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Accurately diagnosing weeds, pests and diseases affecting nursery crops.

Accurately diagnosing weeds, pests and diseases affecting nursery crops can be challenging. If left unchecked these pests can increase costs and reduce productivity. Therefore it is important to take action early to prevent widespread infestations through correct diagnostics.

This months nursery paper was prepared by Andrew Manners* (Senior Entomologist and manager of Grow Help Australia) and John Duff* (Senior Plant Protectionist) as part of the levy funded project 'NY11001 Plant health, biosecurity, risk management and capacity building for the nursery industry'.

* Based at the Queensland Department of Agriculture, Fisheries and Forestry

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Fig.1. Bracteantha infected with *Ralsonia solanacearum*. This species cannot be identified without specialist knowledge and diagnostic capacity.

Infestations of pests, diseases and weeds can reduce growth rates and crop uniformity, as well as increase throw-outs and other costs associated with the crop. Active and regular monitoring can reduce the extent and impact of infestations. Once a problem is observed, it is of critical importance to make an accurate diagnosis. For very obvious symptoms, e.g. presence of spider mites, aphids, caterpillars, etc, a field diagnosis is possible. However, identical symptoms can be produced by multiple diseases and can sometimes be confused with damage produced by insects or mites. In other cases multiple causal agents may be present and identifying the primary cause of symptoms may not be straight forward (e.g. Fig 1). Incorrect diagnosis can lead to increased costs due to inappropriate treatments and allow the pest or pathogen to spread and infect healthy plants.

Information accompanying a plant submitted for diagnosis

It is critical to send detailed information with any plant sent in for diagnostics. This helps the diagnostician put the symptoms and any pest or disease observed/isolated from the plant into perspective and give the most accurate diagnosis possible. If thorough information does not accompany a sample, incorrect recommendations may be provided. For example, if a plant with a leaf spot symptom is submitted and no pathogen is associated with the spots it

When submitting diagnostic samples provide as much information about the crop as possible:

- Species and variety of plant.
- Where the crop has been grown.
- How the crop is being grown, e.g. in containers or in-ground, containers on the ground or raised on benches or under protected cropping.
- History of the crop, e.g. age of plants, length of time the crop has had symptoms, if your plants have ever experienced these symptoms in the past and how they were successfully and or unsuccessfully managed.
- Symptoms of the crop, e.g. leaf spot, root rot etc.
- The percentage of crop affected and the size of the crop (area, number of plants).
- Treatments that have been applied to the crop (fertiliser, insecticides, fungicides or anything else). Provide an estimate of when these treatments were applied.
- Environmental conditions, e.g. rainfall, temperature, high wind, frost, hail etc.
- Provide a photo of the whole crop.

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could be due to the spot being caused by 1) a physiological reaction associated with environmental or growing conditions, 2) fertiliser burn, 3) pesticide phytotoxicity, 4) recent application of a fungicide which has reduced the pathogen to such an extent that it can no longer be isolated from the leaf spot or 5) some other factor. Without further information an incorrect diagnosis may occur, so it is recommended to include as much detail as possible.

Collecting samples for disease diagnosis

Plant pathogens tend to grow in and on plant material. For most groups, pathogens cannot be readily identified from symptoms and must be isolated from the plant (see exceptions below). This involves taking small pieces of plant material from the advancing margin of pathogen activity (e.g. the leading edge of a stem rot or leaf spot) and placing it on a specific media for the pathogen to grow. Once growing on the media it can be examined in various ways to determine its identity. The pathogen must be taken from the advancing margin as secondary pathogens (bacterial and fungal) rapidly develop on dead plant material. For this reason, dead plants are not suitable for the diagnosis of plant diseases as secondary pathogens are likely to mask the primary pathogen. Not all pathogens can be isolated in this way. Some pathogens will not grow on specialist media and spores must be collected and identified directly from plant tissue, e.g. powdery mildew, downy mildew and rusts.

Plant selection can greatly impact a diagnostician's ability to isolate and accurately diagnose the causal agent. It is therefore extremely important that plants with advancing symptoms be presented to diagnosticians. If possible, send in multiple plants so diagnosticians can observe which symptoms are consistent. Having plants with early, intermediate and advanced symptoms (but never dead plants) is beneficial and gives the best chance of isolating the causal agent.

Provide your diagnostic provider a photo of the entire crop and individual plants. This can assist your diagnostician in the diagnosis by putting the symptoms in perspective. It can be beneficial to email photos prior to sending the sample, particularly when whole plants can not be submitted.

It can be tempting to submit only symptomatic plant parts, particularly in cases of stem or leaf dieback. While these symptoms can be caused by pathogens that may be isolated from above ground parts it is also possible that the causal agent is acting upon the roots of the plant. It is always better to submit whole plants and allow the diagnostician to determine from which areas to isolate, however, for large plants this is not always possible. In such cases, send in symptomatic parts of the plant, along with soil and root samples.

Molecular techniques are increasingly part of diagnosing plant pathogens, particularly viruses. Isolations often are able to determine the genus of a pathogen from the morphology of spores and other structures. Determining the species of a pathogen from morphology can be difficult and time consuming. Molecular biology can often be used to ascertain the species identity and confirm initial morphological examinations.

Collecting insect and mite samples

Insects and mites tend to be easier to identify than plant pathogens, at least to a common group e.g. caterpillars, spider mites, aphids, scarab beetles etc. Species level identifications can often require laborious preparations and may not be possible for groups for which diagnostic keys do not exist. However, often knowing the group of insect is sufficient for nursery production managers to put in strategies to reduce the impact of arthropod pests. Sometimes this is not the case, particularly when one species is resistant to insecticides, e.g. western flower thrips or green peach aphid, and other species may have no or differing levels of resistance. In such cases it can be advisable to gain a species level identification. Contact the diagnostic service you plan to use prior to sending insect or mite samples for species level identification as certain organisms have special requirements, e.g. flies cannot be identified using larvae and spider mites must have males and females for identification. In general, it is easiest to submit plants infested with pests as opposed individual insects or mites (Fig. 2). This allows the diagnostician to pick which individuals will be selected for closer examination and avoids sending preservatives in the mail which are most often considered dangerous goods, e.g. 70% ethanol, methanol or other substances.

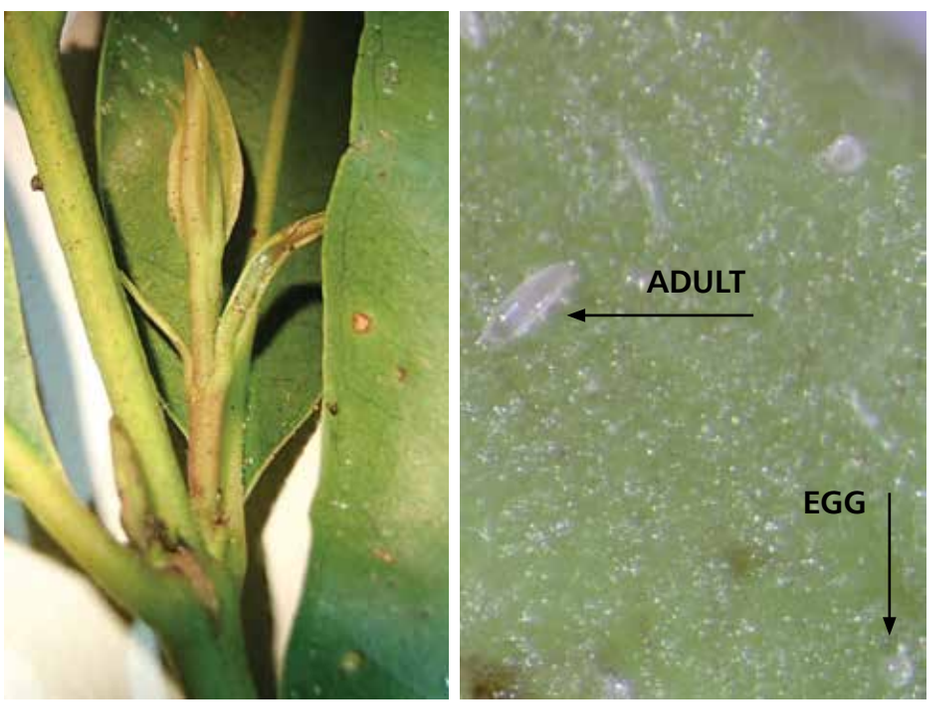

Fig.2. Waterhausia with stunted growing tips (left) caused by eriophyid mites (right). Eriophyid mites are not visible to the naked eye and require at least x20 magnification to be observed. Adults are about 0.1mm in length, eggs about a third of this size.

Diagnosing pathogens in growing media and water

Many plant pathogens are spread through the movement of growing media and/or in water. As such, it is extremely important to purchase and use pathogen free growing media and appropriately disinfested water. However, pathogens may still occur in growing media and water sources and could therefore require testing. Since many saprophytic fungi and bacteria are often present in growing media and water, testing for these pathogens must be specific. These tests, commonly called baits, should only be undertaken when you suspect a particular pathogen, or when it has been isolated from plants during previous tests. Baiting growing media and water may then serve to determine where the infection has or has not originated. Baiting involves using a seedling, leaf or other plant material from a species which is particularly sensitive to a specific pathogen. Such plant material is the 'bait' from which the pathogen can then be readily observed and isolated.

Soil and growing media

Phytophthora is the most commonly baited soil, growing media and water-borne pathogen, though many pathogens can be baited using different methods. To facilitate your diagnostician completing a *Phytophthora* bait, collect a number of sub-samples of soil or growing media and roots (up to a depth of 15cm) beneath each plant in a certain location. Multiple plants may be bulked together to make a representative sample for each location. For each sample, include about 500g soil or growing media and roots from plants with early and advanced symptoms. It is particularly important to include roots in the sample as this will increase the accuracy of the test. Include several samples if practical to narrow down which areas are being affected. Soil or growing media and roots are then sent to a diagnostic laboratory for analysis.

As mentioned above, many other baits can be completed to test for specific pathogens. For example *Cylindrocladium*, and allied genera, can be baited using caster oil leaves, black root rot (*Thielaviopsis sp.*) using carrots and *Pythium* and *Phytophthora* can be baited using a variety of leaves including lemon, umbrella tree, azalea, avocado and apple flesh. Refer to your diagnostic service provider if you would like a specific test completed.

Irrigation and dam water

The same principles apply to pathogen baiting in water used for irrigation. Many fungal and bacterial pathogens can be spread in water including *Pythium*, *Phytophthora*, *Fusarium*, *Cylindrocladium*, etc. The same baits can be used for baiting water as used for growing media ; they are simply left in irrigation water for a period of time and examined for fungal activity. For example, for *Phytophthora*, poke holes in semi-mature umbrella tree leaves and place them inside plastic bottles. Thoroughly cleaned milk containers work well as they have an easy handle from which a string can be tied. The bottle can then be 'floated' in irrigation water such that the entire bottle remains under the surface for 1-2 days before being sent to a diagnostic provider for further testing. This may require a small weight to be attached to one part of the bottle so that the opening remains under the water, but the entire bottle is still at the surface. Testing water can be beneficial, however rainfall and other events can drastically alter the species present in irrigation and dam water over short periods of time.

Nutrient analyses

Growing media and water is of utmost importance to growing high quality plants. It is recommended to monitor such parameters as EC, pH and other nutrients on a regular basis to ensure that growing conditions are optimal. For more information on sampling water and growing media for nutrient analysis refer to the 'Sampling for Analysis' nursery paper, September 2011. Relatively inexpensive commercial EC and pH meters are readily available through many scientific equipment suppliers.

Weeds

In simple terms, a weed is a plant out of place. Weeds are able to spread rapidly and have unwanted economic, environmental or social impacts. Weeds can be very difficult to identify, and may be confused with plants that are not weeds, including native or endangered species. Sometimes weeds look very different between their juvenile and mature stages.

It is important to correctly identify a weed to ensure that control methods are effective and appropriate. Some factors to consider when identifying a weed are where and when the weed grows, its shape, size, leaf form and flower colour. There are several online tools to help you identify weeds on your property.

The Biosecurity Queensland edition of the Weeds of Australia identification tool and the A-Z listing of weeds help to easily identify a weed based on the features of a particular plant. The tool includes over 1000 current and potential weeds. Once you have confirmed the identity of a weed, you can then access management information. Another Australian Weed identification tool provides a detailed summary of major weeds specific to each regional area of each state and territory.

If you cannot identify the plant using online tools or weed identification publications, you can send a sample to your state herbarium for analysis (this usually incurs a fee – check their website for details). Their websites provide information on collecting and preparing weed specimens for identification. Information or the ability to submit plants or photos of weeds is often available through your state department of agriculture or primary. For more information from local community groups refer to the National Landcare Directory.

Packaging considerations

Regardless of the type of material you are sending, be it plants for pest and disease diagnosis, weed or insect identification, water or growing media, it is important to ensure that your sample gets to its destination in good condition (Fig. 3). Samples that become crushed, overheated or stay in transit for long periods can become too degraded for analysis. For this

reason it is recommended to use express post or overnight couriers whenever possible. Wrap plant material in paper towels or newspaper, which can be lightly dampened to prevent desiccation (important for seedlings). If containerised plants are being posted, wrap containers so that growing media does not contaminate the entire sample (Fig. 4). Provide adequate support so that plants cannot move or become damaged if tipped. Alternatively, bare root plants (bag roots if significant amounts of growing media cannot be removed easily) and provide about 500g of growing media bagged separately within the package. For seedlings and delicate plants, it is recommended to package plants in a box to prevent squash damage. Remember to pack with enough padding so that plants do not move around.

For insect or mite pests, send in whole plants (though roots may not be required for above ground pests). It is often important to include growing tips as damage often occurs in this region, even if it is not evident until leaves grow-out. Place samples in a sealed plastic bag or unbreakable container. Sending samples in ethanol or other preservative is not recommended due to current regulations associated with posting dangerous goods; refer to your diagnostic service provider if this is required.

Label each sample clearly with a waterproof marker or with pencil and paper within each bag. Most diagnostic laboratories require a sample submission form to be submitted with the sample. If this form is not submitted, samples will either be delayed or not completed at all. Each laboratory is likely to have slightly different guidelines, refer to your service provider for more detail.

Finally, ensure that you use the correct address. Incorrect addresses can result in samples going missing for days or

Fig.3. Begonias delivered to diagnostic service provider in plastic bags for disease diagnosis. Such a sample would require hand delivery or be packaged in a box such that plants could not move or be damaged in transit.

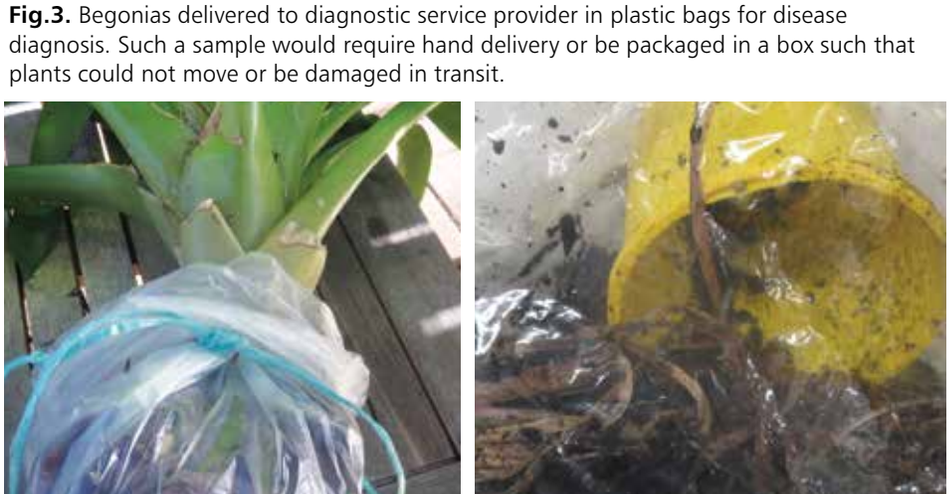

Fig.4. Plants should be packaged to preserve the current state of the plant (left), not so it can fall out of the pot and become contaminated with soil (right).

even weeks, particularly if the diagnostic laboratory is part of a large, multi-organisational facility. In many cases this can result in a sample becoming too degraded for analysis. When in doubt, contact your diagnostic service provider.

Diagnostic services

Diagnostic samples can be sent to your local Department of Agriculture, Primary Industries or Biosecurity branch and sometimes to your state herbarium. Private consultants are also available. Each service is slightly different, offering different tests and with different costs. In addition, as part of a nursery levy funded project, Grow Help Australia provides contract rates to all nursery producers.

References

Grow Help Australia http://www.daff.qld.gov.au/plants/health-pests-diseases/grow-help
Weed Identification tool http://www.weeds.org.au/weedident.htm
Nursery Paper September 2011 Sampling for Analysis

Further Information

Refer to your State Departments of Primary Industries, Biosecurity Authorities or Herbariums

Compiled and edited by Chris O'Connor NGIA Technical and Policy Officer; banner photography by Anthony Tesselaar.