Specificity of Cytoplasmic and Cell-wall Antigens from Four Species of *Phytophthora*

By DOROTHY M. HALSALL

Division of Plant Industry, CSIRO, Canberra, A.C.T. 2601, Australia

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SUMMARY

Cytoplasmic and cell-wall antigens and antisera were prepared from four *Phytophthora* species, and cell-wall antigens were prepared from two *Pythium* species. Immunodiffusion of the *Pythium* and *Phytophthora* cell-wall antigens showed that the two *Pythium* species did not cross-react with the *Phytophthora* cell-wall antisera. Immunodiffusion analysis of both cell-wall and cytoplasmic antigens of *Phytophthora* revealed some degree of specificity between species but not between A1 and A2 mating types in *Phytophthora cinnamomi*. Species specificity was improved by using indirect fluorescent antibody techniques and by the use of cross-absorbed sera. Agglutination and quantitative precipitation techniques did not significantly improve specificity.

It was possible to distinguish serologically between *Phytophthora cinnamomi* and *Phytophthora cambivora* and the *Phytophthora cryptogea*-*Phytophthora drechsleri* group. The absence of consistent serological variation between *P. cryptogea* and *P. drechsleri* is consistent with the suggestion (Bumbieris, 1974) that *P. cryptogea* and *P. drechsleri* should be considered as one species.

INTRODUCTION

Serological methods have been used widely in the taxonomy of the bacteria, but less widely in fungal taxonomy. Some work has been done on the serological differentiation of *Pythium* from *Phytophthora* (Morton & Dukes, 1967) and on comparing several species of *Phytophthora* by gel-diffusion methods (Burrell *et al.*, 1966; Merz, Burrell & Gallegly, 1969). In a study of six *Phytophthora* species, Merz *et al.* (1969) found *P. cinnamomi* to be serologically distinct, while the other five species resolved into two groups. However, some results were anomalous: two isolates of one species, *Phytophthora palmivora*, were placed in different groups. These observations suggest that results obtained by serological methods may conflict with the traditional morphological classification. More detailed serological studies were needed to establish the most suitable techniques and to provide a basis for the grouping or amalgamation of existing species.

Merz *et al.* (1969) used soluble cytoplasmic components as the antigens for the preparation of their antisera and in the immunodiffusion tests. But evidence from an examination of *Ophiobolus graminis* Sacc. (Choo & Holland, 1970; Holland & Choo, 1970) suggests that cell-wall antigens and antisera may be more specific than cytoplasmic antigens, so it was considered important to include these in our investigation of *Phytophthora* species.

This paper describes a serological study of several different species of *Phytophthora*, using cytoplasmic and cell-wall antigens and antisera, to determine the degree of specificity obtainable with these classes of antigens. Their possible value in the identification of *Phytophthora* species was also investigated.

Table 1. *Origin and host or plant association of Phytophthora and Pythium isolates*

Isolate	Origin*	Host/plant association
<i>Phytophthora cinnamomi</i> Rands		
CIN15	W.A.	<i>Eucalyptus marginata</i> Donn. ex Sm.
CIN114	Dwellingup, W.A.	Eucalyptus association
CIN410	Naghi, N.S.W.	Eucalyptus association
CIN83	East Gippsland, Victoria	Eucalyptus association
CIN111	Sterling, S.A.	Eucalyptus association
CIN291 A1	Ourimbah, N.S.W.	<i>Eucalyptus globoidea</i> Blakely
<i>P. drechsleri</i> Tucker		
DRE17	Black Mountain, A.C.T.	Eucalyptus association
DRE128	Northern Tasmania	Eucalyptus association
DRE111	Black Mountain, A.C.T.	Eucalyptus association
DRE181	Jindabyne, N.S.W.	Eucalyptus association
DRE149	Jindabyne, N.S.W.	<i>Eucalyptus stellulata</i> Sieb. ex DC.
<i>P. cryptogea</i> Pethybridge and Lafferty		
CRY102	ex V. Bumbieris, S.A.	Host unknown
CRY120	ex V. Bumbieris, S.A.	Host unknown
CRY121	ex V. Bumbieris, W.A.	Eucalyptus association
<i>P. cambivora</i> Petri		
CAM107	Western Australia	<i>Malus sylvestris</i> (L) Mill
<i>Pythium irregulare</i> Buisman		
PY25, CMI151841	Botanic Garden, A.C.T.	Host unknown
<i>Py. acanthicum</i> Drechsler		
PY11, CMI151836	Perth, Tasmania	Host unknown

* W.A., Western Australia; N.S.W., New South Wales, Australia; S.A., South Australia; A.C.T., Australian Capital Territory.

Phytophthora drechsleri Tucker and *Phytophthora cryptogea* Pethybridge and Lafferty were selected for study because they are very similar both morphologically and in growth tests at different temperatures (Bumbieris, 1974) and it has been suggested that they should be considered as one species. *Phytophthora cinnamomi* Rands was selected as a well-defined and separate species.

METHODS

Organisms. Four species of *Phytophthora* and two of *Pythium* were studied. In certain cases several different isolates of each species were included for comparison. These isolates were selected from widely diverse sources to detect the degree of diversity within each species (Table 1).

Media and culture of fungal mycelium. For both cytoplasmic and cell-wall preparations, the fungal cultures were grown in a defined liquid medium (Hendrix, Guttman & Wightman, 1969) containing either peptone or casein hydrolysate as the nitrogen source. Inoculum cultures (50 ml) were grown in 500 ml flasks for 5 days at 25 °C in static culture. The mycelium was macerated in a sterile Omnimix cup (Sorvall Inc., Norwalk, Connecticut, U.S.A.) and inoculated into either Roux bottles (100 ml) or penicillin flasks (250 ml) for rapid growth at 25 °C for 3 days. The mycelium was harvested by Buchner filtration and washed thoroughly with distilled water before antigen preparation.

Preparation of cytoplasmic antigens. For injection into rabbits, the mycelium was suspended in a minimum amount of 1 M-phosphate buffer pH 7.2, chilled in a CO₂-acetone mixture and macerated in a tissue grinder (Dual, 50 ml). The cell-wall debris was removed by centrifuging (2500 g) the thawed suspension, and the preparation was stored at -18 °C.

Cytoplasmic antigens for use in serological tests were prepared by the method of Merz *et al.* (1969) with the addition of EDTA (4 g l⁻¹) to the phosphate-buffered saline. Initial breakage was achieved using a Sorvall Omnimix (5 min), the slurry was chilled in ice, twice sonicated for 3 min (Biosonik, Bronwill Scientific, Rochester, New York, U.S.A.) and the cell debris removed by centrifuging (12 000 g). The preparations were stored at -18 °C but, because antigenic activity decreased after 2 months, the cytoplasmic antigens were prepared as required.

Preparation of cell-wall antigens. The washed mycelium from three penicillin flasks was combined and macerated in an Omnimix cup for 3 min with 20 ml phosphate-buffered saline. The slurry was centrifuged (12 000 g) and washed once. The cell material was broken finely by sonication (2 × 6 min), centrifuged (12 000 g) and washed three times with phosphate-buffered saline. The final suspensions were checked under a light microscope for the presence of cytoplasmic contents in any section of hypha, and stored at -18 °C.

Antisera. Rabbits were immunized with a primary intramuscular injection of an emulsion containing Freund's complete adjuvant (0.5 ml; Difco) plus the prepared antigen (0.5 ml). Four weeks later a second injection of the antigen alone (0.2 ml) was given subcutaneously. The rabbits were bled from the ear 5, 7, 9 and 12 days after the second injection, taking 10 and 20 ml volumes at alternate bleedings. After incubation at 37 °C for 2 h and storage overnight at 4 °C, the antisera were separated from the clots and stored, without the addition of preservative, at -18 °C. Bleeds were tested separately by gel diffusion for reaction with the homologous antigen. All bleeds of the antisera to the cytoplasmic antigens were similar and so were pooled. The different bleeds of the cell-wall antisera were not uniform, hence the bleed with the greatest activity was used for all work reported here.

Immunodiffusion. Several buffers were tested for their suitability for immunodiffusion with *Phytophthora* antigens: the most suitable was phosphate-EDTA buffer, pH 7.0 (0.067 M) (Allison & Humphrey, 1960) solidified with 0.75 % agar (Ion Agar no. 2, Oxoid). Gels were prepared 3 mm thick, with 5 mm diam. wells placed 4 mm apart; diffusions were carried out at 4 °C and examined after 4, 7 and 9 days.

Cross-absorption of antisera. Cell-wall antigens (1 ml) were washed with phosphate-buffered saline pH 7.0 (0.01 M, 5 ml), centrifuged (2000 g) and the pellets resuspended in buffered saline (0.2 ml). Undiluted antiserum (0.5 ml) was added and mixed thoroughly; the tubes were covered, incubated in a water bath at 37 °C for 2 h and refrigerated overnight. The antigen-antiserum mixture was diluted to 5 ml and mixed thoroughly. The complexed antigen-antibody was removed by centrifuging (2000 g), and the cross-absorbed antiserum decanted and stored at -18 °C (1:10 dilution of antiserum).

Agglutination tests. Cell-wall antigens were diluted (1:10) with phosphate-buffered saline pH 7.2 (0.07 M); antisera were diluted in the same buffer to give final concentrations of 1:20, 1:40, 1:80, 1:160, 1:320 and 1:640. Diluted antiserum (0.1 ml) and diluted antigen suspension (0.1 ml) were added to each agglutination tube and the two components mixed thoroughly. Tubes were incubated at 37 °C for 2 h and stored overnight at 4 °C.

Indirect fluorescent-antibody staining. Cell-wall antigens were diluted 1:5 and 1:10 with phosphate-buffered saline pH 7.2 (0.07 M) and antisera were diluted in the same buffer to give final concentrations of 1:10, 1:20, 1:40, 1:160 and 1:320. Fixing and staining were as described by Walker, Batty & Thomson (1971), acetone fixation being preferable to heat fixation. Three antigen smears were used per slide and 30 min allowed for both antigen-antiserum reactions. Fluorescein isothiocyanate-conjugated goat anti-rabbit antiserum (Microbiological Associated Inc., Bethesda, Maryland, U.S.A.) was used for the indirect labelling technique (Beutner, 1961). Controls were set up omitting the specific

anti-serum or the fluorescent antiserum, or using normal non-specific serum. Slides were examined by transmitted light fluorescence microscopy (Leitz Laborlux) and evaluated for green fluorescence which was readily distinguished from the blue autofluorescence.

Quantitative precipitation analysis for antibody. The method detailed by Maurer (1971) was followed. Preliminary experiments were carried out to determine the optimal volume of antigen suspension required to completely bind the antibody in 0.1 ml antiserum. Immediately before use, the antigens were washed with saline and the antisera centrifuged to remove denatured protein and lipid. Where heated antigens were used, the antigens were placed in a boiling water bath for 10 min and then cooled before washing. For each experiment controls were set up of antigen and antisera alone. Antigen (1.0 ml) and antiserum (0.1 ml) were mixed thoroughly in 8 ml tapered centrifuge tubes, incubated at 37 °C for 1 h then stored in the refrigerator for 5 days to maximize binding (Maurer, 1971). During this period, the contents of the tubes were mixed thoroughly each day. The antigen-antibody complex was centrifuged at 2000 *g* in a swing out head centrifuge (Mistral 6 L), then washed four times with chilled phosphate buffered saline pH 7.2 (0.07 M). The complex was treated with NaOH (5 mM, 2.5 ml) at 37 °C for 30 min to release the antibody, centrifuged (2000 *g*) and the supernatant retained. Samples (0.5 ml) of the supernatant were taken and the protein content was estimated by the method of Lowry *et al.* (1951); reagent A was modified to allow for the concentration of NaOH used in release of the antibody.

RESULTS

Immunodiffusion with cytoplasmic antigens

Antisera to cytoplasmic antigens of *P. drechsleri* DRE17 and *P. cinnamomi* CIN15 were prepared and used in the immunodiffusion analysis of cytoplasmic antigens of *P. drechsleri* DRE128, DRE111, DRE181; *P. cinnamomi* CIN114, CIN410, CIN83; *P. cryptogea* CRY102, CRY120, CRY121; and *P. cambivora* CAM107. Preliminary experiments showed that the undiluted antigen or a 1:2 dilution gave the maximum number of bands with satisfactory definition. Undiluted antigen preparations were used to compare the antigenic properties of these four species (Fig. 1*a,b,c*). *Phytophthora cinnamomi* antiserum formed more precipitin bands with each of the antigens tested than *P. drechsleri* antiserum (cf. Fig. 1*a,b*).

Cytoplasmic antigens of *P. cinnamomi* diffused against *P. cinnamomi* antiserum formed three distinct bands with a faint fourth band; two of these bands were common to the other three species examined but a single unique band formed a marked spur distinguishing *P. cinnamomi* (Fig. 1*a,c*). *Phytophthora cambivora* cytoplasmic antigens formed three distinct bands, one of which was unique to that isolate (Fig. 1*c*). *Phytophthora cryptogea* and *P. drechsleri* could not be distinguished from each other by immunodiffusion against *P. cinnamomi* antiserum.

Phytophthora drechsleri antiserum formed two indistinct bands with cytoplasmic antigens from all species tested. No species-specific bands were evident.

Heat-treated cytoplasmic antigens from all four *Phytophthora* species (Fig. 1*d*) produced only one common band with antisera to both species. The heat lability of the specific antigens suggests that they are probably proteins.

Antigens were prepared from opposite mating types of *P. cinnamomi* (A1, CIN291; A2, CIN410) and diffused against *P. cinnamomi* antiserum (A2, CIN15). There were no apparent antigenic differences between the different compatibility or mating types.

Fig. 1. Gel diffusion of cytoplasmic antigens of four *Phytophthora* species against antisera to two *Phytophthora* species. Antisera: (a, c, d) antiserum to *P. cinnamomi*; (b) antiserum to *P. drechsleri*. Antigens: (a, b, c) untreated and (d) heat-treated antigens of *P. cinnamomi* CIN410, CIN83, CIN114; *P. drechsleri* DRE128, DRE181, DRE111; *P. cryptogea* CRY102, CRY120, CRY121; and *P. cambivora* CAM107.

Immunodiffusion with cell-wall antigens

Antisera to cell-wall antigens of *P. drechsleri* DRE128, *P. cryptogea* CRY102, and *P. cinnamomi* CIN410 were prepared. Cell-wall antigens of *P. drechsleri* DRE128, DRE111, DRE181, DRE149, *P. cryptogea* CRY102, CRY120, CRY121, *P. cinnamomi* CIN410, CIN114, CIN111, CIN83 and *P. cambivora* CAM107 were used in comparative immunodiffusion studies.

Heat-treated and untreated antigens (Fig. 2a,b) gave similar results. If the precipitin bands were more clearly visible with heat-treated antigens, heat treatment was used in subsequent examination of these antigens by immunodiffusion.

Comparison of the precipitin bands formed by antigens of the different species diffused against *P. cinnamomi* antiserum (Fig. 2c) revealed a single clearly-defined band which lacked specificity. With both *P. drechsleri* (Fig. 2d) and *P. cryptogea* (Fig. 2b) antisera, a single indistinct band was formed to *P. cinnamomi* antigens. Against the same two antisera, all isolates of *P. cryptogea* and *P. drechsleri* formed a single distinct band, but two isolates of *P. drechsleri* (DRE128, DRE181) and one isolate of *P. cryptogea* (CRY102) formed an additional

Fig. 2. Gel diffusion of cell-wall antigens of four *Phytophthora* species against antisera to three *Phytophthora* species. Antisera: (a, b) antiserum to *P. cryptogea*; (c) antiserum to *P. cinnamomi*; (d) antiserum to *P. drechsleri*. Antigens: (a) untreated and (b, c, d) heat-treated antigens of *P. cinnamomi* CIN410, CIN83, CIN114, CIN111; *P. drechsleri* DRE128, DRE181, DRE111; *P. cryptogea* CRY102, CRY121, CRY120.

band indicative of a more slowly diffusing antigen (Fig. 2*b,d*). This second band was very strong in DRE128 and CRY102 but only faint in DRE181. The single isolate of *P. cambivora* also formed both precipitin bands although the second band was less well developed than for CRY102 and DRE128.

When cell-wall antigens of *Pythium irregulare* and *Pythium acanthicum* were diffused against each of the *Phytophthora* antisera, there was no reaction to any of the cell-wall antisera from either untreated or heat-treated antigens (Fig. 3*a,b*).

Fig. 3. Gel diffusion of cell-wall antigens of two species of *Pythium* and four species of *Phytophthora* against antiserum to *Phytophthora*. Antiserum: (a, b) antiserum to *P. cryptogea*. Antigens: (a) untreated and (b) heat-treated antigens of *P. cinnamomi* CIN410, *P. drechsleri* DRE128, *P. cryptogea* CRY102, *P. cambivora* CAM107, *Py. irregulare* PY25, *Py. acanthicum* PY11.

Table 2. *Agglutination reactions between homologous and heterologous cell-wall antigens and antisera of three Phytophthora species*

Inverse of maximum antiserum dilution factor giving positive agglutination reaction
(mean value of 2×2 replicates).

Antigen	<i>P. cinnamomi</i>	<i>P. drechsleri</i>	<i>P. cryptogea</i>
<i>P. cinnamomi</i>	640	80	60
<i>P. drechsleri</i>	80	480	320
<i>P. cryptogea</i>	320	320	320

Agglutination tests

Agglutination tests were scored as positive or negative at different dilutions of antiserum to a constant dilution of antigen. Agglutination of *P. cinnamomi* antiserum with the homologous antigen occurred at a dilution of 1:640, whereas with heterologous antigens, agglutination could not be detected below 1:320 dilution (Table 2). With *P. drechsleri* and *P. cryptogea* antisera, the results were less clear cut: in both cases agglutination with *P. cinnamomi* antigen did not occur below 1:80 dilution. However, there was little or no difference between these two species in the serum dilution at which agglutination occurred with either the homologous or heterologous antigen (Table 2).

Indirect fluorescent staining

The antiserum dilution at which fluorescence could be detected was greater for the homologous antigen-antiserum complex (Table 3) for each species, but there was a considerable degree of cross-reaction particularly between *P. drechsleri* and *P. cryptogea*. To reduce cross-reaction, a portion of each serum preparation was cross-absorbed with antigen prepared from isolates of each of the other two species. This resulted in dilution of the antiserum 1:10 and hence the dilution series used was modified accordingly. Cross-absorbed sera fluoresced to a lower dilution than the untreated sera but the specificity was

Table 3. *Indirect fluorescent-antibody staining of homologous and heterologous antigen-antisera reactions of three Phytophthora species*

Inverse of maximum antiserum dilution factor giving a positive fluorescent reaction (mean value of three replicates).

Antigen	<i>P. cinnamomi</i>	<i>P. drechsleri</i>	<i>P. cryptogea</i>
<i>P. cinnamomi</i>	160	60	80
<i>P. drechsleri</i>	80	160	160
<i>P. cryptogea</i>	80	80	80

Table 4. *Indirect fluorescent-antibody staining of homologous and heterologous antigenic reactions with cross-absorbed antisera for three Phytophthora species*

Inverse of maximum antiserum dilution factor giving a positive fluorescent reaction (mean value of three replicates).

Antigen with which cross-absorbed	<i>P. cinnamomi</i>		<i>P. drechsleri</i>		<i>P. cryptogea</i>	
	<i>P. drechsleri</i>	<i>P. cryptogea</i>	<i>P. cinnamomi</i>	<i>P. cryptogea</i>	<i>P. cinnamomi</i>	<i>P. drechsleri</i>
<i>P. cinnamomi</i>	20	20	1	1	1	1
<i>P. drechsleri</i>	1	1	80	20	80	20
<i>P. cryptogea</i>	1	1	20	20	80	40

considerably improved (Table 4). *Phytophthora cinnamomi* antiserum which had been cross-absorbed with either *P. drechsleri* or *P. cryptogea* antigens was specific to *P. cinnamomi* antigen after indirect fluorescence staining. *Phytophthora cryptogea* and *P. drechsleri* antisera cross-absorbed with *P. cinnamomi* antigen gave no fluorescence with *P. cinnamomi* antigen, but in each case cross-reaction between *P. drechsleri* and *P. cryptogea* was still evident. When *P. drechsleri* was cross-absorbed with *P. cryptogea* or vice versa fluorescence was evident only in the lower dilutions (1:20), indicating that the greater portion of the antibody had been removed, but specificity was not improved in the antibody activity remaining in the absorbed antiserum.

Quantitative precipitin analysis

Preliminary runs using this method for comparing the amount of antibody protein bound by an excess of insoluble antigen suggested that it might be possible to measure the differences between *P. drechsleri*, *P. cryptogea* and *P. cinnamomi*. It was found that heated antigen was preferable as this reduced the amount of non-specific protein reacting in the final assay. When the experiment was extended to a number of different isolates of the same species (Table 5), variation within any species was considerable. Within the four isolates of *P. cinnamomi*, the antibody protein bound from *P. cinnamomi* antiserum ranged from 100 to 82 %, whilst with the same antiserum, *P. drechsleri* antigens bound from 92 to 56 % and *P. cryptogea* from 100 to 80 %.

This high degree of variation within species would preclude the use of this method for distinguishing between species on the basis of the percentage cross-absorption of different antibodies.

Table 5. Quantitative precipitation reactions between multiple cell-wall antigens from three *Phytophthora* species and antisera to the same species

Antigens	Antibody					
	<i>P. cinnamomi</i> CIN410		<i>P. drechsleri</i> DRE128		<i>P. cryptogea</i> CRY102	
	Antibody protein (μ g)*	(%)†	Antibody protein (μ g)*	(%)†	Antibody protein (μ g)*	(%)†
<i>P. cinnamomi</i>						
CIN410	67	100	56	81.2	64	81
CIN114	56	83.6	51	73.9	65	82.3
CIN83	55	82.1	62	89.8	62	78.5
CIN111	61	91.0	65	94.2	62	78.5
<i>P. drechsleri</i>						
DRE128	54	80.6	69	100	66	83.5
DRE111	44	65.7	65	94.2	70	88.6
DRE181	62	92.5	61	88.4	86	108.9
DRE149	38	56.7	70	101.5	69	87.3
<i>P. cryptogea</i>						
CRY102	68	101.5	77	111.6	79	100
CRY121	54	80.6	69	100	74	93.7
CRY120	54	80.6	69	100	85	107.6

* Antibody protein absorbed by 1 ml antigen suspension.

† Weight of antibody protein absorbed by 1 ml homologous antigen = 100 %.

DISCUSSION

Cytoplasmic antigens of the *Phytophthora* species examined were heat-sensitive and unstable in storage at -18°C , which indicates the involvement of a protein component. Using gel-diffusion techniques, differences could be detected between *P. cinnamomi* and the single isolate of *P. cambivora* examined, and between these and a group consisting of *P. cryptogea* and *P. drechsleri*. No differences could be detected between *P. drechsleri* and *P. cryptogea*.

Cell-wall antigens of the four species were heat stable and stable in storage at -18°C , suggesting a polysaccharide composition. The pythiaceous fungi are reported to have 80 to 90 % carbohydrate in their cell wall (Novaes-Ledieu, Jiménez-Martínez & Villaneuva, 1967; Bartnicki-Garcia, 1966) with small amounts of protein and lipid. Some of this protein forms a glucan-protein complex (Novaes-Ledieu & Jiménez-Martínez, 1969) but possible differences between the cell-wall structure of *Pythium* and *Phytophthora* species are suggested by the more drastic treatment required to release the glucan-protein from *Pythium* cell walls (Novaes-Ledieu & Jiménez-Martínez, 1969). This suggestion is supported by the serological evidence, since cell-wall antigens from the two species of *Pythium* did not develop precipitin bands to any of the *Phytophthora* antisera. Hence, this method could be used to distinguish *Pythium* from *Phytophthora* species where differentiation is difficult by morphological methods.

Amongst the *Phytophthora* species, it was possible to distinguish *P. cinnamomi* and *P. cambivora* (although only one isolate of the latter species was examined) from the *P. cryptogea*-*P. drechsleri* group by employing gel-diffusion, agglutination or fluorescence-antibody techniques. The use of cross-absorbed sera increased the sensitivity of these methods.

However, *P. cryptogea* and *P. drechsleri* cannot be reliably distinguished from each other

by any of the methods employed here. Individual isolates can be distinguished by refined methods, such as quantitative precipitation of antibody or indirect fluorescent staining of cross-absorbed sera, but the variation within each species shown in the quantitative precipitation experiments made it impossible to distinguish between the two species. Bumbieris (1974) has indicated that these two species cannot be readily distinguished by morphological features or by growth at different temperatures in the presence of malachite green, and suggested they should be regarded as one species, i.e. *P. cryptogea* Pethybridge and Lafferty. This conclusion is supported by the degree of similarity between the isolates when examined by a variety of serological methods.

If *P. cryptogea* and *P. drechsleri* are considered as one species, then the differences observed between *P. cinnamomi*, *P. cambivora* and *P. cryptogea* indicate that techniques such as fluorescent labelling of cross-absorbed sera may be of value in taxonomic work with Phytophthora. The method also has potential for distinguishing Phytophthora from other fungal species on a root surface or in root sections.

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