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Using a *Phytophthora*- specific Immunoassay Kit to Diagnose Raspberry Phytophthora Root Rot

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Abstract. **A commercially available serological assay kit (flow-through enzyme-linked immunosorbent assay, Phytophthora F kit) was compared to a culture-plate method for detecting *Phytophthora* spp. in apparently diseased (phytophthora root rot) and apparently healthy red raspberry *(Rubus idaeus* subsp. *strigosus* Michx.) plants. During 4 years of testing, 46 tests were conducted on apparently diseased roots. All diseased plants gave a strong positive reaction, a result indicating that *Phytophthora* spp. were present. Of the 46 plants that tested positive, *Phytophthora* spp. were recovered from all but one using a selective medium for *Phytophthora* and the culture-plate method. When the same test was conducted on 27 apparently healthy plants, all had a negative reaction for the presence of *Phytophthora* except one sample, which had a slight positive reaction. No *Phytophthora* spp. were isolated from any apparently healthy plants. Our results indicate that the serological test kit enables rapid, dependable, on-site diagnosis of raspberry phytophthora root rot.**

Red raspberry decline and dieback, caused by several *Phytophthora* spp. (Wilcox, 1989), is a common problem in Ohio. The disease is most severe in areas where soil types, topography, and environmental conditions result in regular or extended periods of excessive soil moisture or flooding (Ellis et al., 1991; Wilcox, 1989). These conditions are common in Ohio, and raspberry phytophthora root rot seems to be increasing (M.A. Ellis, unpublished data). Although the disease has been present in Ohio for many years, it was not diagnosed until 1985. Traditionally, plants affected by phytophthora root rot have been diagnosed as suffering from root asphyxiation in wet soils ("wet feet") or from various types of injury, such as those caused by low temperature (winter injury) or excessive herbicide application. Improved techniques for isolating the pathogen from raspberry roots to diagnose the disease have helped determine the extent to which the disease occurs in the state.

Using selective media (Schmitthenner, 1973) has increased the ability to identify the pathogen correctly and diagnose the disease. However, positive diagnosis still requires sophisticated materials and equipment for preparing selective media and considerable effort and time.

Fungicides [*N* -(2,6 -dimethylphenyl) -N-(methoxyacetyl) alanine methyl ester (metalaxyl; CIBA-GEIGY, Greensboro, N.C.); aluminum tris (*o* -ethyl phosphorate) (Fosetyl-AL; Rhone-Poulenc, Research Triangle Park, N.C.)] for controlling raspberry phytophthora root rot have been developed and registered. Obviously, these fungicides should not be used unless phytophthora root rot is diagnosed.

Using an enzyme-linked immunosorbent assay (ELISA) to detect plant pathogens in diseased plant tissues can facilitate the rapid and specific diagnosis of disease (Benson, 1991; McDonald et al., 1990; Miller and Martin, 1988; Miller et al., 1988, 1990). An immunoassay Phytophthora F Kit (Agri-Diagnostics Assoc., Cinnaminson, N.J.) allows commercial growers and plant health professionals to diagnose plant diseases caused by *Phytophthora* spp. rapidly without using time-consuming, culture-plate methods.

The purpose of this study was to evaluate the efficacy of the commercial immunoassay Phytophthora F kit for diagnosing raspberry phytophthora root rot.

In 1988, plants in several commercial red raspberry plantings in Ohio developed typical phytophthora root rot symptoms (Wilcox, 1989). Excessive rainfall resulted in ideal conditions for root rot development, especially in low, poorly drained areas. In a l-ha commercial planting of 'Heritage' (fall-bearing) red raspberries near Wooster, Ohio, ≈ 25% of the plants developed severe root rot symptoms, and most affected plants died before the end of the growing season (M.A. Ellis, unpublished data). Most affected plants were located in a poorly drained area of the field where the soil was excessively moist.

Thirty plants showing typical phytophthora root rot symptoms and 30 apparently healthy plants from different areas of the same field were collected on 25 Aug. 1988. Plants were dug to include major lateral roots with the crown. Cones were discarded. The crown and roots of each plant were placed in individual plastic bags in an ice chest and transported to the laboratory. All samples were stored at 4C until used. No samples were stored more than 4 days before tests were conducted. Twenty samples each from healthy and infected plants were tested for the presence of *Phytophthora* spp. using the culture-plate method and commercial Phytophthora F kit. Each test was conducted on the same location on each plant.

Crowns with major roots from 10 healthy and 10 infected plants were washed under running tap water to remove most of the soil. After being washed, the main taproot and crown region of each plant were examined for lesions caused by *Phytophthora.* Typical lesions were areas of discoloration (reddish brown to black) with a sharp line of demarcation between healthy (white) and diseased tissue. The epidermis was removed by scraping with a scalpel, and discolored tissue samples were taken from near the edge (line of demarcation) of the lesion. No fewer than 25 tissue pieces (five pieces per plate) were placed on pentachloronitrobenzene-benomyl-neomycin-chloramphenicol medium (Schmitthenner, 1973), a selective medium for *Phytophthora* spp. Plates were incubated at 24C for 5 to 7 days. The recovery of *Phytophthora* spp. was recorded from day 5 through 7. Representative isolates were made of the different fungi recovered for identification.

Roots from healthy plants showed no signs of discoloration, and samples were taken from about the same region on the main taproot that they were taken from diseased plants. Tissues from healthy roots were cultured in the same manner as those from infected plants.

The experiment was repeated on 10 additional infected and healthy plants as previously described, except that the epidermis was not removed after the samples were washed in tap water.

The Phytophthora F kit contained all materials needed to perform the assays. Immunoassay were conducted on the same location on each root where tissue samples were removed for the culture-plate method. The area tested was rubbed vigorously with the abrasive pad, then the pad was placed in a bottle containing art extraction buffer and shaken thoroughly. A filter tip was attached to the bottle and the sample was filtered. The rapid assay uses a device with three closely spaced wells on the surface through which the sample and reagents flow. One well is an internal positive control, one is a negative control, and one is the sample well. Root extract and kit reagents were applied to the flow-through device according to instructions supplied with the kit.

A blue color in the sample well indicates a positive reaction and the negative control remains white. A negative reaction (no color change) was observed in the negative control and sample wells. Tests were considered valid if the internal positive control well turned blue

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and the negative control well remained white. Intensity of positive color reactions was measured on a visual color scale provided with the kit and ranged from 1 (lightest) to 5 (darkest). Any blue color indicated a positive reaction. The color intensity indicated the amount of fungus mycelium present in the sample. Color intensity was recorded for each assay.

In 1989, the procedures described above were conducted on additional diseased and healthy 'Heritage' red raspberry plants from two Ohio counties—Wayne and Medina. Tests were conducted on five diseased and two healthy plants on 10 July 1989 from the same planting used in 1988. In addition, five diseased and two healthy plants from a 0.25-ha commercial planting in Medina County were tested on 25 July 1989. The epidermis was removed from all plant samples tested in 1989.

Additional tests on 'Heritage' plants were conducted as in 1989 on 10 diseased plants (five from a commercial planting in Medina County on 3 Aug. and five from a commercial planting in Warren County on 15 July) in 1990 and six diseased plants (all from a commercial planting in Geauga County on 30 June) in 1991. Tests also were conducted on one apparently healthy plant from each location.

In 1988, all test kit reactions for apparently diseased roots were positive (Table 1). None of the positive reactions had a color reading below 4 on the color scale. *Phytophthora* spp. were isolated from all diseased roots except for one sample. The test was repeated on this sample and a positive result was obtained. However, repeated attempts to isolate *Phytophthora* spp. from this sample were unsuccessful. *Phytophthora megasperma* var. *megasperma* Waterhouse, *P. cactorum* (Lebert & Cohn) J. Schröt, and *P. cryptogea* Pethybr & Lafferty were isolated from diseased raspberry roots with isolation frequencies of 68%, 25%, and 7%, respectively. Of the 20 apparently healthy plants tested in 1988, only one gave a slight positive reaction (reading of 1 on the color scale). The test was repeated on this plant sample and the same reaction was observed. No *Phytophthora* spp. were recovered from any apparently healthy roots.

Sampling instructions provided with the test kit state that the epidermis of the sample should be scraped away before the test is conducted. In our studies, leaving the epidermis in place had no apparent effect on the efficiency of the test. We noted that even a thorough washing of the sample seldom removed all visible soil from the root surface. Thus, small amounts of soil present on the root surface had no apparent effect on the results. However, the epidermis was removed in all subsequent tests.

Results of tests conducted in 1989 were almost identical to those of 1988 (Table 2). Of 10 tests conducted on apparently diseased plants, all gave a positive reaction of 4 or higher, and *Phytophthora* spp. were isolated from all plants that gave a positive reaction. The two apparently healthy plants tested from each location gave negative reactions, and *Phytophthora* spp. were not recovered from any such sample.

Table 1. Reaction of immunoassay test kits to and recovery of *Phytophthora* spp. from apparently healthy and infected red raspberry roots in 1988.

Apparently healthy roots[z]			Diseased roots[y]		
Sample	Reaction[x]	Isolation[w]	Sample	Reaction	Isolation
1	0	—	21	5	+
2	0	—	22	5	+
3	0	—	23	5	+
4	0	—	24	5	+
5	0	—	25	5	+
6	0	—	26	5	+
7	0		27	4	+
8	0		28	5	—
9	0	—	29	5	+
10	0	—	30	5	+
11[v]	0		31"	5	+
12	0		32	5	+
13	0	—	33	5	+
14	0	—	34	5	+
15	0		35	5	+
16	0	—	36	5	+
17	0	—	37	4	-t
18	0	—	38	4	+
19	1	—	39	5	+
20	0	—	40	5	+

[z]Roots taken from apparently healthy plants. Roots were washed and the epidermis was scraped away with a scalpel. Test was conducted on scraped area.
[y]Roots taken from plants showing typical phytophthora root rot symptoms; i.e., wilting and collapse of plant and brown to brick-red lesions on roots. All tests were conducted on discolored tissue adjacent to lesion margin.
[x]Color reaction (blue = positive, white= negative) based on a scale of 0 to 5, where 5 = darkest blue and O = white.
[w]Isolations were made from the same area on roots where the serological test was conducted. Isolations were made on pentachloronitiobenzee-benomyl-neo-mycin-chlormphenicol medium. Positive (+) isolation indicates that a *Phytophthora* species was isolated. Negative (–) isolation indicates no fungus recovered.
[v]For samples 11 to 20 and 31 to 40, tests were conducted as described above; however, the epidermis was not removed.

Table 2. Reaction of immunoassay test kits to and recovery of *Phytophthora* spp. from apparently healthy and infected red raspberry roots in 1989.

	Apparently healthy roots[z]			Diseased roots[y]		
county	Sample	Reaction[x]	Isolation[w]	Sample	Reaction	Isolation
Wayne	46	0	—	41	5	+
	47	0	—	42	5	+
				43	5	+
				44	5	+
				45	5	+
Medina	53	0	—	48	4	+
	54	0	—	49	5	+
				50	5	+
				51	4	+
				52	4	+

[z]Roots taken from apparently healthy plants. Roots were washed and the epidermis was scraped away with a scalpel. Test was conducted on scraped area.
[y]Roots taken from plants showing typical *Phytophthora* root rot symptoms; i.e., wilting and collapse of plant and brown to brick-red lesions on roots. Plants were washed and the epidermis was removed with a scalpel. All tests were conducted on discolored tissue adjacent to lesion margin.
[x]Color reaction (blue = positive, white = negative) based on a scale of O to 5, where 5 = darkest blue and O = white.
[w]Isolations were made from the sample area on roots where the test was conducted. Positive (+) isolation indicates a *Phytophthora* species was isolated. Negative (–) isolation indicates no fungus recovered.

In tests conducted in 1990 and 1991 on apparently diseased plants, all gave positive reactions except one from Warren County in 1990. The test was repeated on this sample and a negative result was obtained. *Phytophthora* spp. were recovered from all diseased plants in both years of testing, except for samples giving a negative test result. Healthy plants tested from all locations gave negative test reactions, and no *Phytophthora* spp. were recovered from any apparently healthy plants.

Our results suggest that the flow-through Phytophthora F kit effectively detects the presence of *Phytophthora* spp. in diseased red raspberry roots and crowns. These kits enable rapid, dependable, on-site disease diagnosis. Individual tests can be conducted in <15 min at the growers' locations compared to a minimum of 4 to 7 days for the culture-plate method. In addition, the culture-plate method requires a sophisticated laboratory for medium preparation, isolation, and incubation. Isolating the pathogen is desirable in most plant disease diagnostic situations; however, based on our

results, we are confident that the Phytophthora F kit is sufficiently reliable as a basis for recommending fungicides to be used on raspberry plantings where visible disease symptoms are present and positive test results are obtained.

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