Diagnosis of *Phytophthora* Using ELISA Test Kits

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New ELISA test kits specific to the genus *Phytophthora* were evaluated in the Plant Disease Clinic at Oregon State University. All pure cultures of *Phytophthora* spp. tested produced a positive kit result. However, some *Pythium* sp. and *Peronospora* sp. also produced a positive kit result. Other common root-rotting fungi such as *Rhizoctonia* sp., *Armillaria* sp. and *Cylindrocladium* sp. resulted in negative kit reactions. The type and location of tissue sampled was critical for correct kit results and interpretation. Approximately 50% of the samples sent to the Plant Disease Clinic tested positive for *Phytophthora* and were diagnosed as having a *Phytophthora*-related disease.

OBJECTIVE

Recent advances in serology have resulted in new ELISA test kits for fungal and bacterial diseases (Kim, 1988; Miller, 1988). An ELISA test kit specific for the genus *Phytophthora* was recently released for commercial use (Peterson et al., 1990). The objectives of this research were 1) To evaluate the specificity of *Phytophthora* kits to various fungal genera, species, and isolates of *Phytophthora;* and 2) To determine the usefulness of these kits in the diagnosis of plants suspected as having a *Phytophthora* disease.

MATERIAL AND METHODS

A total of 17 species of *Phytophthora* including 18 isolates each of *P. cinnamomi* and *P cactorum* collected from throughout the world were evaluated for reaction to a *Phytophthora*-specific immunoassay (Test kit E, Agri-Diagnostics Associates, Cinnaminson, NJ). Isolates were grown on a glucose yeast peptone agar, ground in sterile sand with extract solution, and boiled for 10 min prior to using the test kit. At least two tests in each of two different experiments for each isolate were compared to sterile sand controls. Pure cultures of several *Pythium* sp., *Fusarium* sp , *Rhizoctonia* sp., and *Cylindrocladium* sp. were also evaluated using the new test kit

The test kits were used to aid in the diagnosis of plant samples sent to the Oregon State University Plant Disease Clinic over a two-year period. The following data were collected for each plant sample suspected by the grower, county agent, field representative, or specialist of having a *Phytophthora* disease· field history, plant symptoms, significant fungi observed microscopically or isolated on selective media and *Phytophthora* test kit results.

Diseased plant tissue was prepared by grinding pieces of root, crown, or leaf tissue between two small sheets of abrasive paper, called extract pads, provided in each kit. For small samples such as seedlings, the entire root system was rubbed against the extract pad rather than grinding subsamples An extract pad was rubbed directly against discolored or cankered areas of large samples such as woody ornamentals All subsequent steps were followed as outlined in kit directions.

Several large trees of *Chamaecyparis lawsoniana* infected with *P. lateralis* were sampled to determine where a positive color reaction was strongest. Extract pads were rubbed against the discolored area of the cambium, the zone between healthy and discolored cambium, and above the discolored area on healthy appearing cambium. The outer phloem, cambium, and inner xylem of core samples were also tested using the kits.

RESULTS AND DISCUSSION

All pure cultures of *Phytophthora* spp. used in this study reacted positively with the new test kits (Table 1); however, there were differences between and within species. For example, *P cinnamomi* gave the lowest color reaction when compared against all other species (Table 1). Color reactions were also quite variable among the 17 isolates of *P. cinnamomi*, but among isolates of *P. cactorum*, kit reactions were not variable.

Table 1. Absorbance of different *Phytophthora* species[a]

Species	Absorbance[b]	S E.[c]
Phytophthora boehmeriae	2 95	0 024
P cambivora	2 32	0.025
P cinnamomi	0.20	0.027
P. cinnamomi	0.40	0.037
P citricola	2 82	0 075
P cryptogea	2 91	0 024
P drechsleri	> 3	
P erythroseptica	2.62	0 034
P fragariae	> 3	
P gonapodyides	2 72	0 007
P ilicis	2 97	0 023
P lateralis	2 87	0 024
P megasperma	1 04	0 105
P megasperma	2.88	0.033
P megasperma	2 77	0 187
P megasperma	2 89	0 008
P. palmivora	2 17	0 010
P pseudotsugae	2.86	0 018
P vignae	2 99	0 006
P wyringae	2 97	0 014

[a]An equivalent of 1 ug dry wt mycelium was tested on Agri-Diagnostics Phytophthora Kit E

[b]Absorbance at a wavelenght of 405 nm This was a 0-3 scale where 0 3 was considered as the positive-negative threshold

[c]S E. = Standard Error based on at least 2 wells in each of 2 different experiments

Cross reactivity occurred only with some (not all) *Pythium* sp. and several *Peronospora* species. *Pythium middletonii*, isolated from rotted juniper roots, and other *Pythium* species reacted with the *Phytophthora* kit E but negatively to a similar kit specific for the genus *Pythium*. Were the junipers infected by *Phytophthora*, which could not be isolated, or was the disease caused by *Pythium*? Blackberry and raspberry crowns can be infected by either *Phytophthora fragariae* or *Peronospora rubi*. The cross reactivity exemplified by these cases makes positive kit interpretation difficult.

The test kit was a useful aid in the diagnosis of plant problems sent to the Plant Disease Clinic Over 200 samples representing 46 plant genera were tested Many of these plants (88%) were suspected by someone as having a *Phytophthora* disease but only half of these reacted positively to the test kit. Some representative data for a few plant species are contained in Table 2. Kits were particularly useful in mid-summer when *Phytophthora* routinely failed to be cultured from dried, infected plants.

Table 2. Expected and actual kit[1] results from plants suspected as having a *Phytophthora* disease

Plant Genus	Number of samples where diagnosis suspected as *Phytophthora*	Number of samples yielding *Phytophtora* culture	Number of samples tested positive with kits
Abies	8	4	7
Juniperus	7	0	4
Pieris	4	1	2
Pinus	10	0	5
Rhododendron	36	6	9

[1] Kit E, Agri-Diagnostics Associates

A positive result only occurred for an infected plant when discolored or rotted portions of a plant sample were tested. Strongest color reactions were obtained from *Chamaecyparis lawsoniana* when cambial tissue at or below the line between healthy and discolored tissue was tested. Negative color reactions were obtained from samples taken above the discolored tissue or within the xylem toward the center of the trees. Other studies have shown that greater than 1% infected tissue must be present to obtain a positive reaction (Benson, 1991; MacDonald et al., 1990) Therefore, the type and location of tissue sampled is critical to obtain an accurate test kit result.

Use of these kits does not preclude the need to obtain a wide variety of information to diagnose root and crown rot problems. Many of the samples that tested negative did not have field histories, topography, or symptoms consistent with a *Phytophthora* disease. Alternatively, even though all the evidence indicated a *Phytophthora* diagnosis, it may not be the major underlying problem since *Phytophthora* infected plants were found in situations where the major problem was due to winter injury, excessive fertility, or nematode control failure.

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