and dead organisms.	$7-$13 per test	Various suppliers. For example, Immunostrip at www.agdia.com. ALERT-LF at http://plant.neogeneurope.com. Pocket Diagnostic at www.pocketdiagnostic.com.	Presence/absence
Total density of bacteria and fungus	Laboratory Onsite: Protocols being developed to use 3M Petrifilms for heterotrophic bacteria and yeasts and molds.	Indicator of biological load. May be useful to test biofilm risk, and pre- and post-treatment. Majority of micro-organisms likely to be benign or beneficial.	3M Petrifilms $1-$2 per sample in packs of 25 and up.	Microbiology laboratories 3M Petrifilms: Find online (e.g. www.sciencekit.com) or contact 3M, (800) 515-8114.	More than 10,000 heterotrophic bacteria colony forming units/ml is recommended to avoid clogging of emitters.*
Algae	Laboratory (preferred) Onsite: Lamotte Algae kit AWL # 6662.	Clogging, wetting of growing substrate, aesthetic, safety, shore flies	Lamotte kit, about $140	Water testing labs specializing in algae. For example, www.greenwaterlab.com Test kit: www.lamotte.com	Lab-specific. Chlorophyll, or identification and quantification.
Human pathogens (culturing or nucleic acid analysis)	Laboratory (preferred) Onsite: 3M Petrifilm exist for some human pathogens (use as an indicator, not regulatory).	Contamination of water with fecal wastes or other sources of pathogens is health risk with edible crops and drinking water supplies.		Microbiology labs and public health department labs.	Check state health regulations for different water uses.

*Bacterial count recommendation from D.H. Rogers, F.R. Lamm and M. Alam. 2003. Subsurface Drip Irrigation Systems Water Quality Assessment Guidelines. Kansas State University Agricultural Experiment Station and Cooperative Extension Service, MF-2575.