Immunoenzymatic Detection of *Phytophthora fragariae* in Infected Strawberry Plants

Eugénie Amouzou-Alladaye, Jean Dunez, and Michel Clerjeau

INRA—Station de Pathologie Végétale, Centre de Recherches de Bordeaux, BP 131, 33140 Pont de la Maye, France.
Financial support for this research was provided by INRA and the Aquitaine Region Council.
We wish to thank K. D. Mayo for a careful revision of the English of the manuscript.
Accepted for publication 22 February 1988 (submitted for electronic processing).

ABSTRACT

Amouzou-Alladaye, E., Dunez, J., and Clerjeau, M. 1988. Immunoenzymatic detection of *Phytophthora fragariae* in infected strawberry plants. Phytopathology 78:1022-1026.

Antiserum obtained by injecting rabbits with mycelial protein extracts of one strain of *Phytophthora fragariae* had a dilution end point of 1/64 in double diffusion and 1/512,000 in indirect enzyme-linked immunosorbent assay (ELISA). This serum could detect 11 different strains of *P. fragariae* in pure culture and the pathogen in naturally infected or inoculated roots. Although the sensitivities of direct double antibody sandwich and indirect ELISA were comparable, the direct double antibody sandwich ELISA was more specific for the detection of *P. fragariae* in strawberry roots. The antiserum failed to react with 18 fungal species isolated from underground parts of strawberry but reacted with some strains of *P. cactorum*, which parasitized only rhizomes but not roots, and *Pythium middletonii*, which was isolated sometimes in association with *P. fragariae* from strawberry roots. In inoculated strawberry roots, *P. fragariae* was detected reliably by ELISA several days before oospores were found and before symptoms developed. Thus, direct double antibody sandwich ELISA may be useful for early detection of infection and for the detection of latent infections of strawberry plants by *P. fragariae*.

Additional keywords: red core, root flora, serology.

Although *Phytophthora fragariae* Hickman (5), the agent of red core or red stele in strawberry, is probably present in France, it had not caused any serious damage until 1981, when it probably was introduced on contaminated plants (14).

Red core traditionally has been a disease of the northern regions of Europe: Great Britain, The Netherlands, Germany, and Sweden (12). The recent introduction in France and subsequent disease development prompted the establishment of sanitary nursery procedures and control of imported plants. Reliable and rapid methods of detection are needed for such procedures.

Generally, it is quite easy to identify the disease in infected plants from the nursery. When plants are pulled up, root tips are missing and principal roots have brown extremities. This is the rat tail symptom. Longitudinal root sections reveal a central cylinder of red brick color, sometimes extending as far as the rhizome, giving the disease its name. Microscopy of the tissues normally reveals the oospores that are responsible for fungus survival.

These symptoms are not sufficient for detection of all infected plants. Latent infection by *P. fragariae* occurs, particularly in dry periods. Moreover, the oospores, which are often the best indicator of the parasite's presence, are not always visible.

The causal organism is difficult to culture even on specific media (12). Thus, a serological detection method seems best suited to meet the requirements of rapid detection.

Although still not widely used with fungi, serological techniques have been quite successful in recent years. Detection of *Eutypa armeniacae* (15), *Phytophthora cinnamomi* (10), *Verticillium lecanii* (3), *Phoma tracheiphila* (13), *Phoma exigua* (1), etc., and the taxonomy and precise identification of certain other types of *Phytophthora* (11), *Fusarium* (6), *Verticillium* (19), and *Armillariella* (9) genera are examples of the use of serological techniques. Preliminary results recently have been obtained with *P. fragariae* (20).

Our aim was to produce antibodies for serological procedures to enable early detection of any infection by *P. fragariae* and to determine if antibody specificity is such that no cross-reaction occurs with healthy plants or other pathogenic agents of strawberry. Production of an antiserum to *P. fragariae* and its use in the ELISA test are described. The reagents thus obtained had excellent specificity. They reliably and reproducibly detected the fungus in plants infected for 15–20 days.

MATERIALS AND METHODS

Plant material. Five cultivars of strawberry plants were used: Red Gauntlet, Aliso, Belrubi, Gariguette, and Gorella. They were derived from in vitro micropropagation and were provided by the CTIFL (Centre Technique Interprofessionnel des Fruits et Légumes, Centre de Balandran, 30127 Bellegarde, France). They were grown in a greenhouse in pasteurized humus. These cultivars were used as inoculated and as healthy test controls, after field infection, as naturally infected plants.

Fungus culture. Serum was produced against strain AE 584 of *P. fragariae*, isolated in Aquitaine (France). This strain, other isolates (Table 1) of *Phytophthora* sp., and other fungi frequently isolated from the root flora of strawberries were maintained in petri dishes on a malt agar medium (16), except *P. fragariae*, which was maintained on a lima bean agar medium (12) and *P. infestans*, which was maintained on rye seed-dextrose agar (17).

Production of *P. fragariae* mycelium in liquid medium. *P. fragariae* was cultured in 500-ml flasks containing lima bean liquid medium. The medium was inoculated with a dozen disks taken from the periphery of *P. fragariae* cultures on agar medium. After 15 days of incubation at 22 C under 12 hr of light, the mycelium was collected on filter paper in a Büchner funnel, washed with distilled water, and freeze dried. The freeze-dried mycelium was ground, and the resulting powder was stored over calcium chloride.

Preparation of the antigenic suspension. The serum was produced against a soluble protein fraction. Two grams of mycelium powder from *P. fragariae* strain AE 584 was ground with a Polytron blender in the presence of 40 mg/ml of polyvinyl polypyrrolidone (PVPP) in 50 ml of extraction buffer, pH 8.8, containing: 0.03 M Tris, 0.005 M $MgCl_2$, 0.001 M Na_2EDTA, 0.001 M dithiothreitol (DTT), 0.001 M ascorbic acid, and 20% glycerol. After centrifugation at 10,000 rpm for 15 min, the supernatant was collected and centrifuged in a Beckman 60 Ti rotor at 35,000 rpm for 90 min. The supernatant was concentrated

by dialysis against 20% polyethylene glycol 20,000. This concentrated fraction was diluted in phosphate-buffered saline (PBS). The protein content was determined by the technique of Lowry (8), and the solution was stored at −20 C. Protein solutions prepared in this manner were used as immunogens or as antigens.

Immunization technique. A rabbit was immunized by intradermal injection (about 5–10 dorsal punctures) with 0.8 ml of a solution containing 2.5 mg of protein per each of three injections over 15 days, and then three monthly boosters. The first injection included an equal volume of Freund's complete adjuvant with subsequent injections in incomplete adjuvant. Blood was sampled weekly, and antibody titer was evaluated by agar double diffusion (0.8% agarose in veronal buffer, pH 8.6 [LKB]) and by indirect ELISA tests. The IgGs for direct double antibody sandwich (DAS) ELISA were isolated by affinity chromatography on a protein A Sepharose column.

Preparation of samples. Three types of samples were analyzed with the ELISA test. Sample A: solutions of *P. fragariae*-soluble proteins (0.1 μg/ml); sample B: supernatant from centrifuged mycelium culture extracts prepared by grinding 100-mg disks of young mycelium culture in agar medium per milliliter of PBS buffer; sample C: infected strawberry extracts obtained by grinding and centrifuging 1 g of the root extremities of healthy or infected strawberry plants in 10 ml of PBS buffer. Supernatant of these samples were diluted 1/10 with carbonate buffer for indirect ELISA, and with PBS-Tween-PVP for DAS-ELISA.

Three trials were performed on inoculated plants: trial no. 1 in October 1985, no. 2 in February 1986, and no. 3 in September 1986. Healthy strawberry plants were planted in a greenhouse in the following conditions: in steam pasteurized humus to which was added a culture of *P. fragariae* on a bran-vermiculite substrate moistened with 100 ml of the lima bean liquid medium for trials no. 1 and 2 (the inoculum substrate was applied in proportion of 1:3 by pot) and in naturally infested soil for trial no. 3. One hundred-fifty plants per variety were used, 75 infected plants and 75 healthy controls distributed with 15 plants per pot.

Strawberry plants were grown in a growth chamber at 10 C with 12 hr of light and received daily sprinkling for maximum moistness.

From the eighth day after inoculation (D8), lots of six plants were sampled every other day. They were carefully cleaned, and the state of the root system was observed both visually and under the microscope. Roots were cut in small fragments for two-thirds of their length starting from the distal end. Root fragments from inoculated and healthy plants were subdivided in three parts: 1) surface sterilized and placed on a selective agar medium to isolate the fungus; 2) surface sterilized and put in a calcium nitrate solution (1 g/L) to induce sporulation in petri dishes at 10 C; 3) rinsed, weighed, and ground in PBS for analysis by ELISA. Serum specificity was evaluated by testing against several isolates of *P. fragariae*, several isolates of *Phytophthora* sp., and other fungi frequently isolated from the root flora of strawberry (Table 1).

Enzyme-linked immunosorbent assays. Two techniques were used: DAS-ELISA described by Clark and Adams (4) and an indirect technique (7). Antigen extracts for the indirect technique were diluted in a carbonate buffer, pH 9.6, and introduced in plates (250 μL per well). After a 4-hr incubation at 37 C and three 5-min washings in PBS-Tween, the IgG was added at a concentration of 3 μg/ml of PBS-Tween-PVP buffer, then incubated overnight in the refrigerator. After washing, conjugate composed of goat anti-rabbit immunoglobulin coupled with alkaline phosphatase (Nordic), diluted at 1/4,000, was added to plates and incubated for

TABLE 1. Fungus[a] isolates tested with the antiserum obtained from *P. fragariae* strain AE 584[b]

Phytophthora species	Absorbance	Fungi from the root flora of strawberry	Absorbance
P. fragariae	0.326–0.861[c]	*Acremonium* sp.	<T
Other *Phytophthora*[d]		*Alternaria alternata*	<T
P. cactorum		*Aureobasidium* sp.	<T
27 isolates	<T[e]		
3 strawberry isolates	0.221, 0.254, 0.289	*Botrytis cinerea*	<T
P. infestans		*Coniella fragariae*	<T
16 Israel isolates			
2 France isolates	<T	*Cylindrocarpon destructans*	<T
P. cinnamomi		*Cylindrocarpon obtusisporum*	<T
8 isolates	<T		
		Cephalosporium sp.	<T
P. cambivora			
3 isolates	<T	*Fusarium roseum*	<T
P. capsici		*Fusarium* sp.	<T
4 isolates	<T		
		Mucor saturninus	<T
P. parasitica			
3 isolates	<T	*Papulospora* sp.	<T
P. citrophthora		*Penicillium* sp.	<T
2 isolates	<T		
1 isolate	0.129	*Pythium* sp.	<T
		2 strawberry isolates	
		1 non-strawberry isolate	
P. palmivora			
2 isolates	<T		
1 isolate	0.144	*Pythium middletonii*	0.278
P. syringae		*Rhizopus* sp.	<T
2 isolates	<T		
1 isolate	0.144	*Stemphylium* sp.	<T
P. citricola		*Verticillium dahliae*	<T
3 isolates	<T		
		Zythia fragariae	<T
P. megasperma			
3 isolates	<T		

[a] Antigenic extract obtained with sample B extraction procedure (see Material and Methods).
[b] Absorbance values at 405 nm obtained by DAS ELISA.
[c] Reactions exceeding the threshold.
[d] Strains obtained at the INRA Station Pathologie Végétale Antibes and Bordeaux (France).
[e] T = Threshold for positive reaction.

4 hr at 37 C. Paranitrophenyl phosphate (1 mg/ml) was then added and incubated 1 hr at 37 C. Plates for DAS tests were coated with *P. fragariae* specific IgG diluted to 3 μg/ml in carbonate buffer, and incubated for 4 hr at 37 C. Antigen extracts prepared as described above and diluted in PBS-Tween-PVP buffer were added and incubated overnight at 4 C. Conjugate, prepared by coupling *P. fragariae* specific IgG with alkaline phosphatase in the presence of glutaraldehyde (2), was used at a 1/4,000 dilution and 4 hr at 37 C incubation. The substrate, paranitrophenyl phosphate, at a concentration of 1 mg/ml in diethanolamine, was incubated 2 hr at room temperature, the absorbance was measured with an automatic Titertek Multiskan MCC reader at 405 nm.

RESULTS

Serum characteristics and in vitro detection of *P. fragariae*. Antiserum titer was 1/64 by double diffusion in agarose, and 1/512,000 with indirect ELISA with an antigen consisting of 0.1 μg of *P. fragariae* protein extract per milliliter.

Comparison between the DAS and indirect techniques revealed no significant difference in sensitivity (Fig. 1). Antibodies obtained with strain AE 584 of *P. fragariae* were tested against 11 strains of *P. fragariae* from different sources: S46 from A. Bolay (Switzerland); SZ 168, 169, 171, 172, 173, and 293 from Montgomerie (Scotland); and AE 385, 584, and 984 from our collection. Reactions were obtained with all these strains. Absorbance values with undiluted antigenic extracts (0.1 g of mycelium in 1 ml of PBS buffer) obtained with the sample B extraction procedure (Materials and Methods) ranged from 0.326 to 0.861.

Comparison of ELISA techniques for detecting *P. fragariae* in strawberry. The two immunoenzymatic methods enabled the identification of *P. fragariae* both in pure culture and from roots 2 mo after inoculation (Table 2). The indirect ELISA method was less specific for detecting *P. fragariae* in roots; a positive reaction also occurred with healthy roots. For this reason, DAS-ELISA was used in later trials. The A_{405nm} value considered as the positivity threshold for the reactions was obtained by adding the mean value for eight healthy controls to 3.5 times the value of the standard deviation of the healthy controls.

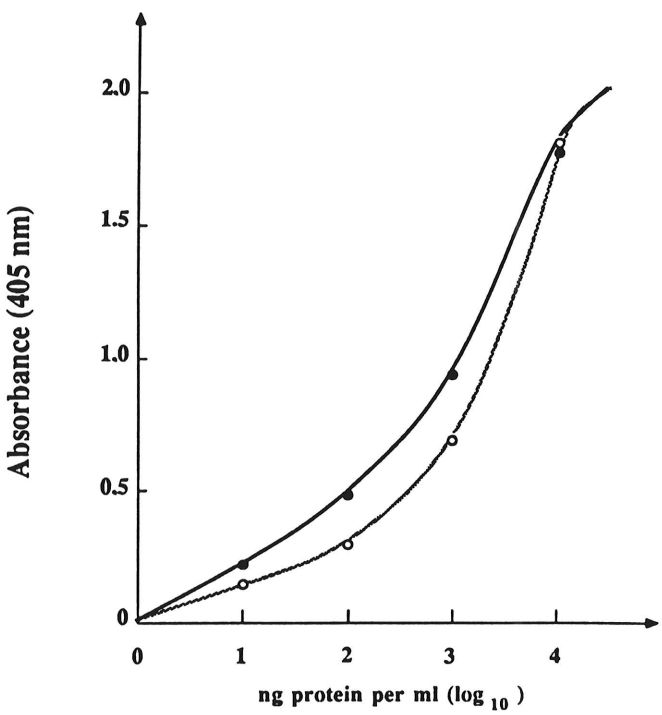

Fig. 1. Variation of absorbance in relation to concentration of protein extract from *P. fragariae* strain AE 584 (Indirect ELISA: ●; DAS-ELISA: o).

Serum specificity on various fungal isolates. To determine the potential for nonspecific cross-reactions, antigens from several *Phytophthora* sp. and different fungi usually found in the root flora of strawberry were tested by DAS-ELISA (Table 1). The serum to AE 584 was very specific with regard to *P. fragariae*. In the 30 strains of *P. cactorum* tested, only three strains isolated from strawberry reacted positively. This reaction does not constitute a handicap in the search for *P. fragariae* in strawberry roots, since *P. cactorum* is a parasite of the rhizome and not of the roots of strawberry.

Among other fungi evaluated, reactions were negative except for four Pythiacae isolates: a strain of *P. citrophthora* isolated from Seville orange, a strain of *P. palmivora* isolated from Hevea, and a strain of *P. syringae* isolated from apple. These species are not generally described as direct parasites of the strawberry. *Pythium middletonii*, which is less frequently isolated from strawberry than other *Pythium* sp., is normally isolated in association with *P. fragariae*.

Application of DAS-ELISA in the early detection of *P. fragariae* in artificially infected strawberry roots. Results of analysis are shown in Figure 2. Infection by *P. fragariae* was detectable by DAS-ELISA in all three trials and in all varieties. In all cases, the first positive reactions were detectable about 15 days after inoculation. In trials 1 and 2, the first positive reactions were minimal, and their values increased progressively with time. In trial 3, some values obtained during the first sampling were well above the threshold. These results in the five varieties may be explained by the difference in inoculation for trial 3, which consisted of infested soil in which the level of inoculum could not be measured. This apparently led to rapid invasion of the plants by the pathogen. Afterwards, these values began to fall, and after 25 days were similar to those observed in trials 1 and 2. No consistent differences among the five varieties could be established. Overall absorbance values with Gariguette and Aliso were the highest, slightly exceeding those obtained with Belrubi, Gorella, and Red Gauntlet.

The other detection methods (Table 3) confirm that infection was the most rapid in trial 3. Indeed, the observation of symptoms on Belrubi and Aliso, the development of sporangia, and the observation of oospores in Gorella were possible from day 12 to day 17 only in trial 3. In general, there was good correlation in time between ELISA results and the oospore and sporulation tests. With only rare exceptions, symptoms were not visible before the period between days 28 and 37, i.e., about 2 wk after the first positive results given by ELISA. Apart from the successful attempt with Red Gauntlet at day 11 in trials 2 and 3 (data not shown), the fungus could not be isolated on agar medium.

Preliminary applications of DAS-ELISA to field indexing. Nine batches consisting of 10–27 strawberry plants collected from nurseries were tested by ELISA and the sporulation test (in calcium nitrate). Each plant was inspected and tested individually; DAS-ELISA was carried out with extracts prepared according the sample C extraction procedure. Results are presented in Table 4. From symptom observation, only one batch out of nine appeared to be infected and one showed dubious symptoms. The sporulation test detected *P. fragariae* in five batches, while ELISA was positive in seven batches. In the different batches, the percentage of plants

TABLE 2. Comparison of the two enzyme-linked immunosorbent assay (ELISA) techniques in the detection of *Phytophthora fragariae* (absorbance values at 405 nm represent the mean of two trials)

	Antigenic solution[a]			
	Mycelium culture	Strawberry roots[b]		
ELISA technique	*P. fragariae* AE 584	Healthy	Inoculated	Naturally infected
ELISA indirect	0.330	0.178	0.104	1,619
ELISA sandwich	0.311	0.024	0.840	1,374

[a] The antigenic extracts were obtained by grinding 100 mg of mycelium culture in 1 ml of PBS buffer and 1 of mg root extremities in 10 ml of PBS buffer (b).

TABLE 3. Results from microscopic observation of oospores, development of sporangia in $CaNO_3$, and the appearance of red stele symptoms

No. of trial	Root analysis technique	Period																			
		D0-D11					D12-D17					D18-D27					D28-D37				
		A[a]	B	G	Go	RG	A	B	G	Go	RG	A	B	G	Go	RG	A	B	G	Go	RG
1	Red stele symptoms	–[b]	–	–	–	–	–	–	–	–	–	–	–	–	–	+[c]	+	+	–	+	+
	Oospores observation	–	–	–	–	–	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+
	Sporulation test	–	–	–	–	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
2	Red stele symptoms	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+
	Oospores observation	–	–	–	–	–	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+
	Sporulation test	–	–	–	–	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
3	Red stele symptoms	–	–	–	–	–	+	+	–	–	–	–	–	–	–	+	+	+	+	+	+
	Oospores observation	–	–	–	–	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
	Sporulation test	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[a] A = Aliso; B = Belrubi; G = Gariguette; Go = Gorella; RG = Red Gauntlet.
[b] – = absence of symptom, of oospore, or of sporulation.
[c] + = presence of symptoms, of oospores, or of sporulation.

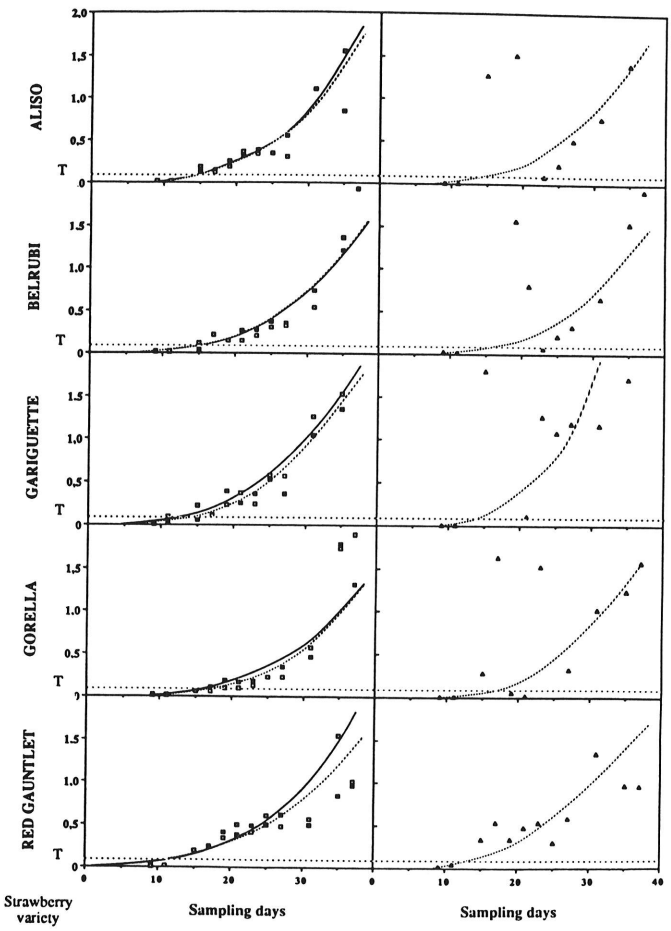

Fig. 2. Variation of absorbance (at 405 nm) in relation to day of sampling after inoculation. (■ = trial 1; □ = trial 2; ▲ = trial 3). The dotted line is the threshold between positive and negative reactions.

positive by ELISA varied from 10 to 100; even if some absorbance values (405 nm) appeared to be rather low, the very low level of nonspecific reaction (0.051–0.085) allowed easy differentiation of the positive reactions.

DISCUSSION

The antibodies obtained with a strain of *P. fragariae* enabled the detection in pure culture of 11 different strains of this pathogen. Detection of the fungus in pure cultures was comparable to DAS-ELISA and indirect ELISA. The two methods showed comparable sensitivities with extracts from infected roots, but DAS-ELISA was more specific. The absence of nonspecific cross-reactions with most fungi of the root flora also represented an advantage in the search for *P. fragariae* in the roots. The results with inoculated plants indicated that using the specific IgG of *P. fragariae* with the DAS-ELISA constituted a method of early detection of the fungus in the roots of inoculated plants. For the five varieties studied, *P. fragariae* could be detected between days 15 and 25 after inoculation. The characteristic root symptoms (red stele) generally appear only after 1 mo of infection. Our results supported the preliminary data briefly reported by Werres and Casper (20), especially in terms of precocity of detection compared to microscopic observations. In addition, we provided more information on the specificity of the antiserum and the reliability of the test.

The practicality and difficulty of isolating *P. fragariae* on a specific agar medium was confirmed. Isolation is only possible soon after infection, as already reported (12). Progression of infection leads to the formation of oospores, which are responsible for the survival of the fungus in the infected roots, at which time the fungus can no longer be isolated.

The reliability of ELISA was shown by the reproductibility of the results in the three trials carried out with *P. fragariae*

TABLE 4. Comparative testing for *Phytophthora fragariae* of strawberry plants, collected in the nursery, by enzyme-linked immunosorbent assay (ELISA) and the sporulation test

Cultivar/batch no. (no. of plants)	Presence of root symptoms (no. of plants with symptoms)	ELISA test[a] no. of positive plants (absorbance range for positive samples)	Sporulation test (no. of positive plants)
Red Gauntlet/424 (26)	0	0	0
Splendida/449 (20)	0	2 (0.101–0.145)	0
Honeoye/451 (27)	12	17 (0.113–0.621)	12
Honeoye/616 (13)	?[b]	13 (0.174–0.497)	9
Gorella/423 (12)	0	0	0
Gorella/448 (20)	0	19 (0.106–1.059)	17
Gorella/550 (10)	0	1 (0.362)	0
Elsanta/696 (22)	0	7 (0.095–0.278)	6
Gariguette/436 (16)	0	16 (0.223–0.735)	16

[a] Absorbance values (405 nm) were observed after a 2-hr incubation of the substrate; the threshold for positive reactions was calculated by adding the mean absorbance value of eight healthy samples to 3.5 times the value of the standard deviation.
[b] Presence of dubious symptoms on some plants.

inoculated plants. Of 100 samples analyzed after the appearance of the first positive result, only three were negative. The latter samples probably correspond to root extremities that were either not invaded or insufficiently invaded by the fungus. The homogeneity of the results shows that, in general, infection of the roots by *P. fragariae* is uniform. Slight differences were observed with the five varieties; nevertheless, none is known to present any particular resistance to *P. fragariae*. Scott et al (18) have mentioned a tolerance of the Red Gauntlet variety to two Scottish races of the parasite. The five varieties used in this work exhibited similar degrees of sensitivity to the strain AE 584.

The ELISA technique therefore seems to be an extremely valuable tool for the early detection of plants infected by *P. fragariae*. Although not offering any advantage in terms of earliness of detection when compared to the production of sporangia and the appearance of oospores, it does have the merit of easy implementation and application to numerous samples. Recent preliminary application for indexing of field samples has demonstrated the superiority of ELISA to the sporulation test and to visual observation of roots. These very encouraging results confirm the value of the technique for field testing. A large-scale indexing is now necessary to specify conditions for application of this ELISA technique for routine testing.

LITERATURE CITED

1. Aguelon, M., and Dunez, J. 1984. Immunoenzymatic techniques for the detection of *Phoma exigua* in infected potato tissues. Ann. Appl. Biol. 105:463-469.
2. Avrameas, S. 1969. Coupling of enzyme to proteins with glutaraldehyde. Use of conjugates for detection of antigens and antibodies. Immunochemistry 6:43-52.
3. Casper, R., and Mendgen, K. 1979. Quantitative serological estimation of a hyperparasite: Detection of *Verticillium lecanii* in yellow rust infected wheat leaves by ELISA. Phytopathol. Z. 94:89-91.
4. Clark, M. F., and Adams, N. A. 1977. Characteristics of the microplate method of enzyme linked immunosorbent assay for detection of plant viruses. J. Gen. Virol. 34:475-483.
5. Hickman, C. J. 1940. The Red Core root disease of the strawberry caused by *Phytophthora fragariae*. J. Pomol. Hortic. Sci. 18:89-119.
6. Hornock, L. 1979. Comparison of *Fusarium accuminatum* and *Fusarium culmorum* isolates by means of tandem-crossed immunoelectrophoresis. Antonie Van Leenwenhoek 45:293-302.
7. Koenig, R. 1981. Indirect ELISA methods for the broad specificity detection of plant viruses. J. Gen. Virol. 55:53-62.
8. Lowry, O. H. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 102:265-275.
9. Lung-Escarmant, B., and Dunez, J. 1979. Differentiation of *Armillariella* and *Clitocybe* species by the use of the immunoenzymatic ELISA procedure. Ann. Phytopathol. 11:515-518.
10. Malajczu, N., McComb, A. J., and Parker, C. A. 1975. An immunofluorescence technique for detecting *P. cinnamomi* Rands. Aust. J. Bot. 23:289-309.
11. Merz, W. C., Burell, R. G., and Gallegly, M. E. 1969. A serological comparison of six heterothallic species of *Phytophthora*. Phytopathology 59:367-370.
12. Montgomerie, I. G., and Kennedy, D. M. 1983. An improved method of isolating *Phytophthora fragariae*. Trans. Br. Mycol. Soc. 80:178-183.
13. Nachmias, A., Bar-Joseph, M., Solel, Z., and Barash, I. 1979. Diagnosis of mal secco disease in lemon by enzyme linked immunosorbent assay. Phytopathology 69:559-561.
14. Nourrisseau, J. G., Malato, G., and Baudry, A. 1983. Une nouvelle maladie fraisier en France: Le coeur rouge des racines. Phytoma 350:31-35.
15. Price, T. V. 1973. Serological identification of *Eutypa armeniacae*. Aust. J. Biol. Sci. 26:389-394.
16. Rapilly, F. 1968. Les techniques de mycologie en pathologie végétale. Ann. Epiphyties 19 n° H.S.
17. Ribeiro, O. K. 1978. A Source Book of the Genus *Phytophthora*. J. Cramer, ed. Ganter Verlag, FL 9490 Vaduz. 417 pp.
18. Scott, D. H., Draper, A. D., and Galetta, G. J. 1984. Breeding strawberries for Red Stele resistance. Plant Breed. Rev. USA 2:195-214.
19. Teranisiki, J., Figueiredo, M. B., Cardoso, G. R. M., and Namekata, T. 1973. The value of serological techniques for the differentiation between *Verticillium albo-atrum* and *V. dahliae*. Arq. Inst. Biol. S. Paulo 40:45-51.
20. Werres, S., and Casper, R. 1987. Nachweiss von *Phytophthora fragariae* Hickman in Wurzeln der Erdbeerkultursorte "Tenira" mit Hilfe des enzyme linked immunosorbent assay (ELISA). J. Phytopathol. 118:367-369.