Sensitivity and Clinical Use of *Phytophthora*-Specific Immunoassay Kits

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ABSTRACT

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The sensitivity of a *Phytophthora*-specific immunoassay kit was tested on 17 species of *Phytophthora* collected throughout the world, including 18 isolates each of *P. cinnamomi* and *P. cactorum*. Kits were also used in the diagnosis of plant specimens with symptoms characteristic of *Phytophthora* infection, which were sent to Oregon State University's Plant Disease Clinic. All *Phytophthora* isolates tested produced a positive reaction with the immunoassay kit. The lowest absorbencies relative to other species were obtained from *P. cinnamomi* and *P. megasperma* (originally isolated from cherry). Variation in absorbance was high among isolates of *P. cinnamomi* but low among *P. cactorum*. Clinic samples with typical symptoms of *Phytophthora* infection produced a positive reaction with the immunoassay, as did pure cultures of *Phytophthora* sp. isolated from these samples. Cross-reactions occurred with several *Pythium* spp. isolated from clinic samples and with several specimens infected with *Peronospora* spp. Other samples without typical *Phytophthora* symptoms but associated with other pathogens did not produce a positive reaction with the immunoassay. Cross-reactivity with some *Pythium* spp. made interpretation difficult, but when kit results were combined with field histories and symptomology, the immunoassays proved to be a useful tool in clinical diagnosis.

Additional keywords: blackberry downy mildew

Phytophthora diseases are important on many crops and native plants grown in Oregon (8). Heavy rain during the fall, winter, and spring, combined with poorly-drained soils, is favorable for *Phytophthora* development in western Oregon. Infected plants may collapse during the summer months from the added stresses of heat and lack of rainfall. Many diseased plant samples are submitted to Oregon State University's Plant Disease Clinic during the summer when these severe symptoms develop. These plant samples are generally in a deteriorated condition, which makes isolation or baiting of the pathogen difficult (14).

Recent advances with monoclonal antibody techniques have led to the development of enzyme-linked immunosorbent assays for bacterial and fungal detection (7,10). The technology is available in kits, in which all the materials necessary for a test are contained in a small package. New kits from Agri-Diagnostics (Cinnaminson, NJ) have been developed for a variety of pathogens, including a genus-specific test for *Phytophthora* (11).

Phytophthora type D kits were found to react weakly to *P. cinnamomi* Rands (9). Phytophthora type E kits were developed and evaluated for *P. cinnamomi* on azalea (2). Both previous studies were concerned with early detection prior to extensive symptom development or comparison with more traditional isolation procedures. Recently infected plants are rarely, if ever, sent to the Plant Disease Clinic, especially if aboveground symptoms have not developed. Clinical diagnosticians are asked to determine whether a rotted specimen died from a *Phytophthora* disease or not.

The newer kit E consists of two monoclonal antibodies and has increased sensitivity to *P. cinnamomi* compared to that obtained with kit D (11). The antigen that these antibodies react to is heat-stable and persists within dried and dead infected plants. This makes for an ideal test for the clinic, particularly when diseased plants are in poor condition for *Phytophthora* isolation. Cross-reactivity with other genera of fungi, most notably *Pythium* spp., is also a concern.

Our objectives were to 1) evaluate the cross reactivity of kit E to various genera of fungi, 2) test the sensitivity of this kit to a number of *Phytophthora* species, 3) determine if variation in reactivity occurs between isolates of the same species from a large geographic host range, and 4) determine how these factors could impact the usefulness of these kits as a diagnostic tool in a plant disease clinic.

MATERIALS AND METHODS

Culture collection evaluation. A total of 17 species of *Phytophthora* (Table 1), including 18 isolates each of *P. cinnamomi* (Table 2) and *P. cactorum* (Lebert & Cohn) J. Schröt. (Table 3) collected from throughout the world, were evaluated for reaction to a *Phytophthora*-specific immunoassay (kit E, Agri-Diagnostics). Isolates were grown in liquid glucose-yeast-peptone media (4) for 7–10 days at 19 C. A 20-mg sample of aspirated mycelia was oven-dried at 32 C for 24 hr and then ground in sterile sand with 2 ml of "extract solution" supplied in each kit. The ground mycelial suspension was boiled for 10 min and diluted so that an equivalent of 1 μg dry weight of mycelia (or 2 μg dry weight for all *P. cinnamomi*) was tested. Dilutions were placed into the polystyrene wells, and all subsequent steps were as outlined in the kit protocol. Absorbance of at least two wells in each of two different experiments at 405 nm was compared to an "extract solution" control also ground in sterile sand.

The sensitivity of the kits to *P. sojae* (Kauf. & Gerd.) Han & Max and *P. cinnamomi* were evaluated by loading an equivalent of 0.01–50 μg dry weight of mycelia onto the multiwell assay. Cultures were grown and prepared as described above.

Pure cultures of other fungi, isolated from clinic samples, were evaluated for cross-reactivity to the multiwell assay. Fungi tested included cultures of *Pythium* spp. (18 isolates from diseased plant material representing at least six species), *Fusarium* sp. (one isolate), *Rhizoctonia* sp. (one isolate), *Cylindrocladium* sp. (one isolate), *Cylindrocarpon* sp. (two isolates), *Phomopsis* sp. (one isolate), and *Botrytis cinerea* Pers.: Fr. (two isolates).

Clinic samples. The multiwell assay was used to aid in the diagnosis of diseased plant samples with root or crown rot symptoms sent into Oregon State University's Plant Disease Clinic between September 1989 and May 1991. Other samples with foliar symptoms of *Phytophthora* diseases were also tested. The following data were collected for each specimen: field history, plant symptoms, significant fungi observed microscopically or isolated on selective media, and the resulting immunoassay absorbance at 405 nm of both plant tissue and fungal cultures isolated from diseased plant tissue.

Diseased plant tissue was prepared by grinding pieces of root, crown, or leaf tissue between two small "extract pads" (sheets of abrasive paper) that were provided in the kit. However, the entire root system of small samples, such as seedlings, were rubbed against the larger extract pad instead of having subsamples ground. The pad was rubbed against the

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discolored or cankered area of large samples such as woody ornamentals. All subsequent steps were followed as outlined in kit directions. The time to complete a multiwell assay was greatly reduced by using an eight-well plate washer (model 26300, Corning Glass Works, Corning, NY).

Portions of each diseased plant sample were also plated on a modified PARP media (6): cornmeal agar (17 g/L) amended with ampicillin (250 ppm), rifampicin (10 ppm), and pimaricin (25 ppm) (CARP). All CARP plates were incubated in the dark at 15 C. *Phytophthora* and *Pythium* cultures isolated from diseased plants were then tested on the immunoassay in the same way that plant tissue was tested, except for a 10-min boiling water bath after extract pads had been removed from the extract solution.

Sporangial germination was observed for each isolate to confirm genus identification of *Pythium* spp. or *Phytophthora* spp. A 1-cm plug of the CARP plate culture was placed in 20 ml of pea broth (5) and incubated in the dark at room temperature (approximately 22 C) for 3–5 days. The pea broth was drained off, and the culture was rinsed three times in distilled water and then flooded with 20 ml of soil extract water. Soil extract water was prepared by placing a mixture of soil and water (1:1, v/v) for 24 hr and then passing it through a cheesecloth or paper filter (5).

Several plants with symptoms and signs of downy mildew (caused by *Peronospora* sp.) were tested for cross-reactivity with the multiwell assay. Several leaves of pigweed (*Amaranthus* sp.) with white rust (caused by *Albugo bliti* (Biv.-Bern.) Kuntze) and mycelial fans of *Armillaria mellea* (Vahl:Fr.) P. Kumm. from a Bing cherry sample were also tested.

Table 1. Spectrophotometric absorbance and source of different *Phytophthora* species[a]

Species[b]	A_{405}[c]	SE[d]	Host source	Place of origin	Culture source(s)
P. boehmeriae Sawada (371)	2.95	0.024	*Ficus* sp.	New Guinea	Riverside (P1257) (ATCC 46717)
P. cambivora (Petri) Buisman (150)	2.32	0.025	*Abies procera*	Oregon	P. B. Hamm
P. cinnamomi Rands (362)	0.20	0.027	Unknown	Oregon	R. G. Linderman (P-19)
P. cinnamomi Rands	0.40	0.037	*Araucaria cunninghamii*	Papua New Guinea	Riverside (P3659)
P. citricola Sawada (232)	2.82	0.076	*Abies* sp. (Fraiser fir)	Minnesota	G. C. Adams
P. cryptogea Pethybr. & Lafferty (385)	2.91	0.024	Unknown	Netherlands	C. M. Braiser (P197)
P. drechsleri Tucker (263)	>3	...	*Prunus* sp. (cherry)	California	Riverside (P1813)
P. erythroseptica Pethybr. (452)	2.62	0.034	*Solanum tuberosum*	Great Britain	Riverside (P3451)
P. fragariae C. J. Hickman (431)	>3	...	*Rubus idaeus*	New York	Riverside (P3306)
P. gonapodyides (Petersen) Buisman (266)	2.72	0.007	*Pseudotsuga menziesii*	Oregon	R. G. Pratt (47)
P. ilicis Buddenhagen & R. A. Young (802)	2.97	0.023	*Ilex* sp. (holly)	Oregon	P. B. Hamm
P. lateralis Tucker & Milbrath (643)	2.87	0.024	*Chamaecyparis lausoniana*	Oregon	E. M. Hansen
P. medicaginis Hansen & Maxwell (2)	2.77	0.187	*Medicago sativia*	Washington	Christen (WI)
P. megasperma Drechs. (63)	1.04	0.105	*Prunus* sp. (cherry)	California	W. Wilcox (216S-1)
P. palmivora (E. J. Butler) E. J. Butler (484)	2.17	0.010	Cocoa pod	Jamaica	C. M. Brasier (P78)
P. pseudotsugae P. B. Hamm & E. M. Hans (268)	2.86	0.018	*Pseudotsuga menziesii*	Oregon	R. G. Pratt (WF 7)
P. sojae Kauf. & Gerd. (92)	2.88	0.033	*Glycine max*	Indiana	Lavollete (5-58)
P. syringae (Kleb.) Kleb. (583)	2.97	0.014	*Rhododendron* sp.	Oregon	J. Britt (PC 1193)
P. trifoli Hansen & Maxwell (32)	2.89	0.008	*Trifolium* sp.	Mississippi	R. G. Pratt (105)
P. vignae Puras (811)	2.99	0.006	*Vigna unguiculata*	Sri Lanka	D. Fernando

[a] An equivalent of 1 μg dry weight of mycelium of each isolate was tested on Agri-Diagnostics Phytophthora kit E immunoassay.
[b] Number in parentheses refers to culture collection number of Hansen and Hamm, Oregon State University, Corvallis 97331-2903.
[c] Absorbance at 405 nm wavelength.
[d] SE = Standard error based on at least two wells in each of two different experiments.

Table 2. Spectrophotometric absorbance and source of different isolates of *Phytophthora cactorum* (Lebert & Cohn) J. Schröt.[a]

Culture collection number[b]	A_{405}[c]	SE[d]	Host source	Place of origin	Culture source(s)
222	2.44	0.058	*Malus sylvestris*	Great Britain	Riverside (P3128)
176	1.98	0.058	Soil	New York	W. Wilcox (NY 287)
224	2.81	0.002	*Malus sylvestris*	South Africa	Riverside (P1725) (ATCC 46998)
227	2.99	0.003	*Fragaria* × *ananassa*	Germany	Riverside (P3138)
216	2.54	0.011	*Gypsophila* sp.	Massachusetts	Riverside (P3083)
379	2.86	0.002	*Fragaria* × *ananassa*	Nova Scotia	H. S. Pepin (BC 871)
188	2.25	0.019	*Malus sylvestris*	Quebec	W. Wilcox (NY 195)
615	2.93	0.031	*Daphne* sp.	Oregon	R. G. Linderman (P-35)
220	2.63	0.016	*Malus sylvestris*	Poland	Riverside (P1724) (ATCC 34124)
228	2.75	0.013	*Syringae vulgaris*	California	Riverside (P0714) (ATCC 10091)
203	2.72	0.021	*Abies procera*	Minnesota	G. C. Adams (N^F)
197	2.91	0.004	*Rubus idaeus*	New York	W. Wilcox (NY 327)
381	2.59	0.002	*Prunus persica*	British Columbia	H. S. Pepin (BC 874)
190	2.27	0.0	*Fragaria* × *ananassa*	New York	W. Wilcox (ST-19)
225	2.91	0.006	*Juglans* sp.	California	Riverside (P1354)
219	2.46	0.020	*Pyrus* sp.	Australia	Riverside (P1991)
377	2.80	0.009	Unknown	Scotland	H. S. Pepin (R-21) (BC 842)
348	2.69	0.081	*Pseudotsuga menziesii*	Washington	P. B. Hamm
178	2.30	0.325	*Malus sylvestris*	Missouri	W. Wilcox (P770)
Average overall	2.62	0.062			

[a] An equivalent of 1 μg dry weight of mycelium of each isolate was tested on Agri-Diagnostics Phytophthora kit E immunoassay.
[b] Of Hansen and Hamm, Oregon State University, Corvallis 97331-2903.
[c] Absorbance at 405 nm wavelength.
[d] SE = Standard error based on at least two wells in each of two different experiments.

Table 3. Spectrophotometric absorbance and source of different isolates of *Phytophthora cinnamomi* Rands[a]

Mating type	A_{405}[b]	SE[c]	Host source	Place of origin	Culture source(s)
A1	0.48	0.170	Unknown	Oregon	R. G. Linderman (P-19)
A1	0.55	0.160	*Persea americana*	California	Riverside (P2138) (ATCC 38581)
A1	1.26	0.017	Soil	Taiwan	Riverside (P2322)
A1	2.00	0.230	*Leucodendron orgenteum*	South Africa	Riverside (P2540)
A1	2.22	0.281	*Ocotea bullata*	South Africa	Riverside (P2543)
A1	1.72	0.129	*Nothofagus* sp.	Papua New Guinea	Riverside (P3656)
A1	1.70	0.324	*Araucaria cunninghamii*	Papua New Guinea	Riverside (P3659)
A1	2.42	0.231	*Castanopsis araucarih*	Papua New Guinea	Riverside (P3661)
A1	1.43	0.042	*P. americana*	Papua New Guinea	Riverside (P3663)
A1	1.29	0.142	*Eucalyptus* sp.	Australia	Riverside (P3664)
A1	1.49	0.370	*Camellia* sp.	California	Riverside (P6382)
A1	1.51	0.121	*Camellia* sp.	China	Riverside (P6492)
A2	1.16	0.121	*P. americana*	Venezuela	Riverside (P2392)
A2	0.57	0.084	*P. americana*	California	Riverside (P2457)
A2	0.80	0.019	*P. americana*	California	Riverside (P2490)
A2	1.53	0.034	Soil (azalea)	China	Riverside (P3232)
A2	0.80	0.172	*P. americana*	Indonesia	Riverside (P6304)
A2	1.36	0.296	Soil	Taiwan	Riverside (P6377)
A2	1.61	0.343	*Macadamia integrifolia*	Taiwan	Riverside (P6380)
A2	0.96	0.225	*P. americana*	Florida	Riverside (P6490)
Average overall	1.34	0.117			

[a] An equivalent of 2 μg dry weight of mycelium of each isolate was tested on Agri-Diagnostics Phytophthora kit E immunoassay.
[b] Absorbance at 405 nm wavelength.
[c] SE = Standard error based on at least two wells in each of two different experiments.

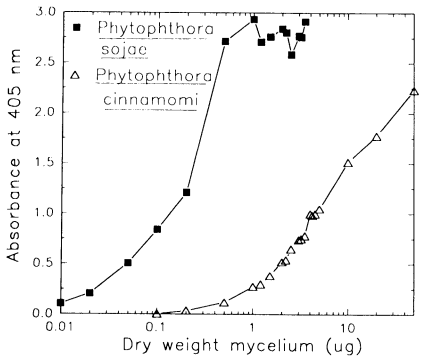

Fig. 1. Absorbance at 405 nm wavelength of Agri-Diagnostics *Phytophthora*-specific kit E immunoassay with mycelial preparations of *P. sojae* and *P. cinnamomi* (isolate 362). A 20-mg sample of aspirated mycelia was oven-dried at 32 C for 24 hr and then ground in sterile sand with 2 ml of "extract solution" supplied in each kit. The ground mycelial suspensions were boiled for 10 min and diluted to the quantity indicated.

The immunoassay was evaluated by the absorbance at 405 nm. The positive–negative threshold was set at an absorbance of 0.3 above the absorbance of the negative control supplied with the kit. Most sample readings were well above or below this level. A positive–negative threshold three times higher than the absorbance of a healthy control (12) was seldom used, since healthy plants were rarely sent to the plant disease clinic.

A final diagnosis was obtained using all information available. Some field visits and resampling were conducted as necessary for a complete diagnosis.

RESULTS

All *Phytophthora* isolates produced a positive reaction with the immunoassay (Tables 1–3). The lowest absorbencies relative to other species were obtained from *P. cinnamomi* and *P. megasperma* Drechs. (originally isolated from cherry). Variation in absorbance was high among isolates of *P. cinnamomi* (Table 2) but low among isolates of *P. cactorum* (Table 3) collected from several plant species worldwide.

Although the absorbance reading for one *P. cinnamomi* isolate was below the 0.3 threshold, higher absorbencies were obtained when more mycelia were tested (Fig. 1). Standard absorbance curves for *P. sojae* and *P. cinnamomi* were at least one order of magnitude different. At a dry weight equivalent of 1 μg, *P. sojae* was at the top of the assay range, and *P. cinnamomi* was at the low end of the sensitivity range.

Several pure cultures of *Pythium* sp. isolated from 11 different hosts were tested with the *Phytophthora* assay. These isolates represented at least six different species, according to cultural and sporangial characteristics; however, only three isolates were identified to species. Four of the six species were positive when tested on the kits, including *Pythium middletonii* Sparrow (identified by J. D. MacDonald, Davis, California) and *P. undulatum* Petersen. Two of the six species tested negative, including *P. intermedium* de Bary. All other fungal genera tested negatively to the assay kits.

Clinic samples with typical symptoms and field histories of Phytophthora root or crown rot produced a positive reaction with the immunoassay, as did pure cultures of *Phytophthora* sp. and some *Pythium* sp. isolated from these samples. Other samples without typical *Phytophthora* symptoms or field histories indicative of *Phytophthora* spp. and associated with various other pathogens did not produce a positive reaction with the immunoassay.

A total of 46 plant genera were evaluated through the clinic (Table 4), and many did not have a *Phytophthora* disease as a final diagnosis. Many of the plants tested (88%) were suspected by either the grower, county extension agent, field representative, or extension specialist of having such a disease. Only 13% of these samples yielded a culture of *Phytophthora* on CARP media. However, half of the samples reacted positively with the test kit and had a *Phytophthora* disease as a final diagnosis.

Other specimens (7.7%) were either healthy or not suspected to have a *Phytophthora* disease. All but one of these samples had a negative reaction with the test kit and had a *Phytophthora* disease as a final diagnosis. The remaining specimens, including poor samples (4.3%), did not have a preliminary diagnosis associated with them and tested negative on the kits.

A positive test result occurred only when discolored or rotted portions of a plant were tested. When healthy-appearing tissue on a diseased plant was tested, the results were negative. For example, symptoms of *Phytophthora lateralis* Tucker & Milbrath infection of Port Orford cedars are distinctly demarcated between white, healthy cambium and a cinnamon brown, invaded cambium. Samples taken from discolored areas gave absorbencies greater than 3.0, whereas samples from healthy areas gave absorbencies less than 0.1.

Five samples were positive for Phytophthora root rot according to symptoms, isolation on CARP, and test kit results but were found to have other, more fundamental, problems. Each of

these samples was from a site that had case histories or field characteristics that revealed other causes for the major problems, which were not seen in the clinic. For example, winter injury, excessive fertility, and nematode-control failure were among these diagnoses.

Only *Pythium* spp. could be isolated from 18 plant samples, despite their occurrence in fields with topography, history, and symptoms characteristic of a *Phytophthora* disease and with high absorbance readings. For example, a root- and crown-rotted *Juniperus horizontalis* Moench. 'Wiltonii' was collected from standing water in a container nursery. Rotting roots tested on the kit gave a 1.13 absorbance, and the culture initially isolated on CARP gave a 2.9 absorbance. This culture was later identified as *Pythium middletonii* and gave a negative reaction when tested on a *Pythium*-specific test kit provided by the same manufacturer.

Cross-reaction to several *Peronospora* sp. on several different hosts was observed (Table 5). Highest absorbance was from a blackberry leaf infected with *Peronospora rubi* Rabenh. ($A_{405} = 2.5$). Only symptomatic tissue tested positive. The kit did not cross-react to *Albugo bliti* on pigweed ($A_{405} = 0.09$) or to mycelia fans of *Armillaria mellea* ($A_{405} = 0.05$).

Table 4. Plant genera tested on Agri-Diagnostics Phytophthora kit E immunoassay

Plant genus	Samples (no.)	Positive isolation[a]	Positive ELISA[b]
Abies	8	4	7
Acer	2	0 (1)	1*
Alnus	1	0	1
Arctostaphylos	1	0	0
Buxus	2	1	0
Calocedrus	1	0	1
Castanea	1	0	0
Cedrus	4	0 (1)	0
Chamaecyparis	15	1	13
Cornus	2	0	0
Cotoneaster	1	1	1
Cupressus	1	0	0
Erica	1	0	0
Fragaria	2	1 (1)	2
Gaultheria	1	1	1
Hamamelis	1	0	1
Hebe	1	0	0
Hypericum	1	0	0
Ilex	4	2	2
Juniperus	11	0 (1)	4*
Kalmia	1	0	1
Laurus	1	0	0
Lycopersicon	3	0	3
Mahoberberis	1	0	1
Mahonia	1	0	0
Malus	18	0 (1)	11*
Medicago	2	1	1
Microbiotica	1	1	1
Nandina	1	0	0
Picea	3	0 (1)	1
Pieris	4	1	2
Pinus	10	0 (3)	5
Pleurothallis	1	0	0
Prunus	4	0	1
Pseudotsuga	12	4	4
Pyrus	4	0	0
Rhododendron	38	6 (4)	9
Rosa	3	0	1
Rubus	13	1 (3)	5
Solanum	7	2	6
Stewartia	2	1	0
Thuja	7	0	1
Tsuga	2	0 (1)	2*
Vaccinium	6	0 (1)	1
Viburnum	2	0	1
Vitis	2	0	0
Total	209	28 (18)	91

[a] Number of samples in which *Phytophthora* (or *Pythium*, in parentheses) was isolated on cornmeal agar (17 g/L) amended with ampicillin (250 ppm), rifampicin (10 ppm), and pimaricin (25 ppm).

[b] Number of samples that gave an absorbance ≥0.3 and had a *Phytophthora* disease as the final diagnosis. Asterisk indicates at least one sample in which *Pythium* was isolated and pure cultures reacted positively to *Phytophthora*-specific kit E immunoassay.

DISCUSSION

The kit E immunoassay reacted to worldwide isolates of *Phytophthora* spp. tested here, as implied by the manufacturer. However, there were differences of sensitivity between and within species. The kit reacts to *P. cinnamomi*, as has been previously reported (2), but, as in this study, with varying degrees of sensitivity, and certainly not as well as with other species such as *P. sojae* or *P. cactorum*. If these results hold for plant tissue, then although a kit can detect an infection by *P. cinnamomi*, it may not be able to adequately detect early infections, depending on the isolate present. This may explain some of the variation observed by other researchers (2). In general, the kit consistently and with high sensitivity identified a wide range of *Phytophthora* species to genus.

The observed cross-reactivity of the immunoassay to *Pythium* sp. and *Peronospora* sp. could make interpretation of kit results difficult at times. For example, what should the diagnosis be, when all available evidence indicates a *Phytophthora* infection, but only a *Pythium* is isolated? Was *Phytophthora* in the sample but not easily isolated, or is the disease caused by *Pythium*? Other workers have encountered similar interpretation problems when detecting zoospores in recirculation water (1). It may not be necessary to obtain an exact diagnosis, since the cultural and chemical controls for both fungi are presently the same. The development of biological or more specific chemical controls in the future may necessitate a more accurate diagnosis.

Cross-reaction with *Peronospora* sp. may not be a problem in most cases. Normally, one would not test for a root-rotting pathogen aboveground nor a foliar pathogen belowground. Additionally, the diseased or symptomatic part of the tissue infected with *Peronospora* must be tested to get a positive reaction. Since both *Phytophthora* and *Peronospora* can be present in the crown area of blackberries and raspberries (13), interpretation of positive kit results from tissue tested from this area is difficult.

A sample with an absorbance near the positive–negative threshold also makes test kit interpretation difficult. Absorbance values above 0.6 or below 0.1 are easily interpreted as positive and negative, respectively. Absorbance values that fall between this range, and especially between 0.2 and 0.4, can only be interpreted when other evidence is available. Our experience helped set the 0.3 threshold, but other researchers have had variable thresholds ranging from 0.1 to 0.6, depending on the experiment (1,2,9).

The immunoassay was a useful aid in the diagnosis of problems on a wide variety of plant genera (Table 4). Although a *Phytophthora* diagnosis was expected many times, only half of the samples yielded that diagnosis. A negative kit result helped the diagnostician pursue other possible causes of the diseased plant. Kits were also useful in midsummer when *Phytophthora* spp. routinely failed to be cultured from dried, infected plants. Although this situation has been considered an "unconfirmed positive" (9), other information can be used to make a diagnosis.

As with many plant diseases, a kit is not necessary to diagnose *Phytophthora* diseases for all plants. For example, Phytophthora root rot of true fir Christmas trees is easily diagnosed by symp-

Table 5. Reaction of *Peronospora* sp. to Agri-Diagnostics *Phytophthora*-specific kit E immunoassay

Plant	Samples (no.)	Portion tested	Average absorbance at 405 nm[a]	*Peronospora* sp.
Alyssum	2	Leaf	0.289[b]	*P. parasitica* (Pers.:Fr.) Fr.
Onion	1	Leaf	0.076	*P. destructor* (Berk) Casp. in Berk.
Pea	1	Leaf	0.366	*P. pisi* Syd.
Corydalis sp.	1	Leaf	0.358	*P. corydalis* de Bary
Rose	1	Leaf	0.214	P. sparsa Berk.
Rubus sp.	8	Leaves	0.768[c]	*P. rubi* Rabenh.
Rubus sp.	5	Fruit	0.866[d]	*P. rubi*

[a] The average is based on the number of samples received and two ELISA plate wells per sample.
[b] Highest absorbance = 0.372 at 405 nm wavelength.
[c] Highest absorbance = 2.503 at 405 nm wavelength.
[d] Highest absorbance = 1.700 at 405 nm wavelength.

toms alone. Few other problems cause symptoms similar to Phytophthora root rot, which can be differentiated using a pocket knife to determine where the discoloration originates (3).

Use of the kit does not preclude the need to obtain a wide variety of information to diagnose many root and crown rot problems. This is particularly true because of kit cross-reactivity, absorbances near the positive–negative threshold, and the need to find the underlying cause of the problem. However, these test kits are another useful and needed tool for the diagnostic process.

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