In-Field Rapid, Portable and Cost Effective Plant Disease Diagnostics©

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INTRODUCTION

The Nursery & Garden Industry Queensland (NGIQ) has identified a plant disease diagnostic product developed by United Kingdom (U.K.) based company, Forsite Diagnostics, that is portable, cost effective, accurate, rapid, and reliable (96% correlation to laboratory based diagnosis). In this paper Queensland Industry Development Manager John McDonald outlines the technology behind and the application of the Pocket® Diagnostic™ test kits relevant to the Australian horticultural industry (Fig. 1).

Fig. 1. The Pocket® Diagnostic™ test kit.

POCKET DIAGNOSTIC™ TEST KIT

The Pocket Diagnostic test kit is a lateral flow device (LFD), also referred to as immunochromatographic strip test that applies a genus or species specific antisera/antibody used for detecting a range of plant pathogens (each test is relevant to one organism either by genus or species and is one time use only). The Pocket Diagnostic test kit is robust and simple to use and gives a result in approximately 3-10 min at a cost of between $10 and $20 per test (approximately 10-20% of the cost for current laboratory based testing). Pocket Diagnostic test kits are available for the following pathogens (Table 1).

Table 1. Pocket Diagnostic™ test kits available for detecting the following pathogens.

Beet virus (two strains)	Potato virus (five strains)
Botrytis (Genus)	*Pythium* (Genus)
Cucumber mosaic virus	*Ralstonia solanacearum*
Erwinia amylovora	*Rhizoctonia* (Genus)
Impatiens necrotic spot virus	Tomato mosaic virus
Orchid virus screen	Tomato spotted wilt virus
Phytophthora (Genus)	*Xanthomonas hortorum*
Plum pox virus	

The published paper in the international journal; *Plant Pathology*, Charles Lane of the Central Science Laboratory (CSL) (Lane et al., 2007) described the testing/evaluation of the LFD Pocket Diagnostic test kit for *Phytophthora* spp. in the U.K. during 2006/2007. CSL (Food & Environment Research Agency-FERA), the body that provides the U.K. government with all of its plant health technical and diagnostic support, tested the kits in its national surveillance for *P. ramorum* and *P. kernoviae*. The results of the trial demonstrated a typical sensitivity and specificity of the test to be between 85-90%. This could also be significantly enhanced to between 95-99% through user training and experience in reading the results, sample selection, and extraction.

DISEASE IMPACTS ON INDUSTRY

Plant diseases (bacterial, viral, fungal, etc.) have a significant cost impact on the production of greenlife across the nursery industry in Australia. The overall cost of plant diseases runs into tens of millions of dollars per year due to crop losses, market access restrictions, crop treatment and industry biosecurity inputs such as labour, equipment and infrastructure. The cost to Australian plant industries of one disease alone (*Phytophthora* spp.) is estimated to be more than $250 million per annum (HAL Report: NY00018) and the World Wide Fund for Nature Australia (2004) forecast that *P. cinnamomi* is likely to cause economic costs of approximately $1.6 billion nationwide over the next 10 years. Furthermore there are the as yet incalculable economic and environmental costs associated with the potential incursions of exotic plant diseases such as *Puccinia psidii* (eucalyptus/guava rust) and *P. ramorum* (sudden oak death) however the recent (2010) incursion of myrtle rust into New South Wales and Queensland is likely to cost more than $18 million per annum.

The management of plant pathogens through prevention, detection, mitigation and remedial activities are resource intense (human and economic) and require a significant skills base to be effective across Australian plant industries. As industry accepts a greater participation and responsibility within the national biosecurity continuum, systems and tools that support industry in the proof of absence or early detection of significant plant pathogens will aid in the overall biosecurity surveillance strategy. A single factor that enhances the effectiveness of the above is the early detection and diagnoses of the relevant pathogen leading to the timely application of the appropriate management strategy.

Diagnostic tools currently used within the horticultural industry are focused on laboratory based processes managed by state government departments of Primary Industries/Agriculture. In most states/territories the nursery industry has had access to either fee for service or free government diagnostic services for many years. Based on the general government policy of “user pays” it is unlikely that free diagnostic services will remain available in the short term.

The current cost structure of disease diagnostic services varies across Australia however an average pathogen diagnostic process has a cost of approximately $100-$150 per sample and is likely to take between 2 to 14 days to provide a result depending on the sample quality, pathogenic organism and laboratory capacity. The limited number of diagnostic facilities, high cost and extended diagnostic timelines have been a significant impediment to growers for the broad scale uptake of pre-emptive crop testing, monitoring and infected crop diagnostic services. The Queensland Nursery Industry Accreditation Scheme Australia (NIASA) Committee alone invests approximately $11,000 per annum in laboratory based diagnostic services underpinning the NIASA program in the state.

The misdiagnoses of plant diseases by growers (due to in-house “diagnoses”) has resulted in high crop losses and disease spread through poor disease management strategies, overuse/incorrect selection of plant protection products (e.g., fungicides) and inoculated plant products dispatched to end users. The plant industries in Australia need access to a reliable, rapid, portable, cost effective and accurate plant disease diagnostic tool that can be applied by industry technical support and grower’s on-farm.

Technology behind the Pocket Diagnostic Test Kits and the LFD

The technology that sits behind the Pocket Diagnostic™ Test Kits and the LFD is based on a pathogen specific antisera/antibody binding to an identified pathogen specific protein (antigen). The pathogen specific antibody is impregnated within the membrane at two locations, one at the point of sample input and one further along the membrane at the "positive" (T Line) site in the result window on the membrane cassette. The pathogen protein is attracted to the antibody and binds to this particle which is bound to blue latex beads. As the solution moves along the membrane carrying the antibody/protein/latex sandwich it encounters a strip of antibody/latex impregnated membrane at the test result site (T line) and binds further to these particles and gives a blue line as a positive test result. The more of the pathogen protein in the sample the "stronger" the blue positive line (T line) appears.

If the sample does not contain the pathogen specific protein the blue latex beads do not move down the membrane therefore no positive blue line appears resulting in a negative test response. To ensure confidence in the test an inert antibody moves within the solution to bind on a second test site (C Line) to show that the membrane is functional. If the control line (C Line) fails to turn blue it can be assumed that the test has failed and needs to be undertaken again with a new test kit.

The LFD consists of a number of membrane based materials bound together in the manufacturing process and utilises capillary action to draw the sample along the membrane strip. The diagram below demonstrates the basic design of a typical LFD.

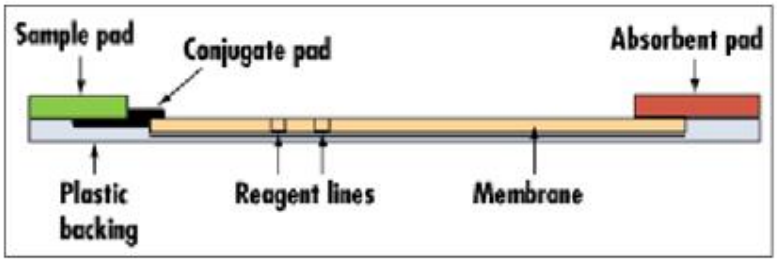

Fig. 2. Diagramatic representation of the Pocket® Diagnostic™ test kit (Source: IVD Technology).

The standard pocket diagnostic test kit consists of:

- A LFD membrane cassette.
- A small bottle containing ball bearings in a buffer solution.
- A simple pipette.

Undertaking a test involves:

- The plant tissue (washed) to be tested is cut into small pieces (a total of no more than 2.5 cm²) and placed into the buffer solution/ball bearing bottle and shaken for a period of between 30 sec and 1 min.
- The ball bearings are designed to smash the plant tissue apart and allow the buffer solution to extract the specific plant pathogen protein which will be suspended within the solution.
- Using the pipette a small amount of solution is withdrawn and 2-3 drops are placed on the membrane device (cassette).
- The liquid moves along the membrane and if the T line and C line turn blue this indicates a positive result. If the C line only turns blue this indicates a negative result, likewise if the T line only turns blue without the C line (re-test).
- Allow no more than 10 min. to attain a result, any blue lines appearing after this period are ignored.

The integrity of the test and ensuring the kit has the best opportunity to deliver the correct result a number of parameters need to be observed and operational considerations applied. These include:

- Too much plant material in the buffer bottle can increase the viscosity of the solution and therefore fail to adequately travel along the membrane (total plant volume should not exceed 2.5 cm^2).
- Excessive organic material in the solution can block the membrane resulting in a failed test.
- Wash all plant material prior to immersion in the buffer bottle.
- Succulent foliage should be shaken (buffer bottle) for 30 sec whereas tougher plant tissue, stems and roots, should be shaken for approximately 1 min.
- Too much solution applied to the cassette will dilute and spill off the membrane and generally give a false negative (2-3 drops only).
- The tissue sample must contain the pathogen therefore an understanding of how pathogens infest a host is useful information in selecting plant tissue for testing.

The Pocket Diagnostic™ Test Kits are capable of detecting plant pathogens from plant material, water and growing media using similar processes to those required for laboratory based diagnoses.

This includes:

- Plant material that has detectable levels of the pathogen (Fig. 3). Unlike baiting or plating in laboratories the kits do not rely on "live" pathogens to return a positive due to the detection of a pathogen specific protein/antigen. All plant material can be used in tests including herbaceous material, woody stems/bark and plant roots.
- Growing media/soil can be assessed (for *Phytophthora* or *Pythium*) through the standard baiting process using the foliage of a likely host variety [e.g., azalea (*Rhododendron* spp.), *Citrus* spp., or Queensland umbrella tree (*Schefflera actinophylla*)] suspended in a diluted sample of the growing media/soil in distilled water. Perforated vegetative plant material is allowed to float in the sample for 48-72 hours and tested using the kit as per normal.

Water sources/drains can be tested (for *Phytophthora* or *Pythium*) through the standard baiting process using the perforated foliage of a likely host type [e.g., azalea (*Rhododendron* spp.), *Citrus* spp., or Queensland umbrella tree (*Schefflera actinophylla*)] suspended in the water for a period of 48-72 h. Upon removal from the water source the vegetative material is processed using the kit as per normal.

UTILISING POCKET DIAGNOSTIC TEST KITS

There is a great potential for the Pocket Diagnostic test kits to serve the Australian horticultural industry on a range of levels including:

Grower's In-Field

- Monitoring of imported greenlife (starter stock plus stock for on-growing).
- Monitoring of "at risk" stock during the growing cycle.
- General crop monitoring at farm level.
- Dispatch monitoring of stock leaving the production nursery.
- Risk management of water and growing media.
- Dispute resolution.

Service Providers

- BMP program technical officers and auditors.
- Private consulting technical officers.
- Pest and disease scouts.
- Government biosecurity surveillance.
- Private and institutional diagnostic laboratories.

Fig. 3. Using the Pocket® Diagnostic™ test kit.

Note: NGIQ has negotiated a "Dealership" agreement with Forsite Diagnostics for the distribution of the various Pocket Diagnostic™ test kits within Australia. Contact NGIQ for an order form on email: nido@ngiq.asn.au.

Literature Cited

Lane, C.R., Hobden, E., Walker, L., Barton, V.C., Inman, A.J., Hughes, K.J.D., Swan, H., Colyer, A. and Barker, I. 2007. Evaluation of a rapid diagnostic field test kit for identification of *Phytophthora* species, including *P. ramorum* and *P. kernoviae* at the point of inspection. Plant Pathol. 56:828-835.