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Comparison of serological, culture, and bait methods for detection of *Pythium* and *Phytophthora* zoospores in water

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Two immunodiagnostic detection assay procedures were compared with two conventional assays for their sensitivity in detecting propagules of *Pythium ultimum* var. *sporangiiferum*, *Pythium* Group F, *Phytophthora cactorum* and *P. cryptogea* in dilution series in sterile distilled water. The most sensitive assay for all four species was the zoospore trapping immunoassay (ZTI). Conventional membrane filtration-dilution plating gave similar results to ZTI with the two *Phytophthora* spp., but was less sensitive in *Pythium* detection. Immunodiagnostic dipstick assays and conventional bait tests showed similar sensitivities in the dilution series, and were generally about two orders of magnitude less sensitive than ZTI. The four techniques were also compared for their detection efficacy with water samples collected from horticultural nurseries and in *in situ* tests of infected root zones of *Chamaecyparis*, tomato and *Chrysanthemum*. In these comparisons, ZTI was again the most sensitive test for water samples, although membrane filtration-dilution plating proved to be a more consistent test. Dipstick and baiting assays were the best techniques for *in situ* testing, and dipsticks provided epidemiologically valuable, quantitative data on pathogen propagule numbers.

Keywords: detection, *Phytophthora* spp., *Pythium* spp., quantification, waterborne inoculum, zoospore trapping immunoassay

Introduction

Many species of *Pythium*, and the closely related genus *Phytophthora*, are economically important plant pathogens. Diseases caused by both *Pythium* and *Phytophthora* species are generally favoured by wet soil conditions, when their rapid dispersal is often achieved by asexual, flagellate zoospores. Both genera are commonly detected in contaminated irrigation water supplies (Bewley & Buddin, 1921; McIntosh, 1966; Shokes & McCarter, 1979; Pittis & Colhoun, 1984; Pottorff & Panter, 1997) and can rapidly spread in hydroponically grown crops (VanVoorst *et al*., 1987; Stanghellini & Rasmussen, 1994; McPherson *et al*., 1995) or in situations where irrigation water is being recycled (Thomson & Allen, 1974; Braune, 1987; MacDonald *et al*., 1994; Strong *et al*., 1997; Pettitt *et al*., 1998).

For effective control measures to be put in place, it is essential that rapid and reliable detection systems are available. Traditionally, standard detection methods have employed baiting, culture plating, or a combination of both (Pittis & Colhoun, 1984). These techniques take several days, require taxonomic expertise, and are often too slow to assist growers in disease management decisions. Technological advances in antibody-based assays have enabled rapid detection of *Pythium* and *Phytophthora* species in plant tissues (Rittenburg *et al*., 1988; Harrison *et al*., 1990; Lyons & White, 1992; Beckman *et al*., 1994); in soil (Miller *et al*., 1992; White *et al*., 1996; Miller *et al*., 1997); and in water samples (Ali-Shtayeh *et al*., 1991; Cahill & Hardham, 1994; Themann & Werres, 1996; Wakeham *et al*., 1997). Test formats vary, and employ either enzyme-linked immunosorbent assay (ELISA), membrane trapping assay, or a dipstick format. A number of these tests are commercially available as kits for on-site use (e.g. Adgen Ltd, Auchincruive, Ayr, KA6 5HW, UK), and enable growers to obtain results within 10 min of sample collection. However, most of these tests are limited by their inability to differentiate between live and dead propagules of target pathogens. Tests developed by Cahill & Hardham (1994) and Wakeham *et al*. (1997) do detect viable propagules and have commercial potential for on-site use. This paper evaluates these two antibody-based methods, and compares them with more conventional methods of detection and quantification of viable and infective *Pythium* and *Phytophthora* inoculum in horticultural irrigation water.

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Materials and methods

Culture of oomycetes and production of zoospores

One isolate each of four species were used in this study: *Pythium ultimum* var. *sporangiiferum* [Horticulture Research International (HRI) Wellesbourne isolate P40 PUVS1]; *Pythium* Group F (HRI Wellesbourne isolate P40 F); *Phytophthora cactorum* (HRI East Malling isolate EMRS 154); and *P. cryptogea* [International Mycological Institute (now CABI Bioscience) isolate IMI 324217]. Zoospores of *Pythium* and *Phytophthora* spp. were produced by different methods. For *Pythium* zoospores, single 0·5 cm discs of actively growing mycelium were inoculated onto 3% V8 juice agar (White *et al*., 1996) in 9 cm Petri plates. After 7 days' incubation in the dark at 25°C, each plate was flooded with sterile distilled water (SDW) and left at room temperature (18–25°C) on the laboratory bench for 2–3 h. For *Phytophthora* zoospores, young mycelial mats were produced by inoculating 15 mL aliquots of clarified V8 broth (Johnston & Booth, 1983) in 9 cm Petri dishes with plugs of mycelium taken from the edge of actively growing cultures on potato dextrose agar (PDA). After 5–7 days' incubation in the dark at 20°C, mycelial mats were transferred to starvation conditions by decanting the V8 broth and rinsing twice with sterile pond water (SPW). Rinsed mycelial mats were then replaced in their Petri dishes with 15 mL SPW and incubated at 20°C for a further 1–3 days to allow the development of sporangia. Synchronous zoospore release was achieved by chilling cultures containing sufficient sporangia at 4°C for 1 h. Suspensions of zoospores were collected and diluted after a further 2 h at room temperature on the laboratory bench.

Assays for detection of zoospores in water samples

Zoospore trapping immunoassay (ZTI)

Using a Nalgene reusable bottle-top filter unit (Fisons Scientific Equipment, Birmingham, UK), 1 L water samples were filtered through 5 μm cellulose nitrate membranes (Whatman International Ltd, Maidstone, UK). Following filtration, each membrane was incubated overnight in a solution containing 0·07 M glucose and selective antibiotics to encourage germination of viable spores of target species, while inhibiting the activities of other organisms trapped on the filter. When *Pythium* spp. were the target organisms, the antibiotics rifamycin (30 mg L^{-1}) and pimaricin (100 mg L^{-1}) (Sigma Aldrich Ltd, Poole, UK) were used. For *Phytophthora* spp., the modified BNPRA mixture [pimaricin 10 mg L^{-1}, rifamycin 10 mg L^{-1}, ampicillin (Sigma) 500 mg L^{-1}, nystatin (Sigma) 25 mg L^{-1}, pentachloronitrobenzene 25 mg L^{-1}, benomyl 10 mg L^{-1}] was used at the same concentration as used for agar plates (Pettitt & Pegg, 1991). Following overnight incubation, membranes were air-dried and processed by immunoassay, as described by Wakeham *et al*. (1997), using a polyclonal antiserum coded 95/10/2. This antiserum had previously been raised to a soluble mycelial fraction collected from five *Pythium* spp. and characterized for reactivity with a range of other fungal spore types (Wakeham *et al*., 1997).

Dipstick immunoassay

Dipsticks, prepared from a sheet of cellulose nitrate membrane (pore size 0·45 μm) and cut into 10 × 60 mm strips, were precoated with 0·1 M L-aspartic acid (Cahill & Hardham, 1994). The cellulose nitrate strips were attached to 10 × 60 mm strips of acetate sheet with double-sided adhesive tape to provide 'handles', allowing their suspension in water samples from the neck of the sample bottle. Two dipsticks were suspended in each water sample tested, incubated at room temperature overnight (16 h), removed from the water and air-dried. The dipsticks were immunodeveloped using the same procedure as described above for ZTI (Wakeham *et al*., 1997). The ability of the dipstick assay method to detect viable propagules could also be improved by using overnight incubation of dipsticks with glucose and antibiotics, as described above, after removal from water samples.

Conventional plating

Water samples (1 L) were filtered through 5 μm cellulose nitrate membrane filters, as outlined above for ZTI. After filtration, filters were removed from funnels using sterile forceps and cut into small pieces (≈ 5 × 5 mm squares). Filter pieces were placed in universal bottles containing 5 mL resuspension medium, and placed on a flask shaker at medium speed for 5 min. The resuspension medium consisted of 0·09% w/v agar dissolved in SDW (Ali-Shtayeh *et al*., 1991) plus the same rates and types of selective antibiotics as used for spore germination on ZTI membranes, as outlined above. Three replicate 1 mL aliquots of resuspension medium were pipetted and spread onto 9 cm Petri plates of PDA, again amended with the appropriate rates and types of antibiotics as outlined above. Stacked plates were left to dry in a laminar flow hood for approximately 1 h and then placed in an incubator at 25°C for 24–36 h. Once visible colonies formed, colony-forming units (CFU) per plate were counted, and from these counts mean numbers of CFU L^{-1} were calculated.

Conventional baiting assays

Bait tests used either 8 mm diameter rhododendron leaf disks or hemp seeds. Using a sterile cork borer, leaf disks were cut from *Rhododendron ponticum* leaves taken from new flush growth, and cleaned by wiping with 75% ethanol. Washed *Cannabis sativa* seeds were autoclaved in distilled water. Ten baits of each type were placed directly in water samples and incubated overnight (16 h) on the laboratory bench. For *in situ* bait tests, baits were placed in nylon mesh bags for easy retrieval and were left in place for 36 h. After incubation, baits were removed from the water, blotted on sterile tissue paper, and plated on PDA plates supplemented with appropriate antibiotics (see above). In each baiting assay of an individual sample, 10 baits (either leaf disks or seeds) were used, and following 36 h incubation on the laboratory bench, the numbers

of baits with identifiable colonies growing from them were counted and expressed as counts out of these 10 baits.

Detection sensitivity

The detection sensitivity of the four individual procedures was determined and compared by preparing dilution series of freshly prepared zoospore suspensions. A zoospore suspension of each test species was first made up to 1 L with SDW, and its concentration was estimated using haemocytometer counts (counts were carried out on 30 replicate samples for each suspension). From these suspensions a 1 L doubling dilution series was prepared in SDW. Each 1 L dilution in a series was further diluted one in four, giving four replicate 1 L aliquots for each dilution in each series which ran 1 : 8, 1 : 16, 1 : 32, and so on. Each replicate aliquot was used for a different propagule detection assay, providing a comparison between ZTI, membrane filtration conventional plating, baiting and dipstick assays over a range of propagule concentrations for the four pathogen species tested.

Comparison of techniques for testing irrigation water samples

Water samples

Water samples were collected in sterile bottles from irrigation reservoirs, water collection tanks, rain gutters and water treatment installations on a number of UK commercial nurseries. Each 4 L sample was divided into 4 × 1 L subsamples, each of these being tested using one of the four detection assays as described above.

Root zone and run-off samples

Run-off feed solution was collected and tested, in a similar manner to the above, from a hydroponic tomato crop growing in rockwool blocks on a commercial nursery, and from an experimental hydroponic chrysanthemum crop growing in sand and inoculated with *Pythium sylvaticum* at HRI Efford. Run-off water was also collected from *Chamaecyparis lawsoniana* plants inoculated with *Phytophthora cryptogea* and growing in a peat-based medium in 2 L pots, using the technique described by Pettitt *et al*. (1998). Using this approach, a suitable volume for comparative tests was obtained by pooling the water collected from 10 individual pots. In situations where the collection of large volumes of water was inappropriate (e.g. testing the root zone of hydroponic crops or the run-off from small individual pots), *in situ* tests were carried out with plant tissue baits and with dipsticks. Where possible in these tests, smaller volumes of water were taken using a 20 mL syringe for comparisons using ZTI and conventional plating assays.

Results

Zoospore trapping immunoassay was the most sensitive technique for detecting zoospores of all four pathogen species assessed (Table 1). Comparison between ZTI and haemocytometer counts was carried out by regression analysis ($y = -55{\cdot}7 + (0{\cdot}98x)$, $r^2 = 0{\cdot}97$). This showed that ZTI was detecting a large proportion of spores present in dilutions, and that this technique was equally sensitive at appropriate dilutions for the four pathogen species under the aseptic conditions of these *in vitro* studies (Table 1). There was a strong correlation ($r^2 = 0{\cdot}95$) between ZTI counts and the results from membrane filtration-colony plating, which was also very sensitive, and was especially good for the detection and quantification of *Phytophthora cactorum* and *P. cryptogea* zoospores (Table 1). Although less sensitive than membrane filtration colony plating and ZTI, conventional baits and dipstick assays were effective at detecting propagules of *Pythium* Group F and *P. ultimum* over the dilutions tested. However, bait and dipstick assays were much less sensitive in their detection of both *Phytophthora cactorum* and *P. cryptogea* (Table 1). Nevertheless, the quantitative estimates of propagule numbers by dipstick assays were correlated with haemocytometer counts ($r^2 = 0{\cdot}98$), colony plating ($r^2 = 0{\cdot}95$), and ZTI assays ($r^2 = 0{\cdot}97$).

In field tests of the four detection techniques, ZTI was also generally the most sensitive test for water samples, although not readily applicable to root zone tests (Tables 2 and 3). Again, estimates of propagule numbers from ZTI and dilution plating were highly correlated ($r^2 = 0{\cdot}88$). However, membrane filtration dilution plating was occasionally more sensitive than ZTI. For example, in two out of three assessments of irrigation reservoir water, ZTI detected 52 and 137 CFU L^{-1}, dilution plating detected 38 and 88 CFU L^{-1}, while in the third assessment ZTI detected only nine CFU L^{-1} and dilution plating detected 54 CFU L^{-1} (Table 2). Dipstick and bait tests were approximately equivalent to one another in being the least sensitive tests, with the dipsticks giving positive detection in five samples where baits failed, and baits giving positives in three samples where dipsticks failed. Whilst less sensitive, the results from dipstick tests were still correlated with those from ZTI ($r^2 = 0{\cdot}72$) and dilution plating assays ($r^2 = 0{\cdot}63$). Bait tests were the only assays of the four assessed that did not give fully quantitative results. The average percentage sensitivities of the three fully quantitative assays (calculated by expressing the number of CFU detected by each test as a percentage of the maximum detection for each sample) were: 86·9% for ZTI; 75·9% for colony plating; and 19·7% for dipsticks. Colony plating was the most dependable assay, with no 'failed' tests in the comparative field experiments (Table 2). The ZTI failed on only one sample, which was taken from recycled tomato hydroponic feed solution. Samples of recycled feed solution from hydroponic tomato crops also caused problems with some dipstick tests (Tables 2 and 3; unpublished data). These test failures were due to the presence of large quantities of what appeared to be lysed cellular material, which became intensely coloured with the fast red immunostaining procedure used, and tended to obscure all other stained structures (germinated spores) on the membrane. When

Table 1 Detection and quantification of viable zoospores of *Pythium* group F (isolate P4O F), *Pythium ultimum* var. *sporangiiferum* (isolate P4O PUVS1), *Phytophthora cactorum* (isolate EMRS 154), and *Phytophthora cryptogea* (isolate IMI 324217) in sterile distilled water employing ZTI, dipstick, conventional plating and baiting methods

Estimated number of zoospores L^{-1}[a]	ZTI count germlings L^{-1}	Dipstick count germlings L^{-1}	Membrane filtration-dilution plating CFU L^{-1}	Baits	
				Hemp seed	*Rhododendron* leaf disk
Pythium Group F (isolate P4O F)					
$1{\cdot}0 \times 10^{5}$ (4746)	[b]	1764	[b]	10	10
$5{\cdot}0 \times 10^{4}$	[b]	940	[b]	10	10
$2{\cdot}5 \times 10^{4}$	[b]	571	[b]	10	10
$1{\cdot}3 \times 10^{4}$	[b]	32	[b]	10	10
$6{\cdot}3 \times 10^{3}$	[b]	17	[b]	10	10
$3{\cdot}1 \times 10^{3}$	[b]	7	[b]	10	10
$1{\cdot}6 \times 10^{3}$	2072	7	[b]	9	9
$7{\cdot}8 \times 10^{2}$	762	1	48	8	3
$3{\cdot}9 \times 10^{2}$	398	3	14	10	9
$2{\cdot}0 \times 10^{2}$	152	4	11	8	6
Pythium ultimum var. *sporangiiferum* (isolate P4O PUVS1)					
$6{\cdot}7 \times 10^{3}$ (12)	[b]	26	[b]	10	5
$3{\cdot}3 \times 10^{3}$	[b]	12	[b]	10	9
$1{\cdot}7 \times 10^{3}$	[b]	5	[b]	10	8
$8{\cdot}3 \times 10^{2}$	768	2	125	6	9
$4{\cdot}2 \times 10^{2}$	377	3	60	2	1
Phytophthora cactorum (isolate EMRS 154)					
$1{\cdot}3 \times 10^{4}$ (210)	[c]	0	[b]		10
$6{\cdot}6 \times 10^{3}$	[c]	5	1062		6
$3{\cdot}3 \times 10^{3}$	[c]	0	613		6
$1{\cdot}6 \times 10^{3}$	[c]	0	200		1
$8{\cdot}2 \times 10^{2}$	[c]	0	147		1
$4{\cdot}1 \times 10^{2}$	70	1	88		0
$2{\cdot}1 \times 10^{2}$	71	0	100		0
$1{\cdot}0 \times 10^{2}$	56	0	45		0
$0{\cdot}5 \times 10^{2}$	26	0	23		0
$0{\cdot}3 \times 10^{2}$	20	0	27		0
Phytophthora cryptogea (isolate IMI 324217)					
$6{\cdot}3 \times 10^{3}$ (13)	[b]	184	[b]		10
$3{\cdot}1 \times 10^{3}$	2844	123	890		7
$1{\cdot}6 \times 10^{3}$	1371	0	413		1
$7{\cdot}8 \times 10^{2}$	501	0	118		1
$3{\cdot}9 \times 10^{2}$	391	1	67		0
$2{\cdot}0 \times 10^{2}$	15	1	67		0
$1{\cdot}0 \times 10^{2}$	64	0	10		0
$0{\cdot}5 \times 10^{2}$	29	0	0		1
$0{\cdot}2 \times 10^{2}$	2	0	2		0
$0{\cdot}1 \times 10^{2}$	7	0	3		0

[a]Values in brackets are standard errors of the mean counts for the initial spore suspensions, $n = 30$.
[b]Zoospores per CFU were too numerous to count.
[c]With faint immunostaining of the zoospore, the overall pink coloration of the membrane made it impossible to determine zoospore numbers.

the potentially anomalous results of tests on samples taken from tomato crops were excluded, the average sensitivities of the three quantitative assays were 94·5% for ZTI; 71·1% for colony plating; and 16·9% for dipsticks.

For *in situ* tests in the root zone of plants otherwise left undisturbed, both ZTI and colony plating assays proved to be inappropriate techniques, as they required the collection of comparatively large water samples that were not reasonably representative of small areas of root zone. Bait tests have often been used in these situations (Ribeiro, 1978; Pettitt *et al*., 1998) and have proven effective in the samples assessed here (Table 3). Baits and dipsticks were broadly comparable in their applicability, and in these root zone tests dipsticks compared favourably with bait tests, giving valuable extra quantitative data on the numbers of propagules (CFU) present over the test periods (Table 3).

Table 2 Comparison of the performance of the two immunodiagnostic tests, ZTI and dipstick tests, with that of conventional baiting and membrane filtration-dilution plating for detection and quantification of propagules of *Pythium* spp. and *Phytophthora* spp. in tests of water samples collected from horticultural nurseries

Source of water sample		ZTI count germlings L^{-1}	Dipstick count germlings L^{-1}	Membrane filtration-dilution plating CFU L^{-1}	Baits (counts out of 10)
Irrigation reservoir[a]		52	4	38	0[f]
Irrigation reservoir[a]		9	4	54	5[f]
Irrigation reservoir[b]		137	61	88	10[f]
Rainwater gutter[b]		22	1	19	0[f]
Rainwater gutter[b]		13	4	10	4[f]
Water storage tank[b]		48	–[i]	33	10[f]
Water storage tank[b]		–[d]	–	277	10[f]
Tomato: recycled feed[a]		4[e]	4	4	0[g]
Tomato: recycled feed[a]		50	3	40	10[g]
Tomato: recycled feed[a]		0[e]	0	38	6[g]
Chrysanthemum[b]		230	18	102	10[h]
Chrysanthemum[b]		112	5	96	10[h]
Chrysanthemum[b]		58	0	43	2[h]
Chamaecyparis[a]		150	58	106	6[f]
Chamaecyparis[a]		381	75	249	10[f]
Chamaecyparis[a]		125	41	66	8[f]
Slow sand filter (HNS)	In[c]	301	24	187	10[f]
	Out[a]	0	0	0	0[f]
Slow sand filter (tomatoes)	In[a]	50[e]	3[e]	147	0[f]
	Out[a]	0[e]	0	0	0[f]
UV treatment (tomatoes)	In[a]	80	22	50	10[f]
	Out[a]	0	0	0	0[f]

[a]Tests were for *Phytophthora* spp. (SCRI PAb, kindly supplied by Dr J. Harrison).
[b]Tests were for *Pythium* spp. (PAb 95/10/2; counting only intensely stained germlings).
[c]Tests were for Pythiacae (PAb 95/10/2; counting all germlings).
[d]Germlings were too numerous to count accurately.
[e]Lysed material on membrane confusing the count.
[f]*Rhododendron* leaf disks.
[g]Tomato leaf disks.
[h]*Chrysanthemum* leaf disks.
[i]Not tested.

Discussion

Baiting, culture plating, or a combination of both are the current standard methods of detection for viable propagules of both *Pythium* and *Phytophthora* species (Pittis & Colhoun, 1984; MacDonald *et al*., 1990). However, technological advances in fungal diagnostics, using either antibody or nucleic acid probes, offer potential for the development of rapid systems to detect and quantify target inoculum of fungal plant pathogens. (Dewey & Thornton, 1995; Beck *et al*., 1996; Kennedy *et al*., 2000; Postma *et al*., 2001). Immunoassays are being increasingly exploited in the development of diagnostic kits because of their speed, simplicity, relatively low cost, and the ability to perform on-site semiquantitative assays (Danks & Barker, 2000). The antibody-based dipstick proved comparable to the conventional plant tissue baits in sensitivity for detection of both *Pythium* and *Phytophthora* spp., and shows potential for *in situ* testing in restricted sampling sites. For example, testing infectivity in localized areas, such as different positions in a channel of a hydroponics system or within a root system. In such tests, dipstick assays had the advantage of giving quantitative estimates of the numbers of propagules present, while bait tests were more economic, relatively easy to carry out in large numbers, and took less time per sample. However, of all the tests both ZTI and colony plating proved most sensitive for the quantitative measurement of viable propagules of *Pythium* and *Phytophthora* species. In a previous study, ELISA was employed for the detection of *Phytophthora* and *Pythium* spp. in irrigation systems (Ali-Shtayeh *et al*., 1991), it was not possible to differentiate between viable and nonviable inoculum. The fact that ZTI provides a direct assay of the number of actual viable propagules, as opposed to just a concentration of antigen present (whether viable or nonviable), has provided a useful tool for demonstrating the efficacy of both slow sand filtration and ultraviolet treatments for killing oomycete propagules (T.R.P. and M.F.W., unpublished results). However, although achieving faster results than

Table 3 Comparison of the performance of the two immunodiagnostic tests, ZTI and dipstick tests, with that of conventional baiting and membrane filtration-dilution plating for detection and quantification of propagules of *Pythium* spp. and *Phytophthora* spp. in tests of infected root zones, carried out *in situ*

Root-zone sampled	ZTI count germlings L^{-1}	Dipstick count germlings L^{-1}	Membrane filtration-dilution plating CFU L^{-1}	Baits (counts out of 10)
Chamaecyparis[a]	150[e]	61	[f]	10[g]
Chamaecyparis[a]	[f]	34	167[e]	10[g]
Chamaecyparis[a]	[f]	130	[f]	6[g]
Chamaecyparis[a]	[f]	7	[f]	10[g]
Chamaecyparis[a]	[f]	4	[f]	4[g]
Chamaecyparis[a]	[f]	40	[f]	6[g]
Chamaecyparis[a]	[f]	143	[f]	10[g]
Tomato[c]	[f]	10	[f]	0[h]
Tomato[c]	[f]	0[d]	[f]	6[h]
Tomato[c]	[f]	2	[f]	8[h]
Chrysanthemum[b]	[f]	35	[f]	10[i]
Chrysanthemum[b]	[f]	3	[f]	7[i]
Chrysanthemum[b]	[f]	118	[f]	10[i]

[a]Tests were for *Phytophthora* spp. (SCRI PAb, kindly supplied by Dr J. Harrison).
[b]Tests were for *Pythium* spp. (PAb 95/10/2; counting only intensely stained germlings).
[c]Tests were for Pythiacae (PAb 95/10/2; counting all germlings).
[d]Lysed material on membrane confusing the count.
[e]Assay carried out on 20 ml sample.
[f]Sampling not feasible for this assay.
[g]*Rhododendron* leaf disks.
[h]Tomato leaf disks.
[i]*Chrysanthemum* leaf disks.

conventional plating assays, even when the germination step is included and sample numbers are large, employing ZTI may not be as rapid as ELISA. Nevertheless, with the high sensitivity achieved using ZTI, and the ability to differentiate between viable and nonviable spores, modification of the test to incorporate lateral flow immunochromatography (Danks & Barker, 2000) may enable the development of rapid on-site tests for use by nonscientific staff.

As observed in previous work employing both ZTI and dipstick assays (Wakeham *et al*., 1997), the sensitivity and specificity of PAb 95/10/2 to zoospore and mycelial components of *Phytophthora* was less than to similar structures of the *Pythium* species tested. Nevertheless, PAb 95/10/2 proved extremely useful in both specific tests for *Pythium* spp., where only intensely stained germlings were counted (these represented the vast majority of CFU in all the *Pythium* tests reported), and in general tests for pythiacaeous propagules, which were used to test slow sand filtration effluents. For the field tests for *Phytophthora* spp., improved sensitivity was attained using a PAb raised to mycelial fragments of *Phytophthora cryptogea* (supplied by J. Harrison, Scottish Crops Research Institute, Dundee, UK). This may explain the relatively lower sensitivity of ZTI, in comparison with colony plating, against *Phytophthora cryptogea* and *P. cactorum* in the *in vitro* comparisons. Where detection of both fungal species is a requirement of the test, the inclusion of both antisera within the same assay format would be a prerequisite for the detection of *Pythium* and *Phytophthora* spp. in general field studies. Where detection of a specific species is required, the use of monoclonal antibodies would be more appropriate, although opting for a DNA-based method such as squash-blot hybridization (Arganoza & Akins, 1995) or real-time quantitative PCR (Heid *et al*., 1996; Schnerr *et al*., 2001) may provide more reliable specificity. Optimization of the existing ZTI test is required to remove the background interference observed when sampling tomato crop residues, as the stained material present on some membranes made counting very difficult and, while not necessarily affecting counts, reduced confidence in their accuracy (Tables 2 and 3; unpublished routine test data). However, this may simply be a result of endogenous alkaline phosphatase activity. Within the existing immunoassay format, 0·6 mM levamisole is used to block endogenous alkaline phosphatase activity (Wakeham *et al*., 1997). In cell and tissue histology, 85–90% reduction in endogenous alkaline phosphatase activity is achieved (Sigma-Aldrich Ltd, unpublished results). Nevertheless, in hydroponic tomato nutrient material where phosphate levels are maintained at 30 p.p.m., a high level of alkaline phosphatase activity may result. To optimize the assay, an increased level of levamisole may be required. Increased background problems may also be associated with low pH (Sigma-Aldrich Ltd, unpublished results). With the exception of the tomato irrigation samples, where a pH of 5·5 was observed, all others were at a pH of 7·0–7·5. For tomato samples, the inclusion of buffering solution may be required to attain a neutral pH throughout the immunoassay process.

Another potential problem with the practical application of ZTI was seen where levels of debris and silt in water were high, making counts of viable propagules difficult. In these cases, counts may be improved by

prefiltering water samples through fine nylon mesh sieves (25–50 μm). Occasionally, water samples can be contaminated with large quantities of material the same size as, or smaller than, oomycete zoospores; in these cases colony plating would be more appropriate. Nevertheless, depending on the quality of water samples, the ZTI and colony plating techniques both provide a reliable insight into the populations of viable propagules of *Pythium*, *Phytophthora* and other oomycete spp. present in water samples.

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