

Comparison of Serological and Culture Plate Methods for Detecting Species of *Phytophthora*, *Pythium*, and *Rhizoctonia* in Ornamental Plants

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ABSTRACT

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Commercially available ELISA (enzyme-linked immunosorbant assay) kits (in multiwell format) were compared to standard culture plating for their ability to detect *Phytophthora*, *Pythium*, and *Rhizoctonia* in a variety of ornamental plants exhibiting symptoms of root disease. When the pathogens were present in roots at levels that yielded ELISA reactions >0.3 absorbance units above the test threshold, there was good agreement between the two methods, particularly for detection of *Phytophthora* spp. Agreement between methods was less when the pathogens were present at apparent low levels, an effect attributed largely to sampling error. In greenhouse experiments, there was no significant difference between ELISA and culture plating in detection of *P. cryptogea* in inoculated sage roots. When extracts from infected chrysanthemum roots were mixed at various ratios with extracts from healthy roots, *P. cryptogea* could be reliably detected ($P = 0.05$) when the amount of infected tissue in a sample was as low as 1%. While meaningful thresholds still need to be established, the tests appear to be a promising method with which growers could rapidly diagnose disease problems.

There are many root pathogens that cause widespread, chronic, and sometimes severe diseases of container-grown plants. To minimize the potential for disease, many growers apply fungicides to plants at regular intervals as prophylactic treatments. If disease outbreaks do occur, a first course of action is to apply fungicide drenches to arrest spread. However, most fungicides have narrow activity spectra, and their effective use requires knowledge of the specific pathogen(s) being targeted. Because species of *Phytophthora* de Bary., *Pythium* Pringsh., and *Rhizoctonia* DC. can spread rapidly in nurseries, growers cannot risk waiting several days or weeks for a diagnosis. Under pressure for quick action, they typically apply a mixture of fungicides, assuming that at least one of the included materials will provide control. In spite of the expense and inefficiency of this approach, it has become standard practice in the nursery industry. However, recent legislation enacted in California to limit the amount of potentially harmful chemicals in the environment virtually assures this practice will be challenged in the future. Indeed, it may eventually become necessary for growers to justify each fungicide applied

to their crops by documenting the presence of specific target organisms.

With the time and technical expertise required for conventional diagnosis, targeted use of fungicides in nurseries has been a practical impossibility. But recent technological advances in assays utilizing monoclonal antibodies may change this situation. Agri-Diagnostics Associates of Cinnaminson, New Jersey, has developed field-usable antibody kits to detect species of *Phytophthora*, *Pythium*, and *Rhizoctonia*, among others. In their newest format, these kits can provide a diagnosis in as little as 15 min (6). While the antibodies were developed and optimized for diagnosis of soybean and turf diseases, species within these genera are among the most important root pathogens affecting nursery crops (2). If these kits could be used reliably to detect their respective pathogens in nursery crops, they could make targeted use of fungicides a practical control strategy.

The purpose of this research was to determine whether these existing antibody kits could be used to diagnose root diseases of nursery crops. The diversity in plant species, potting mixes, management practices, microbial populations, and many other factors in nursery operations could confound test results and interpretation. Our objective was to compare the antibody tests to standard culture-plate methods for their ability to detect potential pathogens. While we worked with kits to detect all three pathogens, experiments emphasized detection of *Phytophthora* spp. Parts of this work have been reported previously (4).

MATERIALS AND METHODS

Antibodies were obtained from Agri-Diagnostics Associates in a standard ELISA multiwell format. To detect *Phytophthora*, the *Phytophthora* D kits were used. The antibodies in these kits react weakly with *P. cinnamomi* Rands but strongly with other common species of *Phytophthora*. These antibodies react only weakly with some species of *Pythium* and thus provide good differentiation. For *Pythium* spp., we used *Pythium* C kits. These give broad genus-level detection, while reacting only weakly with some *Phytophthora* species. For *Rhizoctonia*, we used *Rhizoctonia* A kits, which react strongly with *R. solani* Kühn and *R. cerealis* Van der Hoeven.

Except where noted, infected tissues were ground up for assay with the use of the abrasive pads (Extrak pads) supplied in the detection kits. After grinding, tissue macerate was washed from the pads by agitation for 10–15 sec on a vortex mixer in test tubes containing 4 ml of the kit-supplied extraction buffer. After extracts of all samples to be tested had been prepared, 100 μ l of each was dispensed into the wells of a microtitre plate. A microplate adapter (Scientific Industries, Inc., Bohemia, NY) on a vortex mixer was used to incubate plates for 10 min at room temperature with continuous shaking. Plates were then washed five times with the kit-supplied wash solution. Enzyme-conjugated antibody (100 μ l) was then added to each well, and the incubation and washing process was repeated. This was followed by adding 100 μ l of substrate to each well and, after 10 min incubation for color development, 50 μ l of stop solution. Absorbance values were determined at 405 nm with a Titertek Multiscan 3100 ELISA plate reader (Flow Laboratories, McLean, VA). Negative controls included in each multiwell plate consisted of both the kit-supplied negative controls and tissue extracts from plants known to be healthy. The absorbance values of healthy plant extracts were used to establish positive-negative thresholds. We used the mean absorbance of healthy controls, plus three times the standard deviation, as the threshold value in all tests (9). Non-sensitized wells were always included to detect possible interference.

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Field trials. To compare culture plate and antibody tests on a variety of plant materials, we visited several commercial nurseries (one in northern California and three in southern California) and collected plants exhibiting symptoms of root disease. At each site, 20–30 individual plants were selected for testing (usually two to three individuals of up to 10 different species). With the exception of tam juniper (*Juniperus sabina* L. ‘tamariscifolia’), which was collected at each nursery, we generally collected different species at the various nurseries. This was done to obtain a broad sampling of common nursery plants to determine whether any interfered with the ELISA tests. While plants from the northern California nursery were returned to our laboratory for examination, those from southern California nurseries were processed in small laboratories at the individual nurseries to assure sample freshness.

Plants were removed from containers and root systems were washed with tap water to expose individual roots. Root segments that appeared necrotic but not

decayed were excised and placed on moist paper towels. If necrosis extended up into plant crowns, chips of crown tissue were also excised. There was a deliberate effort to avoid selection of dead roots near the exterior of the root ball, as we assumed these could have died from heat or desiccation stress and were likely to be colonized by secondary organisms.

The tissue collected from each plant was randomly segregated into two subsamples. One subsample was ground up and the appropriate ELISA tests were used to assay it for the presence of species of *Phytophthora*, *Pythium*, and *Rhizoctonia*. The remaining tissue subsamples were surface-disinfested in a 10% bleach solution and cultured on PVP (10) and PVP (5) to detect *Phytophthora* spp. and *Pythium* spp. and on water agar (WA) to detect *Rhizoctonia* spp.

The agar media were incubated in the dark at ambient temperatures and examined for fungal growth 4 days after initial culturing. If no growth was detected, they were allowed further incubation and rechecked at 2-day intervals for up to 2 wk. *R. solani* was identified directly on WA by characteristic hyphal branching patterns. Colonies of potential *Pythium* spp. and *Phytophthora* spp. recovered from PVP or PVP (10) media were transferred to plates of V-8 juice agar (5). As colonies grew out on the V-8 agar, mycelial disks were cut from colony margins and floated in petri dishes containing nonsterile soil extract (3). Disks were incubated in soil extract for 2–4 days to allow sporangium formation, after which they were chilled briefly in a refrigerator. The mycelial disks were then observed under a light microscope to detect the mode of sporangium germination and to identify the cultures to genus level.

Greenhouse experiments. To more directly compare the detection capabilities of the *Phytophthora* kits, sage (*Salvia officinalis* L.) plants were grown from seed in a greenhouse. At the time of use, plants were approximately 6 mo old and were growing individually in 15-cm-diameter pots containing a peat-sand potting mix. To obtain a range of infection severities, plants were inoculated with varying numbers of zoospores of *P. cryptogea* Pethybr. and Lafferty. Zoospores were obtained by placing mycelial disks in soil extract for 4 days and then chilling the extract for 1 hr in a refrigerator. Zoospore concentrations were determined with a hemacytometer, and plants were inoculated by pipetting measured amounts of inoculum onto the soil surface. Inoculum levels were 0, 1×10^2 , 1×10^3 , 1×10^4 , 1×10^5 , and 1×10^6 zoospores per pot, and there were five plants per inoculum level. The potting mix in containers was saturated before adding inoculum and was maintained at saturation for 4 hr after inocu-

lation. Thereafter, plants were watered normally on a daily schedule.

Root samples were collected from all plants at 3, 6, 8, and 11 wk after inoculation by lifting each plant from its pot and cutting a full-depth, pie-shaped wedge from the root ball. After removal of the root sample, plants were placed back in their pots, and fresh potting medium was added to fill the void in the root ball. A pot label was inserted in the sampled zone so it could be located and avoided in subsequent samplings. Each root sample was processed individually for pathogen detection by washing with tap water to remove the potting medium and placing the recovered roots on moist paper towels. Root segments with apparent lesions were excised from the mass and assigned to one of two subsamples. Those in one subsample were ground up and assayed with the antibody kits, while 10 1-cm-long segments from the other subsample were surface-disinfested and placed on PVP medium.

The positive-negative threshold for the ELISA test was determined from absorbance values of healthy-appearing roots recovered from uninoculated plants. In culture plate tests, any detectable growth of *P. cryptogea* from any tissue piece was considered a positive test for the sample.

The entire experiment was repeated once without modification and twice more with modification. The modification consisted of drenching plants with 50 ml of a metalaxyl solution (37.5 mg a.i./liter) 3 wk after inoculation. This was done in an effort to slow disease progression and plant mortality by inhibiting secondary inoculum cycles in the pots. Sampling intervals and root processing were done as described previously.

The objective of these four inoculation trials was simply to provide groups of plants with a range of infection severity for test comparison. The numbers of root samples testing positive or negative by ELISA were compared to culture plate results by means of a chi-square test (8).

Detection sensitivity. To determine the lowest level of root infection that could reliably be detected by the *Phytophthora* antibody tests, we performed a series of tissue dilution experiments. Chrysanthemum cuttings were rooted and grown in aerated half-strength Hoagland’s solution (3). When approximately 2 wk old, several rooted cuttings were inoculated by adding 1×10^6 – 1×10^7 zoospores of *P. cryptogea* into the crocks of nutrient solution. Plants were maintained in the nutrient solution for an additional 5–7 days to allow root lesion development, after which healthy and necrotic roots were excised from uninoculated and inoculated plants, respectively. The healthy and infected root tissues were combined in various ratios to provide root extracts ranging from as high as 10% to as low as 0.07% infected

Table 1. Plant species assayed for the presence of species of *Phytophthora*, *Pythium*, and *Rhizoctonia* by ELISA tests during field trials in southern and northern California

Plant species and cultivar	Plants tested (no.)
<i>Ceanothus</i> L. ‘Concha’	2
<i>Cotoneaster salicifolius</i> Franch.	2
<i>Cotoneaster divaricatus</i> Rehd. & Wils.	2
<i>Cycas revoluta</i> Thunb.	2
<i>Escallonia</i> Mutis ex L. ‘Newport’	6
<i>Fatsyhedera undulata</i>	2
<i>Festuca ovina</i> L. ‘Glaucua’	2
<i>Gardenia jasminoides</i> J. Ellis	6
<i>Grevillea</i> R. Br. ex J. Knight ‘Noellii’	1
<i>Hibiscus rosa-sinensis</i> L. ‘Kona’	6
<i>Hibiscus rosa-sinensis</i> L. ‘Whitwings’	5
<i>Iberis sempervirens</i> L. ‘Snowflake’	2
<i>Ilex crenata</i> Thunb. ‘Helleri’	2
<i>Juniperus chinensis</i> L. ‘Columnaris’	3
<i>Juniperus chinensis</i> L. ‘Gold Coast’	2
<i>Juniperus chinensis</i> L. ‘Hetzi’	2
<i>Juniperus horizontalis</i> Moench ‘Blue Chip’	2
<i>Juniperus procumbens</i> Siebold ex Miq. ‘Nana’	6
<i>Juniperus sabina</i> L. ‘Tamariscifolia’	17
<i>Juniperus scopulorum</i> Sarg. ‘Cologreen’	2
<i>Juniperus scopulorum</i> Sarg. ‘Wichita Blue’	2
<i>Juniperus virginiana</i> L.	3
<i>Pinus thunbergiana</i> Franco	3
<i>Pittosporum tobira</i> (Thunb.) Ait.	6
<i>Podocarpus macrophyllus</i> var. <i>maki</i> (Siebold & Zucc.) Endl.	2
<i>Potentilla</i> L. sp.	3
<i>Punica granatum</i> L. ‘Nana’	2
<i>Rhododendron</i> L. sp. (azalea)	6
<i>Vinca major</i> L. ^a	1
<i>Viola</i> L. sp. ^a	1

^a Plants were seedlings and because of their small size, samples were a composite of three to four individual seedlings.

tissue. In some experiments, extracts containing 1–10% infected tissue were obtained by measuring out appropriate lengths of healthy and infected tissue before grinding. In all experiments, extracts containing less than 1% of infected tissue (and in some experiments, all tissue dilutions) were prepared by grinding healthy and diseased root tissue separately and mixing aliquots together to obtain the desired dilutions. In all cases, tissues were ground in glass tissue grinders, and a ratio of 10 cm of root tissue to 4 ml of kit-supplied buffer was maintained.

In most experiments, the microplates were incubated for 10 min with constant shaking as described previously. In several experiments, however, incubation times were increased to 20 and 40 min to see if this would increase detection sensitivity. We also did experiments in which severely decayed tissue and tissue collected from the margins of lesions were used in dilutions with healthy tissue. The objective of these experiments was to determine if tissue condition significantly affected detection sensitivity. In these experiments, 40-min incubation times were used.

Each of the tissue dilution experiments were repeated at least two times and, depending upon the amount of tissue available, three to five separate extracts were made for each dilution. The results were analyzed with PC-SAS (SAS Institute, Inc., Cary, NC) and a Dunnett's 1-tailed *T* test (8) in which all tissue dilutions were compared against the negative control (healthy root tissue).

RESULTS

Field tests. Our visits to commercial nurseries enabled us to perform ELISA tests on tissue samples collected from a wide variety of plant materials at different locations and stages of growth (Table 1). Of all the plant species tested, we encountered no cases where tissue extracts interfered significantly with the ELISA reaction.

With all three ELISA tests, there were a small number of plants that tested negative (absorbance values at or below the test threshold) but from which the pathogen was recovered on agar media (Fig. 1). There were also a large number of plants (25–30% of the plants tested) in which low-level absorbance values were obtained (0.01–0.10 units above the cutoff) but from which the pathogen was not recovered on agar media (Fig. 1). Many of these unconfirmed positives for species of *Phytophthora* and *Pythium* (Fig. 1A and B) occurred at one nursery where metalaxyl drenches are used routinely to suppress these pathogens. Fungicide drenches had been applied 3–4 wk before our visit, and these may have reduced the efficiency of fungal recovery on the agar media.

When the amount of antigen in root

tissues was sufficient to cause ELISA reactions with absorbance values >0.3, *Phytophthora* spp. were recovered on agar media from 84% of the plants testing positive by ELISA (Fig. 1A). The agreement between culture plating and ELISA was less for species of *Pythium* and *Rhizoctonia*, where the homologous fungi were cultured and verified in only 29–32% of the cases where plants yielded strong ELISA reactions (Fig. 1B and C).

Although all plants tested in the field trials exhibited symptoms of root disease (stunting, chlorosis, wilt, and root necrosis), we were unable to detect *Phytophthora*, *Pythium*, or *Rhizoctonia* by either ELISA or culture plate methods in almost half of the plants (Fig. 1). This suggests that on many plants, the symptoms were caused by other biotic or abiotic factors. We were able to occasionally associate fungi such as *Fusarium* Link:Fr. spp., *Cylindrocladium* Morg. sp., and *Thielaviopsis* Went. sp., but we did not attempt

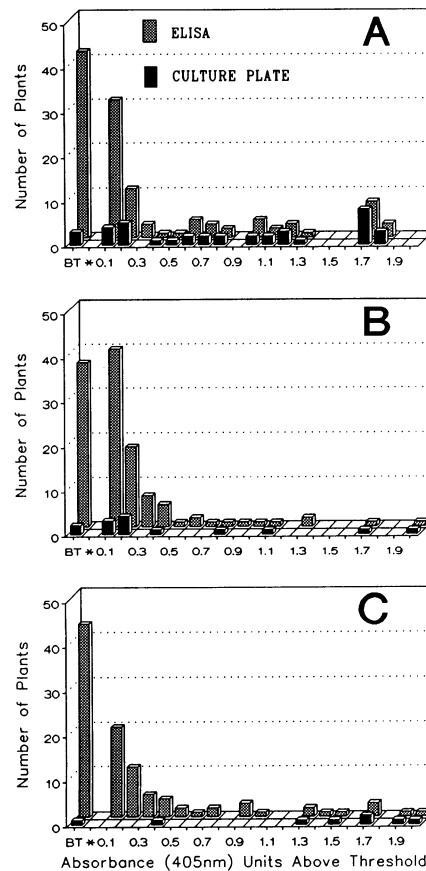


Fig. 1. Comparison of ELISA and culture plate methods for detection of (A) *Phytophthora* spp., (B) *Pythium* spp., and (C) *Rhizoctonia* spp. in nursery plants. Plants tested were those listed in Table 1. Histogram indicates the numbers of plants that gave ELISA reactions of various intensity (in intervals of 0.1 absorbance units). The asterisk on the X-axis indicates the test threshold, and BT indicates plants with ELISA reactions at or below the threshold. Solid bars in foreground indicate the number of plants in each absorbance interval from which the pathogen was recovered on agar media.

pathogenicity tests with any of the fungi we recovered. Such tests were considered impractical because of the large number of fungal isolates involved and the diversity of plant species from which they were cultured (Table 1). Furthermore, the main objectives of these tests were to obtain comparative detection data and determine whether any plant species interfered with the ELISA tests.

Controlled detection experiment. In the four greenhouse experiments, a total of 254 root samples were assayed for *P. cryptogea*. This was less than the potential 400 samples because of severe disease and plant mortality at the highest inoculum levels. In all four trials, there were progressively fewer plants available for sampling at 8 and 11 wk. The metalaxyl treatments applied in the third and fourth trials did not noticeably reduce disease severity or plant mortality. We applied the drenches well after inoculation so they would not inhibit infection, but they were apparently applied too late to slow disease development (1).

Among the inoculated plants, there were some (particularly those exposed to the lowest numbers of zoospores) that never showed symptoms of disease and never gave a positive detection result. This indicates that either many of the inoculations did not result in infection

Table 2. Frequency of detection of *Phytophthora cryptogea* in sage roots by ELISA testing

	Positive	Negative	Total
Observed ^a	118	136	254
Expected ^b	110	144	254

^a Observed values are the numbers testing positive or negative by ELISA.

^b Expected values are those obtained by culture plating on selective media. The chi-square test indicated that ELISA results do not differ significantly from expected results ($\chi^2 = 1.026$).

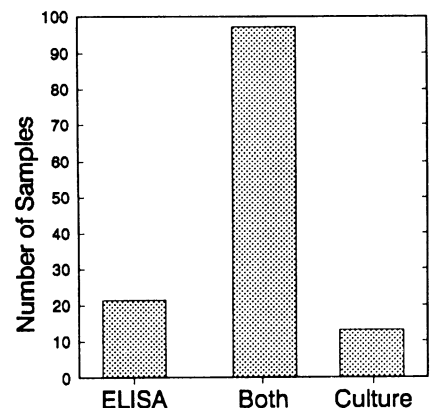


Fig. 2. Comparison of ELISA and culture plate methods for detection of *P. cryptogea* in sage roots. Number of samples that tested positive by ELISA only, culture plating only, or by both methods is indicated. A total of 131 samples (out of 254) tested positive by one or both methods.

or that infection sites were few in number and not easily detected. On the other hand, when disease symptoms (chlorosis and wilt) developed on plants, *P. cryptogea* could always be detected in roots by one or both assay methods.

In these experiments, the ELISA and culture plate assays exhibited similar detection capabilities. Chi-square analysis indicated that the number of samples testing positive or negative by ELISA (observed values) did not differ significantly from the numbers obtained by culture plating (expected values) (Table 2). Culture plate results were used as estimates of expected values in this analysis, because culture plating is the current standard method of detection.

Although the two methods yielded similar numbers of positive detections (Table 2), they did not always detect *P. cryptogea* in the same root samples. The extent of such discrepancy is illustrated in Fig. 2, where the number of samples that tested positive by ELISA only,

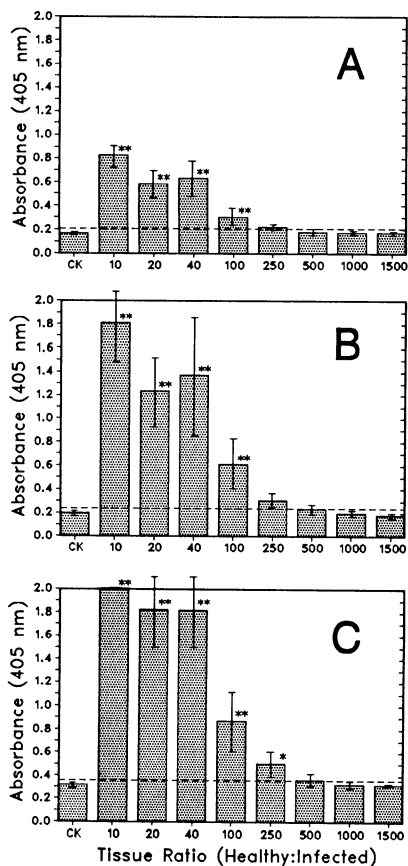


Fig. 3. Sensitivity of ELISA tests in detecting *P. cryptogea* in chrysanthemum root extracts. Healthy and infected root extracts were mixed in various ratios and microtitre plates were incubated for (A) 10 min, (B) 20 min, or (C) 40 min. CK on the X-axis indicates negative controls (extracts from healthy roots), and the horizontal dashed line represents the threshold (the mean absorbance of controls plus 3 SD). Vertical lines indicate ± 1 SD. Dilutions marked with an asterisk indicate absorbance values significantly greater than the threshold (**, $P = 0.05$; *, $P = 0.25$) when compared using Dunnant's 1-tailed *T* test.

culture plate only, or both methods is shown. Of a total of 131 positive detections, the two methods were in exact agreement on 97 samples. ELISA detected *P. cryptogea* in 21 samples missed by culture plating, while the culture plate method yielded 13 detections missed by ELISA (Fig. 2). Disagreement between the methods was most common (21 cases) in root samples originating from plants inoculated with low numbers of zoospores (1×10^2 or 1×10^3) and least common (three cases) in samples from plants inoculated with the highest (1×10^6) numbers of zoospores.

Detection sensitivity. With the hydroponically grown chrysanthemums, it was easy to identify and select freshly infected root tissues for ELISA testing. When various amounts of extracts from roots infected by *P. cryptogea* were added to extracts from healthy chrysanthemum roots, we found that there had to be at least 1% of infected tissue in a sample to yield a significant positive reaction (Fig. 3). While we did obtain some positive test results (where absorbance values exceeded the threshold) in the 1:250 dilution (Fig. 3), the results were variable and not significant at the 95% level. These detection limits were not changed significantly by increasing incubation times from the recommended 10 min to 20 or 40 min. Only if incubation times were extended to 40 min and the confidence level reduced to 75% could the reaction obtained for the 1:250 dilution (0.4% infected tissue) be regarded as significant (Fig. 3C). Thus, in chrysanthemum roots, if 0.4% of the tissue in an extract is infected with *P. cryptogea*, there would be a 75% chance of correctly concluding the roots were infected. The chance of a correct determination increases to 95% if the amount of infected tissue in a sample increases to 1%.

When infected root tissue was separated into two classes (newly colonized

tissue and older, more severely infected tissue), we found that severely infected tissue gave more intense reactions than newly infected tissue at all dilution factors (Fig. 4). However, there was greater variability with the severely infected tissue, and the differences between newly colonized and older infected tissue at each dilution were not statistically significant. Thus, the lower limit of detection (at 95% confidence) was 1% root infection, regardless of how severely individual tissue pieces were infected.

DISCUSSION

We found good agreement between the ELISA and culture plate methods for detection of *Phytophthora* spp. The two methods exhibited similar detection capability in greenhouse experiments with inoculated plants (Table 2, Fig. 2) and in field trials when there was sufficient antigen present in roots to cause moderate to strong ELISA reactions (Fig. 1A). Agreement was less consistent in field trials in plants having low levels of antigen of *Phytophthora* (Fig. 1A), and in tests with species of *Pythium* and *Rhizoctonia* (Fig. 1B and C).

In cases where the methods did not agree (Fig. 1), we recognize several factors that may have contributed to the discrepancies. Some discrepancies are likely the result of culturing or taxonomic errors. For example, growth of *Trichoderma* Pers.:Fr. spp. on WA plates sometimes made direct examination for *Rhizoctonia* spp. difficult. In addition, we were not able to positively identify to genus every fungal culture recovered on PVP and PVPH media. Some isolates formed globose or papillate sporangia, but we were not able to induce zoospore release and observe germination. The culture plate results in Fig. 1 reflect only positive identifications.

With all three tests, there were a large number of plants that gave low-level ELISA reactions (0.1–0.2 absorbance units above the threshold) and negative culture plate results (Fig. 1). This could mean that the plants had low-grade infections that were more effectively detected by ELISA than by the culture plate method. Alternatively, it could be an indication that the ELISA tests were detecting non-viable antigen or that our positive-negative threshold was established at too low a value, resulting in many "false positives" (9). The heterogeneity of plant materials involved in the field trials, however, made threshold evaluation from these data difficult. Our experiments with inoculated sage suggest the threshold we have used is appropriate, at least for that species. Shifting the threshold to a higher value would increase (to approximately 10%) the number of plants with low levels of disease that would be erroneously classified as healthy (*data not shown*).

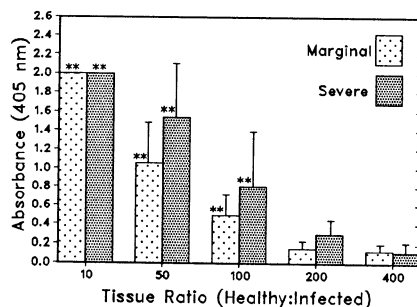


Fig. 4. Effect of tissue condition on the sensitivity of ELISA tests in detecting *P. cryptogea* in chrysanthemum root extracts. Extracts from fully necrotic (severe) tissues or tissues cut from the margins of lesions (marginal) were mixed with extracts from healthy roots in various ratios. The Y-axis indicates absorbance units above the threshold and vertical lines indicate ± 1 SD. Dilutions marked with a double asterisk indicate absorbance values that are significantly greater than the threshold ($P = 0.05$) when compared using Dunnant's 1-tailed *T* test.

Some of the discrepancies in assays for *Pythium* spp., when ELISA absorbance was >0.3 and culture plates were negative (Fig. 1B), actually may have been false positives. Virtually all of these cases involved assays of hibiscus, and the same root extracts yielded very strong reactions (absorbance values >1.0) in the *Phytophthora* kits. Furthermore, *Phytophthora* spp. grew out of all of the root samples cultured on agar media, and no *Pythium* spp. were recovered. We have identified the pathogen associated with hibiscus as *Phytophthora parasitica* Dastur (*unpublished data*), and tests with pure culture extracts showed that it does possess antigens that, when present at high concentrations, cross-react with the antibodies in the *Pythium* C kits. Thus, plants with moderate to severe *P. parasitica* infections (as the hibiscus plants had) could produce false positive reactions with the *Pythium* C kits. If the 11 hibiscus plants are deleted from the data set for *Pythium* spp. (Fig. 1B), there is much greater agreement between the ELISA (at absorbance values >0.3) and culture plate results (62% agreement instead of 29%; *data not shown*).

In addition to the 11 cases where false positives may have clouded the results for *Pythium* spp. (Fig. 1B), there were opportunities for false negatives in the results for *Phytophthora* spp. (Fig. 1A). A large number of the plants included in the field trials were junipers (Table 1), and *P. cinnamomi* is known to infect junipers (7). The antibodies in the *Phytophthora* D kits do not react strongly with extracts from *P. cinnamomi*, and the kits could have failed to detect this species. The newer *Phytophthora* E kits, released by Agri-Diagnostics Associates in June 1989, contain the same antibody used in our field trials, plus an antibody which reacts strongly with *P. cinnamomi*. The E kits were not available at the time this work was done.

While there clearly were cases where false positives and false negatives in the ELISA tests could have contributed to disagreement between the methods, we feel root sampling was a more important factor. In all of the field tests (Fig. 1) and greenhouse experiments (Table 2, Fig. 2), we attempted to recover roots with fresh lesions for pathogen detection. This introduced bias into all root samples, which we felt would maximize detection by both methods. In the greenhouse experiments, however, we could not always discern lesions on plants inoculated with low numbers of zoospores. And in the field trials, we found that the naturally dark root pigmentation of some plants (such as junipers) made it difficult to reliably detect and select infected tissues. Junipers were involved in all cases where *Phytophthora* spp. and

Pythium spp. were recovered on agar media from plants testing negative (below threshold) by ELISA (Fig. 1A and B).

There also was opportunity for error when each root sample was subsampled for separate ELISA testing and culture plating. Separate tissue samples were required because the fungi were killed in the process of grinding roots for the ELISA. Hence, ELISA testing and culture plating could never utilize the exact same tissue pieces. Because of this, the terms "false positive" and "false negative" (which imply immunochemical errors rather than sampling errors) are inappropriate to describe many of the discrepancies between the methods. We have used the terms "unconfirmed positive" (or negative) to describe cases where lack of agreement could have resulted from sampling error.

The opportunity for sampling error in these comparisons is revealed in the tissue dilution experiments (Fig. 3), wherein the ELISA test was able to detect (at low probability) as little as 0.4% tissue infected by *P. cryptogea* in a sample of chrysanthemum roots. This is equivalent to a 1-mm lesion on 25 cm of root tissue and suggests that sampling error is a real possibility with low-grade infections. Indeed, most (65%) of the disagreement between ELISA and culture plate results in our greenhouse experiments (Fig. 2) involved plants inoculated with relatively low numbers (1×10^2 – 1×10^3) of zoospores.

In our greenhouse experiments, *P. cryptogea* was not detected by either method in 123 of 254 root samples from inoculated plants (Table 2). This implies that many inoculations did not result in infection or that infections remained below the detection thresholds of both methods for the duration of the experiments. Of the 131 samples that tested positive, the ELISA method detected 90% of the total, and culture plating detected 84% (Fig. 2). Exclusive use of either method would have missed 10–16% of the infected plants. The discrepancies revealed in Fig. 2 most likely resulted from subsampling error, but the chi-square analysis (Table 2), indicated that errors were evenly distributed between the two methods.

We do not know at this time how widely the detection limits identified with chrysanthemum (Fig. 3) may apply to other examples. Different *Phytophthora* spp. in different plants may produce differing amounts of the specific antigen detected in these tests. By far, the strongest reactions we have obtained in our work have been with *P. parasitica* in hibiscus. Whether this reflects greater fungal biomass per unit of root or greater antigen production per unit of fungal

biomass is unknown.

Our experiments indicate that, although developed for other purposes, these antibody tests have excellent potential as diagnostic tools for nursery crops. There are, however, many questions that must be resolved before the tests can be effectively used and interpreted by nurserymen. For example, the impact of root sampling strategies on the occurrence of unconfirmed positives and negatives, particularly at low levels of root infection, needs to be more fully explored. In addition, more detailed taxonomic studies need to be done to identify the range of *Phytophthora* spp. and *Pythium* spp. common in nurseries so their reactivity with the antibodies in the kits can be more directly compared. Also, the positive-negative threshold for the kits and how it might be influenced by particular host-pathogen combinations needs to be studied. We are very encouraged by our preliminary findings and feel that as more is learned about the capabilities and limitations of these tests, they will dramatically influence disease management decisions in nurseries.

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