NURSERY PAPERS
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BAITING FOR *PHYTOPHTHORA* AND *PYTHIUM* IN PRODUCTION NURSERIES

The production and sale of healthy nursery stock is essential, as production nurseries are the very heart of our ornamental, vegetable, fruit and forestry industries. To manage plant diseases effectively, nursery managers can monitor for specific, high-risk pathogens using baiting techniques. This paper can be used as a guide to conduct different baiting techniques for *Phytophthora*. These methods extend normal crop monitoring, can be implemented easily by nursery managers and assist in resolving urgent disease problems. This nursery paper has been produced by Queensland Department of Agriculture and Fisheries pathologists as part of a nursery levy funded project.

Summary

- Baits are plants that are susceptible to particular pathogens and show infection quickly.
- Other pathogens are sometimes detected on lupin baits, e.g. *Pythium* and *Chalara*.
- Lupin baits can be used to regularly monitor for *Phytophthora* in water and growing media.
- Lupin baiting is cheap and reliable.

Figure 1. Rotting roots infected with *Phytophthora*

INTRODUCTION

Before selecting a baiting method for soil or water, it is essential that growers be aware of the diseases that are likely to attack their crops. As with any diagnostic procedure, the accuracy of the baiting method will depend on selecting a representative sample for testing. In addition, the application of fungicides prior to completing baits may result in a false negative result. Therefore it is important to complete tests on material that has not had pesticides applied for two weeks prior to testing. There are many types of baits that can be used to detect a range of plant pathogens. *Phytophthora* lupin baiting is one of the easiest methods that can be incorporated into a production nursery disease management strategy.

BAITING FOR PHYTOPHTHORA

Some baiting methods can detect different species of *Phytophthora*. Thus the baiting methods for *Phytophthora* involve exploiting plant tissue on which the targeted species produces an easily recognisable, characteristic lesion or rot. Preference for a natural host of a *Phytophthora* species often determines the choice of bait. Most commonly, methods involve inserting soil, growing media or infected plant tissue into a wound or hole in a firm fleshy fruit (e.g. apple), or floating or partially immersing baits in a water and growing media mixture (e.g. lupins).

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Lupin baiting

In Australia, radicles of young New Zealand blue lupin seedlings, *Lupinus angustifolius*, are used extensively in diagnostic laboratories because they detect many *Phytophthora* species. This is an excellent technique where *Phytophthora* rots the radicle within five days; seedlings either do not grow or die completely. Lupins with obvious symptoms can be placed in a Petri dish with water and examined under a dissecting microscope; sporangia will be visible on the root lesion. Sometimes soil bacteria and *Fusarium* sp. can infect lupin baits making it difficult to observe the sporangia of the target *Phytophthora*. *Chalara* spores and rotifers may sometimes be observed on lupin roots but do not cause rotting symptoms. In addition, *Pythium* may also be detected but rarely cause significant rot symptoms. *Pythium* is more likely to cause a superficial root tip rot; spherical sporangia are sometimes able to be observed. A one page description of lupin baiting is outlined on the back page of this nursery paper.

Figure 2. Healthy lupins (left), *Phytophthora* infected lupins (right)

Leaves of the infected host

In most cases, the leaves from the symptomatic host will be attacked by *Phytophthora*. This method follows similar methods to lupin baiting. Instead of placing lupin seedlings in holes in the cup lid (as per methods on page 4), leaves are placed directly in the water. Leaves should be clean and healthy without any evidence of any pathogen infection. Prick or make a small cut in the leaf before placing in the water. Infection should occur within 1-5 days and include rotting tissue, most often around the artificially damaged areas. Sporangia may be observed under a dissecting microscope.

Baiting water sources for *Phytophthora*

The methods for lupin baits and using leaves of the infected host can be easily modified to bait water sources for *Phytophthora*. For lupin baits, fill up three cups entirely with the water source. If there are no plants showing signs of infection, umbrella tree leaves (*Schefflera actinophylla*) can be suspended in water, as per the method described above. Many other host plant species can be used if necessary.

Figure 3. Umbrella leaf infected with *Phytophthora* from infested water

A NUMBER OF FACTORS NEED TO BE CONSIDERED WHEN *PHYTOPHTHORA* IS THOUGHT TO BE THE CAUSE OF DISEASED PLANTS

1. Plants affected by *Phytophthora* spp. may not show above ground symptoms until root or crown rots are well advanced.
2. *Phytophthora* spp. attack only intact plant tissue or fresh wounds and do not invade plant tissue previously colonised by other organisms.
3. *Phytophthora* can be difficult to isolate directly from decayed tissue or soil, because of secondary organisms or those feeding on dead and decaying material.
4. If *Phytophthora* is recovered, it is very likely to be the cause of the disease and may spread rapidly in a production nursery. Inoculum levels of this pathogen can increase from a low level to an extremely high level in a few days.
5. A root disease where *Phytophthora* is the causal agent, may be misdiagnosed and be wrongly attributed to a member of the closely related genus *Pythium*, or a member of the ubiquitous genus *Fusarium*. These two organisms often colonise decayed roots following *Phytophthora* infection, but may not be the cause of the disease.
6. Distinguishing *Phytophthora* from *Pythium* takes practice and must be completed on a compound microscope at high power.

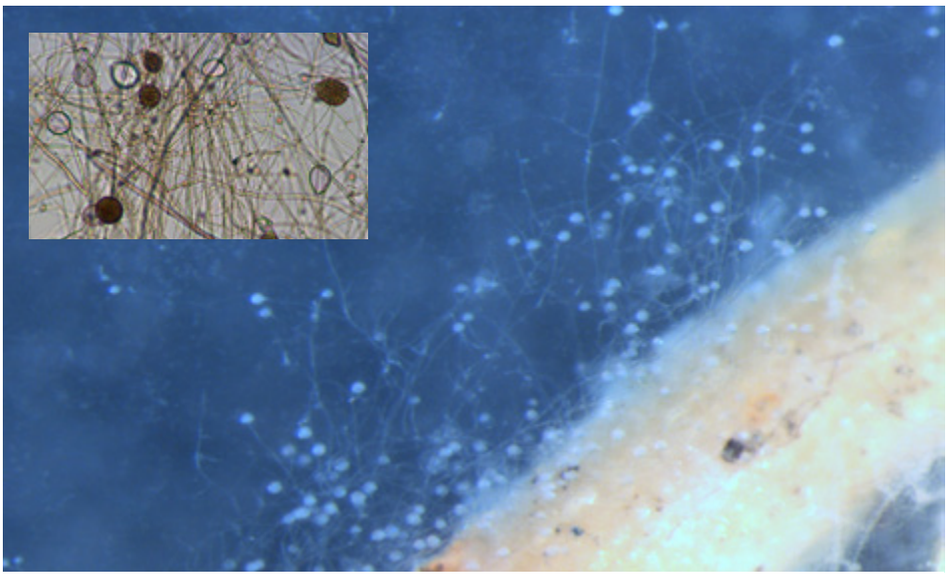

Figure 4. *S*porangia on infected lupin roots and a close up of sporangia under a compound microscope (insert). At this magnification, *Pythium* and *Phytophthora* cannot be distinguished.

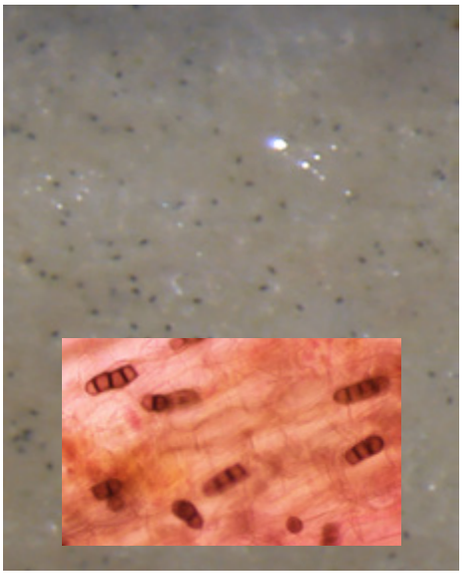

Figure 5. *Chalara* chlamydospores on lupin roots (black dots) and at high resolution (insert).

RECOGNISING *PHYTOPHTHORA* AND OTHER ORGANISMS ON LUPIN BAITS

It takes practice to recognise *Phytophthora* and differentiate it from other similar organisms. In a diagnostic laboratory affected roots are surface sterilised and plated onto a selective medium to culture *Phytophthora*. Typically, *Phytophthora* produces sporangia that are bulbous or balloon-like, but may be elongate for certain species. By contrast, *Pythium* is almost always spherical. However, under a dissecting microscope it can be very difficult to distinguish *Phytophthora* from *Pythium*; a slide is normally required with examination under a compound microscope. *Chalara* may sometimes be observed but rarely cause a rot on lupins; their spores are dark and form relatively short chains (Figure 5). Rotifers are filter-feeding zooplankton that have a balloon like structure on a long stalk. The stalk often moves in spring like actions. Many images are available online.

IMPLICATIONS OF THE FINDINGS FOR THE NURSERY INDUSTRY

Nursery managers have a vital role to play in building resilience and on-farm biosecurity capacity of the Australian horticulture industry.

By implementing the recommended techniques in day-to-day operations, nursery managers can manage plant disease risk effectively, minimise losses and unnecessary costs and enhance their on-farm biosecurity.

CONCLUSION

Baiting for *Phytophthora* is relatively simple and can be completed following an outbreak or as part of a regular monitoring program. There are many possible bait types to suit a range of pathogens. It is always recommended to contact a professional disease diagnostic laboratory to confirm the presence of a disease. Each state has a diagnostic service through their local department of Agriculture/Primary Industries. Finally, always ensure that waste is disposed of hygienically, e.g. deep burial, incinerated or otherwise sterilised.

DO NOT DISCARD BAIT WASTE MATERIAL ON-FARM.

LINKS TO RESOURCES

NIASA Best Management Practice Guidelines, 4th Edition, 2010, Appendix 2.

Phytophthora Diseases – Problematic in the Nursery and Beyond.
http://www.ngia.com.au/Attachment?Action=Download&Attachment_id=1833

Pythium species. A Constant Threat to Nursery Production.
http://www.ngia.com.au/Attachment?Action=Download&Attachment_id=1842

Soilborne Root Pathogens in Production Nurseries. A Pest Management Plan.
http://www.ngia.com.au/Attachment?Action=Download&Attachment_id=1848

This research topic was funded as part of the 'Building the resilience and on-farm biosecurity capacity of the Australian production nursery industry' project (NY15002) funded by Horticulture Innovation Australia Limited using the Australian Nursery Industry levy and funds from the Australian Government. It was undertaken by Dr Andrew Manners, Queensland Department of Agriculture, Fisheries and Forestry.

LUPIN BAITING METHODS

Materials required

- Clear plastic cups (225-250mL) with a tight fitting lid for each sample
- Blue lupin seed; available through Rockfields Pty Ltd (Ph. 0438267312, email: **rockfield@internode.on.net**) and other seed suppliers.
- Vermiculite
- Sterile water – either distilled or boiled and cooled water
- Bleach
- Hole punch
- Microscope. Use of a dissecting microscope is recommended to examine lupins for pathogen structures as per pictures on page 3. Magnification of 40x is sufficient, 60x or higher is ideal.

Methods

1. Surface sterilise lupin seed in 1% bleach solution for 1 minute[1]. Sterilise enough seed so you have plenty to complete the required number of lupin baits.
2. Drain and rinse in sterile water such that no bleach is left on seeds. Then, soak seed in sterile distilled water for 5-60 minutes[2].
3. Pre-germinate lupin seed in a plastic bag containing sterile vermiculite at room temperature in a clean area; don't seal the bags, they need to breath. Add distilled water until the vermiculite is moist enough to germinate; vermiculite shouldn't be completely saturated – damp but not drenched.
4. Agitate the bag to spread moisture. Check daily to ensure that there is sufficient moisture and development of lupins.
5. Store in a clean environment at room temperature. Lupin radicles need to be no longer than 2cm. Lupins with very long radicles are less susceptible to *Phytophthora* infection, therefore it is important to use short lupins.
6. Punch or melt 5 holes each 5-8mm diameter in lids of plastic cups. For each test, it is best to complete three cups. Therefore, if testing two different media or plant types, six cups will be required.
7. Label cups with an appropriate identifier and the date. Place a layer of growing medium, or soil, 3cm deep in the bottom of the plastic cup[3].
8. Fill cup with distilled water. Cap with lids with punched or melted holes.

Figure 6. Size of lupin radicle suitable for use in baits. Do not use lupins with radicles any longer than the six pictured here

9. Place one lupin of appropriate size (Figure 6) through each of the holes in the lid. Ensure that radicles touch the water in the cup; add more water if required.
10. Check health of lupin roots every 1-2 days (Figures 2, 7-9).

[1] Household bleach is normally around 4% bleach, therefore must be diluted 1:3 bleach:water; the exact dilution will need to be modified according to its concentration.

[2] Seed coats will start to appear slightly cracked and have a 'prune like' appearance; soaking the seeds for short periods of time (or not at all) may delay germination by an extra day depending upon environmental conditions.

[3] Do not overfill the cups with the growing medium or soil. One part of growing medium to five to ten parts of distilled water is best for good zoospore production. If the crop has been drenched with a pesticide that is active against *Phytophthora*, e.g. metalaxyl, use about half the volume of growing media in each cup to assist in diluting the pesticide.

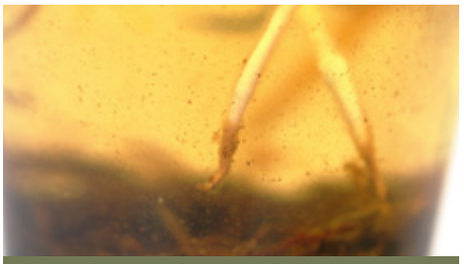

Figure 7. Slightly rotten lupins infected with *Phytophthora*

Figure 8. Severely rotten lupins infected with *Phytophthora*

Figure 9. Slightly rotten lupins infected with *Pythium*

INTERPRETING RESULTS

On the fifth day, if lupin roots are a pearly white colour they are not infected with *Phytophthora* (Figure 2). If there is any evidence of a brown rot, they may be infected with *Phytophthora* or perhaps *Pythium* (Figures 7, 9). Severe root rot is almost certainly an indication of *Phytophthora* infection (Figure 2, 8). Examine roots and try to find sporangia (Figures 4-5). Take pictures of results. If a root rot has been detected, **it is recommended to submit a sample for diagnostic testing** to confirm *Phytophthora*, unless you have considerable experience in detecting *Phytophthora*. Please note, false positives (i.e. a root rot caused by something other than *Phytophthora* or *Pythium*) may occur under certain conditions, e.g. if too much fertiliser is placed in lupin cups. False negatives may occur if the crop has been drenched with certain pesticides (e.g. metalaxyl) or if lupins with a very long radicle are used.

The diagnostic service provider Grow Help Australia provides subsidised diagnostic testing for all production nurseries in Australia and 10 free samples for each NIASA accredited business per year during the life of the levy funded project NY15002. Search for "Grow Help Australia" on your web browser.