FACTSHEET 21/15

Cross Sector

AHDB HORTICULTURE

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Testing water for plant pathogens

Water for irrigation can easily become contaminated with potential plant pathogens and, whether a new source of water is being considered or there are concerns with the current supply such as possibly contaminated storage tanks or the occurrence of suspicious disease outbreaks, water testing is essential for guiding management decisions.

However, the approach to testing can strongly influence the value of the results. This factsheet explains the appropriate testing that is currently available to properly assess the disease risks and outlines interpretation of results, together with the questions to ask a prospective test provider.

Figure 1. Water testing equipment

Figure 2. Water filter

Action points

Why test water for plant pathogens?

- Water can easily be contaminated with plant pathogens and rapidly initiate and spread plant diseases – testing will detect contamination.
- Tests help identify the source of contamination – allowing effective treatment.
- Monitoring the efficacy of water treatment systems.
- Compliance with accreditation.
- There is no reliable way of visually assessing the level of pathogen contamination as water containing large numbers of infective pathogen spores can still appear ‘crystal clear’. A true and reliable measure of the risks can only really be obtained by carrying out microbiological tests.

Types of water tests available for plant pathogens

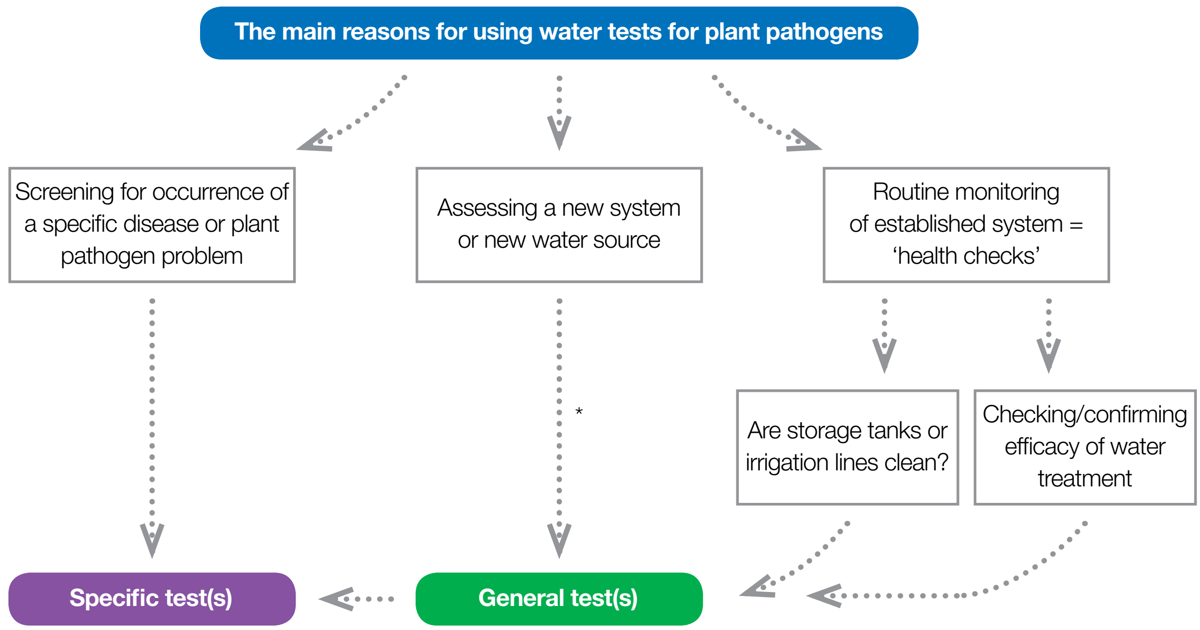
The reasons for needing to test water for potential plant pathogens vary and are summarised in figure 3, which also indicates the most appropriate testing approach for each situation. Tests can either be 'specific', focusing on one genus or even species of pathogen or one particular disease problem, or more 'general', screening for a wider range of pathogens and attempting to use certain, more commonly seen, non-pathogens as indicators.

Specific testing

- Specific testing is very useful when dealing with a known disease problem, especially within a closed irrigation system under protection. It also has a vital role in the detection of notifiable pathogens (eg *Phytophthora ramorum*) and the Animal and Plant Health Agency (APHA) has an array of sophisticated tests available to their inspectors for this purpose.
- In certain circumstances, general tests can identify situations when a specific test is needed as a follow on, especially when testing potential new sources of irrigation water.
- The main potential pitfall to specific testing for single species or genera is the danger of getting a false sense of security from regular negative test results, while actually missing other pathogen species that might be present. Using specific tests as the sole assessment of water-borne disease risks on a nursery is very unwise.

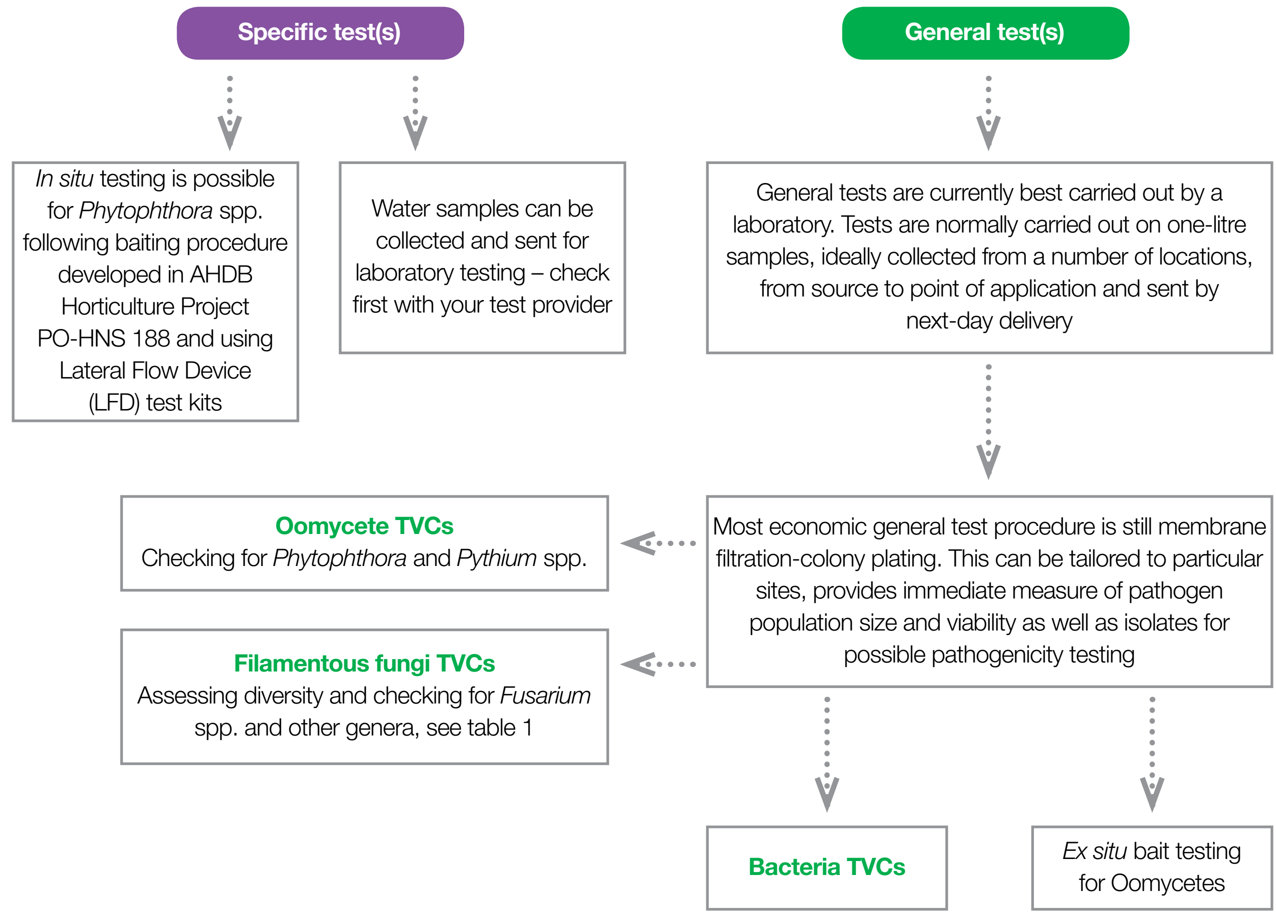
General testing

- General testing is the most appropriate approach for testing the efficacy of water treatment systems on nurseries, where specificity to genus/species is not necessary. Pathogen propagules are usually relatively rare. More commonly occurring non-pathogen relatives can be detected and used as indicator species. For example in the case of *Pythium* and *Phytophthora*, related Oomycete species such as *Saprolegnia ferax*, which lives on debris and insect remains, is very common in untreated irrigation water. An effective water treatment system will remove these propagules in the same way it would remove pathogen spores and so, if they are detected in treated water, this gives an early warning of possible treatment failure.
- General testing is also effective for regular assessments of clean irrigation systems for early signs of contamination.
- The most common causes of system contamination are flooding (eg increased disease risks associated with contamination of boreholes by flooding – table 1) and the damage or removal of storage tank covers.
- Currently, the most economic general testing method is membrane filtration, followed by selective agar plating. General tests normally will include media for total Oomycete propagule counts (this includes many non-pathogens as well as *Pythium*, *Phytophthora* and *Aphanomyces*), for total filamentous fungus counts and for total bacteria counts.
- General tests can be tailored to specific situations, for example, if a nursery has a concern about *Fusarium*, semi-selective agar media for this group can easily be included in the test. Tests can be made specific by subculture of selected colonies and identifications by morphology or molecular methods (although this generally takes more time and incurs greater costs).



*While it can be tempting to go straight for a specific pathogen test (eg testing for *Phytophthora* spp.) as this might be of immediate concern, it is best to initially carry out a set of general tests when assessing a new source of water or changes to an irrigation system as there may be unforeseen pathogen problems present. Also, a general test can identify the likelihood of disease risks by looking at general microbial counts and the types and diversity of species (non-pathogens as well as pathogens) present. This may then lead to specific tests.

Figure 3. Reasons for using water tests for plant pathogens



TVC = Total Viable Count – viability is very important, especially when testing treated water which, if the treatment is working, will often contain traces of dead pathogens but no viable pathogen material.

Figure 4. Types of water tests available for plant pathogens

Figure 5. Water samples should be sent to a laboratory by courier in empty sparkling water bottles – clearly marked with sample numbers on both the lid and the bottle

Figure 6. Filling water bottle from hose

Table 1. Plant disease risks associated with different sources of water for irrigation

Rivers, streams and ditches, as well as outdoor reservoirs and ponds, carry a very high risk of contamination with plant pathogens, as do uncovered storage tanks, especially those positioned out of doors. Often appearing very clean, greenhouse roof water can also be contaminated, especially by species of *Pythium* and *Fusarium*, largely depending on the cleanness of the roofs and the gutters. Mains water and water abstracted from boreholes is generally free from plant pathogens.

Water source		Disease risk
Mains	1) Used directly	Very low–none
	2) Stored in covered tanks	Very low–moderate
	3) Stored in uncovered tanks	Moderate–high
Borehole/well		
A) Clean, uncompromised borehole and extraction equipment	1) Used directly	Low–none
	2) Stored in covered tanks	Low–moderate
	3) Stored in uncovered tanks	Moderate–high
B) Flooded borehole, dirty extraction equipment		High–very high
Open reservoirs/ponds/lakes		High–very high
Rivers/streams/canals/ditches		High–very high
Collected from roofs or paved areas		Moderate–high
Run-off from fields or production beds		Very high
Recirculating nutrient solution		Moderate–very high

Plant pathogens frequently found in water

A wide range of microorganisms are regularly detected in water samples and potential plant pathogens represent only a small proportion of these. In table 2, all of the important Oomycete genera and a selection of other key pathogen groups spread by contaminated irrigation water are listed, together with some frequently encountered non-pathogen species that can be useful as 'indicators' of both pathogen risks and water treatment efficacy (see 'General testing' page 2). Some examples of naturally occurring genera with known disease suppressive qualities (eg *Trichoderma*), that are often recorded in horticultural water samples, are also mentioned. Growers are often interested in their presence and populations, although we unfortunately still know too little of their complex interactions with plant pathogens in water to draw much useful information for disease management purposes from such observations.

Table 2. Examples of pathogenic genera known to be spread in irrigation water

Genera demonstrated to be spread by water	**Other genera that might be spread in water** [those in brackets are not generally pathogens but are either useful indicator species or potential disease-suppressive agents]
Oomycetes	
Aphanomyces	[*Saprolegnia*]
Phytophthora	
Pythium	
Plasmodiophorids	
Plasmodiophora	
Fungi	
Fusarium	*Alternaria*
Phoma	*Ascochyta*
Thielaviopsis	*Botrytis*
Verticillium	*Colletotrichum*
	Didymella
	Rhizoctonia
	[*Coniothyrium*]
	[*Gliocladium*]
	[*Trichoderma*]
Bacteria	
Erwinia	
Pseudomonas	
Xanthomonas	
Viruses	
Cucumber green mottle mosaic virus (CGMMV)	
Pelargonium flower break virus (PFBV)	
Pepino mosaic virus (pepMV)	
Tobacco mosaic virus (TMV)	
Tomato mosaic virus (ToMV)	

Taking samples

The following procedure is straightforward and very effective:

- The optimum sample volume is one-litre. This is a trade-off between the need for water volume to improve test sensitivity and the economics of sample handling and delivery.
- Sample bottles need to be robust for delivery to the test lab. PET (polyethylene terephthalate) bottles for carbonated soft drinks make ideal containers, and bottles used for supermarket economy-branded carbonated water, if used immediately after decanting their fizzy contents, are ready and clean enough for the purpose of collecting samples for Oomycete and fungus testing (see figure 5).
- Each sample bottle needs to be clearly identified and labelled before packing and dispatch to the testing lab, and samples need to arrive at the lab by the day following collection (see figure 5).
- Single water samples carried out in isolation rarely mean very much, even testing water treatment efficacy requires a minimum of one pre and one post-treatment sample to be certain that the treatment is working. Water-borne plant pathogen inoculum is dynamic, changing over time and with movement within a system. It is, therefore, wise to take several samples from different parts of an irrigation system, from the 'raw' water source, its storage, and from the point of delivery to plants.

Frequency of testing

From a strictly disease-management perspective, the more frequently testing is carried out, the better. Even very infrequent testing is better than none.

The frequency at which water sampling is carried out is, ultimately, a question of economics. With an average outlay for a full test of between £100–£150 plus nursery staff time in collection and packing, and sample carriage, it is important to get best value for money. It is difficult to put a cash value on timely disease intelligence or on the peace of mind provided by testing. In certain situations, for example, when accreditation schemes recommend routine storage tank clean-ups, microbiological tests demonstrating that tanks are clean can achieve significant cost savings in avoided disruption and cleaning.

Timing of testing

Sampling times may be dictated by the scheduling of crops or by the availability of staff to complete the task, however, an optimum arrangement would be to carry out tests four to six times per year, at regular intervals. The numbers of pathogen propagules in water do vary significantly with season. For example, Oomycete numbers peak in late spring and again in early autumn while genera such as *Fusarium* peak in late summer/early autumn. If a single test is carried out per annum, the best time to do so would appear to be in late summer/early autumn.

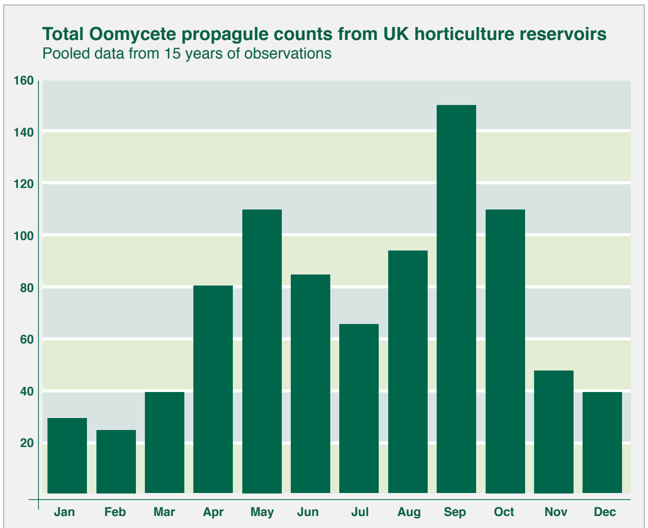


Figure 7. Total Oomycetes propagule counts from UK horticultural reservoirs

Future developments

The techniques available for practical disease diagnostic testing are currently evolving fast with a number of new developments that have recently been reviewed for AHDB Horticulture Project CP 099b. Lower-cost portable DNA-based technologies provide exciting prospects for the future although there is still much development work needed. Meanwhile, further development of immunodiagnostic techniques is underway in AHDB Horticulture Project CP 136 to provide a new range of test kits. The kits will hopefully have the capacity to differentiate between viable and dead pathogen propagules and, thus, be useful in combination with membrane filtration for rapid on site testing of water treatment systems and possibly reducing the need to send samples away to laboratories for analysis.

Further information

AHDB Horticulture factsheets and publications

AHDB Factsheet 22/15: Methods of water treatment for the elimination of plant pathogens.

AHDB Factsheet 23/15: Hygiene and disease avoidance underpin the management of Oomycete stem and root rots.

Instruction sheet for growers: *Pythium* and *Phytophthora* water baiting.

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All of the images contained within this factsheet were provided by Dr Tim Pettitt, University of Worcester.

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