Evaluation of a Rapid Diagnostic Field Test Kit for Identification of *Phytophthora ramorum*, *P. kernoviae* and Other *Phytophthora* Species at the Point of Inspection[1]

C.R. Lane,[2] E. Hobden,[3] L. Laurenson,[4] V.C. Barton,[5] K.J.D. Hughes,[6] H. Swan,[7] N. Boonham,[8] and A.J. Inman[9]

Abstract

Plant health regulations to prevent the introduction and spread of *Phytophthora ramorum* and *P. kernoviae* require rapid, cost effective diagnostic methods for screening large numbers of plant samples at the time of inspection. Current on-site techniques require expensive equipment, considerable expertise and are not suited for plant health inspectors. Therefore, an extensive evaluation of a commercially available lateral flow device (LFD) for *Phytophthora* species was performed involving four separate trials and 634 samples. The assay proved simple to use, provided results in a few minutes and on every occasion a control line reacted positively confirming the validity of the test. LFD results were compared to those from testing a parallel sample, using laboratory methods (isolation and real-time PCR). The diagnostic sensitivity of the LFD (87.6 percent) compared favourably to the standard laboratory methods although the diagnostic specificity was not as stringent (82.9 percent). There were a small number of false negatives, but for statutory purposes where all positive samples must be identified to species level by laboratory testing, overall efficiency was 95.6 percent as compared to visual assessment of symptoms of between 20-30 percent for *P. ramorum* and *P. kernoviae*. This work demonstrates the value of the lateral flow device for diagnosing *Phytophthora* species at the time of inspection and as a useful primary screen for selecting samples for laboratory testing to determine the species identification.

Key words: *Phytophthora ramorum*, *P. kernoviae*, lateral flow device.

[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

[2] Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK. c.lane@csl.gov.uk.
[3] Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK. e.hobden@csl.gov.uk.
[4] Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK. l.laurenson@csl.gov.uk.
[5] Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK. v.barton@csl.gov.uk.
[6] Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK k.hughes@csl.gov.uk.
[7] Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK. h.swan@forsitediagnostics.com.
[8] Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK. n.boonham@csl.gov.uk.
[9] Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK. alan.inman@defra.gsi.gov.uk.

Materials and Methods

Lateral Flow Device

Phytophthora LFD kits designed to recognise all species of *Phytophthora,* including *P. ramorum* and *P. kernoviae,* were supplied by Forsite Diagnostics Ltd, York, UK. Detailed instructions on LFD use were supplied. In summary, several small pieces of leaf showing symptoms were broken up between the thumb and fingers before placed in a plastic bottle containing 5 small (approximately 3mm) ball bearings and extraction buffer. Pieces of suspected diseased tissue transfer into the extraction bottle. The bottle was shaken vigorously for 60 seconds and then the extract taken up in a small disposable dropper. Two to four drops were placed onto an absorbent pad within the kit and left for at least 2 minutes but no longer than 10 minutes before reading. A single blue line developed to indicate the test kit was working (control line) whilst the development of a second blue (target line) indicated the presence of *Phytophthora* spp. A larger sample from the same part of the plant with identical symptoms was submitted for laboratory testing according to a protocol developed at CSL and now part of the EPPO Diagnostic protocol (Anonymous, 2006).

LFD Sensitivity

The sensitivity of the LFDs was evaluated using naturally infected rhododendron leaves submitted as part of routine plant health surveillance and previously tested as positive for *P. ramorum* by isolation and real-time PCR. A small square of necrotic tissue (approx. 12 x 12 mm) was excised from the leaf the wet weight determined. It was then dissected further into smaller portions, the wet weights determined and then tested with an LFD. A similar piece of known healthy leaf tissue (12 x 12 mm) was tested in addition to neat buffer solution. The presence of a test line was visually scored after 5 minutes in addition to quantification of the intensity of the test using an optical reader (Chromatoreader Type 2, Otsuka Electronics Co., Japan). Using the optical reader a negative result is recorded as zero. Lines may be visible at between optical reader values of 4-10, but are easily seen in excess of 15 and may rise to in excess of 100. The experiment was repeated with three unrelated samples.

LFD Specificity

The specificity of the LFD was evaluated using a range of cultures. A small piece of agar (1 cm^2) was excised from the centre of the colony, placed in an extraction bottle, shaken vigorously for 10-15 seconds and then tested with a LFD as described above. Devices were read after 5 minutes and scored visually as either negative or positive.

Comparative Testing

A large number of plant samples submitted by Defra's Plant Health Inspectors were tested using both the LFD and conventional methods as described above. The type and host distribution of samples tested during this trial was representative of material submitted during the UK national survey for *P. ramorum* although the majority of samples tested were rhododendrons.

Results

Sensitivity

A positive reaction was clearly obtained with just a few mg of necrotic rhododendron leaf tissue (equivalent to a few square millimetres) either alone or when mixed with healthy leaf tissue permitting detection in leaf tissue which was less than 1 percent infected by *P. ramorum*.

Specificity

The negative control (agar plug), true fungi (*Alternaria alternata*, *Botrytis cinerea*, *Cylindrocarpon* sp., *Monilinia laxa*, *Pleospora herbarum*, *Trichoderma harzianum*, *Rhizopus* sp.) and isolates of the oomycete *Pythium* also all tested negative. All 13 species of *Phytophthora,* including *P. ramorum* and *P. kernoviae*, tested positive.

Comparative Testing

A total of 634 samples were tested with agreement on 536 occasions (84.5 percent). False positives and negatives were encountered on 70 (11.0 percent) and 28 (4.4 percent) occasions respectively. The diagnostic sensitivity was 87.6 percent and the diagnostic specificity was 82.9 percent (Table 1).

Table 1—Comparison of the Lateral Flow Device with existing diagnostic methods for detection of *Phytophthora* illustrating diagnostic sensitivity ($\frac{A}{A+C}$) and specificity ($\frac{D}{D+B}$)

		Isolation		
		+	-	Total
LFD	+	197 (*A*)	70 (*B*)	267
	-	28 (*C*)	339 (*D*)	367
	Total	225	409	634

Notes

A LFD and comparative both positive;
D LFD and comparative test both negative;
B LFD positive but comparative test negative (false positive);
C LFD negative but comparative test positive (false negative).

Diagnostic sensitivity = 87.60% and Diagnostic specificity = 82.9% .

Discussion

A commercially available lateral flow device for *Phytophthora* (Forsite Diagnostics Ltd, York) identified the presence of *P. ramorum, P. kernoviae* and other *Phytophthora* species on a wide range of plant material as part of plant health inspection and disease management work. The assay was demonstrated to identify a broad range of *Phytophthora* species and did not cross react with other true or lower

fungi. The LFD was shown to be very sensitive and able to detect *P. ramorum* in less than 1 percent infected rhododendron leaf tissue. The assay was simple to use, provided results in 3-5 minutes and on every occasion a control line appeared confirming the validity of the test. LFD results were compared to those from testing a parallel, but not always identical, sample using well-established laboratory methods. For *P. ramorum* this has been extensively evaluated with a diagnostic specificity of 99.3 percent and diagnostic sensitivity of 92.3 percent when isolation was compared to direct real time PCR for a large number of samples (Hughes and others 2006).

These trials demonstrate that LFDs offer a useful decision support tool for the detection of *Phytophthora* spp. at the point of inspection. For statutory purposes, as positive LFD results require laboratory testing to determine the species of *Phytophthora*, the presence of false positives is overcome. Therefore, in this study where all LFD positives were submitted for laboratory testing, an overall efficiency of 95.6 percent was achieved which is a substantial improvement on relying on visual assessment alone. The LFD kits for detecting *Phytophthora* spp. cost from £6 per test so are significantly cheaper than laboratory testing. They have been of considerable value in instructing new plant health inspectors in disease recognition, helping to convince growers and land-owners of the need to sample and hold plants. The simplicity and robustness of these kits makes them ideally suited for all skill levels and their size and weight ideal for varying sites and conditions to obtain a rapid assessment of whether *Phytophthora* may be present. They have the potential to assist plant health organisations manage their disease campaigns in a new way by helping to optimise and target the use of field inspectors, highly skilled diagnostic staff and centralised laboratory services.

Acknowledgments

The authors wish to thank Defra's Plant Health Division for funding this research and Defra's Plant Health and Seeds Inspectorate for their willing assistance in evaluating the LFDs. This work is the coordinated effort of many members of Plant Health Group involved in *P. ramorum* and *P. kernoviae*.

Literature Cited

Anonymous, 2006. EPPO Diagnostic Protocol for regulated pests. EPPO Bulletin 36: 145–155.

Lane, C.R.; Hobden, E.; Walker, L.; Barton, V.C.; Inman, A.J.; Hughes, K.J.D.; Swan, H.; Colyer, A.; 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 Pathology. 56 (5), 828–835. doi:10.1111/j.1365-3059.2007.01615.x.