Specificities of Monoclonal Antibodies to *Phytophthora cinnamomi* in Two Rapid Diagnostic Assays

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ABSTRACT

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Twenty-four monoclonal antibodies (MAbs) previously raised against aldehyde-fixed zoospores and cysts of the fungus *Phytophthora cinnamomi* were screened for their diagnostic specificities. Forty-five isolates of *P. cinnamomi,* 96 isolates encompassing 20 *Phytophthora* species, 17 *Pythium* species, three *Saprolegnia* species, and one isolate each of *Fusarium, Verticillium, Rhizoctonia,* and *Schizophyllum* were tested using an immunofluorescence assay (IFA) and an enzyme-linked immunosorbent assay (ELISA). In the IFA, 11 MAbs reacted with zoospores and cysts of all isolates of *P. cinnamomi* and no other species. These MAbs were thus species-specific in the IFA. One MAb reacted with zoospores and cysts of all the isolates of *Phytophthora* and was thus genus-specific in this assay. Preliminary screening of the 24 MAbs with the ELISA indicated that only 10 MAbs could detect their antigens in mycelial extracts of *P. cinnamomi.* When the 10 MAbs were tested on all fungal isolates, two detected all isolates of *P. cinnamomi* and no other species. These results indicated that these two MAbs were species-specific in the ELISA. No MAbs were found to be genus-specific in the ELISA. Only one MAb, Lpv-2, was species-specific in both assays. No MAbs were genus-specific in both assays.

Phytophthora cinnamomi Rands is a serious plant pathogen infecting over 1,000 different species worldwide (39). In Australia it is responsible for the loss of up to 75% of the native flora in infested areas (35,37). The loss of valuable timber trees and many flowering shrubs has serious economic as well as aesthetic effects. *P. cinnamomi* also causes many other serious diseases in nursery plants (including *Rhododendron* spp. and *Camellia* spp.) and agricultural crops (including avocado and pineapple) (39).

A number of different control strategies can be applied to limit losses due to this pathogen. Early detection and identification is an essential first step in deciding what procedures are required for control. Standard isolation and identification practices involve plating infected roots or soil onto selective media. Once a culture is isolated, it must be grown on standard media and colony morphology examined. The production of both asexual and sexual reproductive structures is also necessary in order for positive identification to be made. This is often a difficult task. Precise identification requires extensive knowledge of the morphology of *P. cinnamomi* and the genus *Phytophthora.*

The development of rapid diagnostic assays for the identification and quantification of *P. cinnamomi,* as well as the genus *Phytophthora,* would be of great value for the control of the diseases caused by these pathogens. Production of antibodies against a wide variety of plant viruses and bacteria has demonstrated that these immunological probes can possess the specificity required for diagnostic assays and kits (17). However, both polyclonal antisera and monoclonal antibodies (MAbs) developed to identify species of fungi often react with more than just the target fungus (8,26,33). For example, MAbs raised against *Humicola lanuginosa* (Griffon & Maublanc) Bunce in rice also react strongly with *Penicillium variable* Sopp (9). MAbs raised against soluble extracts of *P. fragariae* Hickman cross-react with *P. cactorum* (Lebert & Cohn) J. Schröt. and *Pythium middletonii* Sparrow (2). Antibodies raised against cell wall and soluble antigens of *P. cinnamomi* cross-react with *Pythium* species (26). Commercialized kits for the identification of *Phytophthora* species have been developed by AgriDiagnostics Associates (Cinnaminson, NJ) and are now available from Neogene Corp. (Lansing, MI) or Sigma (St. Louis, MO). Recent testing has shown that these kits are, however, not specific for individual species and also cross-react with *Pythium* species (1,33) and *Peronospora* species (33).

Over recent years, a number of MAbs have been raised against zoospores and cysts of *P. cinnamomi* (19–21). Initial screening using immunofluorescence microscopy indicated that some of these MAbs may be specific for *P. cinnamomi* and that others may be specific for the genus *Phytophthora* (18,21). In order to fully evaluate the usefulness of these MAbs for identifying *P. cinnamomi* or the genus *Phytophthora,* we have now screened them against an extensive collection of fungal isolates using immunofluorescence microscopy (IFA) and an enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Fungal isolates. A total of 141 *Phytophthora* isolates representing 21 species, 24 *Pythium* isolates representing 17 species, three *Saprolegnia* species, and isolates of *Rhizoctonia, Fusarium, Verticillium,* and *Schizophyllum* were obtained from several culture collections within Australia (Table 1). All Oomycete cultures were maintained under oil for long-term storage. All cultures were grown on V8 media for routine use. The IFA used zoospores and cysts. They were produced by the method described by Byrt and Grant (6) or by the dilute soil extract method described by Dolan and Coffey (12).

A crude mycelial extract was used for the ELISA. It was obtained by growing cultures in 1/10-strength V8-$CaCO_3$ broth (6) until mycelia covered the bottom of the petri dish. This ranged from 3 to 10 days depending on the growth rate of the fungus. The inoculation plugs were removed from the colony, and excess V8 broth was removed using a Büchner funnel and vacuum. The mycelial mat was then placed in a microcentrifuge tube, frozen in liquid nitrogen, and crushed with a steel rod. The frozen mycelium was mixed with phosphate-buffered saline (PBS; 10 mM sodium phosphate, 100 mM sodium chloride, pH 6.8) and then centrifuged to remove insoluble components. The supernatant was used as the crude extract. The protein concentration in the crude extract was determined using the Bradford reagent assay with bovine serum albumin (BSA) as the standard.

Monoclonal antibodies. Twenty-four MAbs previously raised to aldehyde-fixed zoospores and cysts of *P. cinnamomi* H1000 (6BR, DAR 52646) (19–21) were screened for their diagnostic

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Table 1. List of fungal isolates used, their collection numbers, mating type, host, and location from which isolated and the source of the culture

Species	Isolate	Number	Mating type[a,b]	Host[a]	Location[a,c]	Source[d]
Phytophthora						
batemanensis	H 1014	DAR 50182	NA	*Avicennia marina*	Sydney, NSW	1
	H 1015	DAR 41559	NA	*Avicennia* sp.	Batemans Bay, NSW	1
boehmeriae	H 1026	VPRI 10577	H	NA	NA	3 (#277)
cactorum	H 1016	DAR 37628	H	NA	NA	1
	H 1039	1199	H	NA	NA	3
cambivora	H 1045	1221	NA	NA	NA	3
	H 1163	C5980	NA	Soil	Geraldton, WA	4
cinnamomi	H 1003	A138	A1	NA	Ourimbah, NSW	5
	H 1004	A2114	A2	NA	NA	5
	H 1005	A2150	A2	NA	NA	5
	H 1006	A2156	A2	NA	NA	5
	H 1007	A2347	A2	NA	NA	5
	H 1008	A2374	A2	NA	NA	5
	H 1009	A2420	A2	NA	Ourimbah, NSW	5
	H 1010	A2423	A2	NA	Ourimbah, NSW	5
	H 1011	P293	A2	*Eucalyptus globoidea*	Ourimbah, NSW	6
	H 1012	P397	A1	NA	NSW	6
	H 1113	3266	A1	Soil	Cape Howe, WA	8
	H 1114	3224	A2	*Adenanthos* sp.	Eneabba, WA	8
	H 1115	251N12	A2	*Pinus radiata*	WA	8
	H 1116	480R1	A2	*Banksia* sp.	Molly Island, WA	8
	H 1117	3262	A1	Soil	Cape Arid, WA	8
	H 1118	SC381	A2	*Casuarina fraserana*	Jarrahdale, WA	8
	H 1119	DCE210	A2	*Eucalyptus marginata*	Jarrahdale, WA	8
	H 1000	DAR 52646	A2	NA	Brisbane Ranges, VIC	9, 6BR
	H 1001	A278	A2	NA	NA	5
	H 1060	CR6A	A2	*Castanea sativa*	Norton Summit, SA	10
	H 1064	A14	A2	*Dryandra* sp.	WA	5
	H 1065	A112	A1	*Eucalyptus gummifera*	Kioloa, NSW	5
	H 1066	A148	A1	*Pinus radiata*	Wynabeel, QLD	5
	H 1067	IMI 292083	A1	Soil	Murwillumbah, NSW	A110
	H 1068	A2223	A2	*Banksia marginata*	Grampians, VIC	5
	H 1069	IMI 200344	A1	*Pinus elliotti*	Beerburrum, QLD	A115
	H 1070	A285	A2	*Persea americana*	Alstonville, NSW	5
	H 1071	A2217	A2	*Eucalyptus sieberi*	Nowa Nowa, VIC	5
	H 1072	A11	A1	*Acacia ulicifolia*	Bribie Island, QLD	5
	H 1073	A26	A2	*Casuarina cunninghamii*	Barton, ACT	5
	H 1074	A282	A2	*Persea americana*	Malanda, QLD	5
	H 1077	7587	A2	Soil	Sogeri, PNG	11
	H 1078	7249	A2	Soil	Bulolo, PNG	11
	H 1079	7215	A2	*Rhododendron* sp.	Edie Creek, Wau, PNG	11
	H 1080	7200	A2	*Pinus kesiya*	Aiyura, PNG	11
	H 1081	7096	A2	*Castanopsis* sp.	Mauki, Bulolo, PNG	11
	H 1082	7157	A2	*Nothofagus* sp.	Mt. Kaindi, Wau, PNG	11
	H 1084	7013	A2	*Castanopsis* sp.	Kaindi, Wau, PNG	11
	H 1085	A143	A1	*Rhododendron* sp.	Nara Prefecture, Japan	5
	H 1086	A144	A1	*Rhododendron* sp.	Nara Prefecture, Japan	5
	H 1087	A117	A1	NA	NA	5
	H 1092	7617	A1	*Persea americana*	Kuk, Mt. Hagen, PNG	11
	H 1094	IMI 292089	A1	*Araucaria cunninghamii*	Woitape, PNG	11 (#7369)
	H 1095	IMI 292086	A1	*Castanopsis* sp.	Garaina, PNG	11 (#7126)
	H 1096	7168	A1	*Araucaria cunninghamii*	Oksapmin, PNG	11
citricola	H 1017	P32	H	NA	WA	6
	H 1046	DAR 35047	H	Soil	Eden, NSW	1, ATCC 60564
	H 1049	IMI 334847	H	*Antirrhinum majus*	Nairne, SA	1, DAR 64697
	H 1056	1180	H	NA	NA	3
	H 1131	1221	H	Soil	Kalbarri, WA	8
	H 1132	1450	H	Soil	Walpole, WA	8
	H 1133	3237	H	*Banksia prionotes*	Jurien, WA	8
	H 1134	IMI 329676	H	Soil	Jarrah forest, WA	8 (#1723)
	H 1135	15B-2-6C	H	*Pinus radiata*	WA	8
	H 1136	3253	H	*Banksia attenuata*	Yanchep, WA	8
	H 1137	2952	H	Soil	Nannup, WA	8
	H 1157	6000	H	Soil	Geraldton, WA	4

(*continued on next page*)

[a]NA, not available.
[b]H, homothallic.
[c]ACT, Australian Capital Territory; NSW, New South Wales; NT, Northern Territory; PNG, Papua New Guinea; QLD, Queensland; SA, South Australia; TAS, Tasmania; VIC, Victoria; WA, Western Australia.
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Table 1. *(continued from preceding page)*

Species	Isolate	Number	Mating type[a,b]	Host[a]	Location[a,c]	Source[d]
citrophthora	H 1018	DAR 41736	H	*Citrus sinensis*	Peats Ridge, NSW	2
cryptogea	H 1050	DAR 37579	A2	*Melaleuca* sp.	Wesley Vale, TAS	1, ATCC 36229
	H 1051	DAR 37623	A2	NA	SA	1, ATCC 56226
	H 1120	1462	A2	Soil	South Coast, WA	8
	H 1121	3252	A1	Soil	Cape Arid, WA	8
	H 1122	IMI 329673	A2	Soil	Jarrah forest, WA	8 (#1136)
	H 1123	3338	A2	Soil	Fitzgerald River NP, WA	8
	H 1124	252W12	A2	*Pinus radiata*	Jarrahwood plantation, WA	8
	H 1125	3267	A1	Water	Dwellingup, WA	8
	H 1126	3121	A1	NA	Walpole, WA	8
	H 1025	IMI 129907	NA	NA	WA	6 (P11)
	H 1127	Wong 1	A1	*Banksia attenuata*	Wongonderrah swamp, WA	8
	H 1128	3388	A1	Soil	Roleystone, WA	8
	H 1129	3383	A1	NA	Albany, WA	8
	H 1130	3360	A1	Soil	Busselton, WA	8
erythroseptica	H 1019	VPRI 10578	H	*Solanum tuberosum*	VIC	3 (#278)
	H 1020	VPRI 10581	H	NA	NA	3 (#281)
	H 1101	89.04	H	*S. tuberosum*	University of Adelaide, SA	12
	H 1162	C5856	H	NA	Curtin U., Perth, WA	4
heveae	H 1030	VPRI 10575	H	NA	NA	3 (#275)
meadii	H 1031	VPRI 10570	NA	NA	NA	3 (#270)
megasperma	H 1035	VPRI 10566	H	*Pyrus communis*	Packham, VIC	3 (#266)
	H 1040	VPRI 10562	H	NA	NA	3 (#262)
	H 1043	NA	H	NA	NA	7 (race 2)
	H 1163	C5837	H	*Carya illinoensis*	NA	4
	H 1164	C5858	H	NA	Curtin U., Perth, WA	4
megasperma var. *megasperma*	H 1021	DAR 43045	H	*Brassica rapa* var. *silvestris*	Forbes, NSW	1
	H 1053	DAR 52534	H	var. *napus*	Forbes, NSW	1
	H 1054	DAR 52535	H	var. *napus*	Condobolin, NSW	1
	H 1138	3248	H	Soil	Cape Arid, WA	8
	H 1139	2732	H	NA	Fitzgerald River NP, WA	8
	H 1140	3215	H	*Banksia occidentalis*	Black Point, WA	8
	H 1059	3925	H	NA	NA	7 (race 3)
	H 1052	DAR 66135	H	*Glycine max*	Forbes, NSW	1
	H 1057	1745	H	NA	NA	7 (race 1)
	H 1058	4065	H	NA	NA	7 (race 2)
	H 1141	IMI329671	H	NA	Hopetoun, WA	8 (#1612)
	H 1142	48C3R	H	*Pinus radiata*	Jarrahwood plantation, WA	8
	H 1143	1321	H	Soil	Esperance, WA	8
	H 1144	AHP-11	H	*Eucalyptus caesia*	Ardross, Perth, WA	8
nicotianae	H 1042	VPRI 10559	NA	*Solanum* sp.	NA	3 (#259)
	H 1105	M5595	NA	*Carica papaya*	Innisfail, QLD	12
	H 1106	M5602A	NA	*C. papaya*	Innisfail, QLD	12
	H 1107	M5602B	NA	*C. papaya*	Innisfail, QLD	12
	H 1108	M5627	NA	*C. papaya*	Townsville, QLD	12
nicotianae var. *nicotianae*	H 1032	867	NA	NA	NA	3
	H 1041	VPRI 10451	NA	*Solanum* spp.	WA	3 (#151)
	H 1097	M3049	NA	*Nicotiana tabacum*	NA	12
	H 1098	S1S3	NA	*N. alata*	Melbourne, VIC	12
	H 1100	4320	NA	*N. tabacum*	Walkamin, QLD	12
	H 1102	M4974	A2	*N. tabacum*	Tabaccum, QLD	12
	H 1103	M4975	NA	*N. tabacum*	Tabaccum, QLD	12
	H 1109	M4964	NA	*N. tabacum*	Walkamin, QLD	12
	H 1110	M4936	NA	*N. tabacum*	Tabaccum, QLD	12
	H 1111	M4951	NA	*N. tabacum*	Mareeba, QLD	12
	H 1112	M3034	NA	*N. tabacum*	Paddy's Green, QLD	12
	H 1145	3293	A2	*Banksia leptophylla*	NA	8
	H 1146	3375	A2	NA	NA	8
	H 1147	IMI 329672	A2	*Banksia* sp.	Westfield, Perth, WA	8 (#1621)
	H 1148	GW3R	A2	*Chamelaucium* sp.	South Perth, WA	8
	H 1149	3376	A2	*Banksia attenuata*	Woodvale, Perth, WA	8
nicotianae var. *parasitica*	H 1033	1232	NA	NA	NA	3
	H 1099	M4804	NA	*Dianthus caryophyllus*	Marreba, QLD	12
palmivora	H 1022	1732	NA	NA	NA	7
	H 1044	615	NA	NA	NA	7
	H 1104	M5227	NA	*Carica papaya*	Cairns, QLD	12
polymophica	H 1023	DAR 41562	NA	*Eucalyptus* sp.	Batemans Bay, NSW	1
sojae	H 1169	T10078	H	*Glycine max*	Toowoomba, QLD	13 (race 1)
	H 1170	T10070	H	*G. max*	Toowoomba, QLD	13 (race 4)
species	H 1002	A128	A1	NA	NT	5
	H 1024	AB (5)	NA	*Avicennia marina*	Botany Bay, NSW	2

(continued on next page)

Table 1. (*continued from preceding page*)

Species	Isolate	Number	Mating type[a,b]	Host[a]	Location[a,c]	Source[d]
	H 1089	7099	A1	*Araucaria cunninghamii*	Heads Hump, Bulolo, PNG	11
	H 1091	IMI 292087	A1	Soil	Erave, PNG	11 (#7218) (A124)
	H 1168	C6606	NA	*Chamelaucium uncinatum*	Landsdale, WA	4
syringae	H 1055	DAR 66142	H	*Cymbidium* sp.	Artarmon, NSW	1
vignae	H 1034	901-5	H	NA	QLD	9 (race 2)
Pythium						
acanthicum	H 212	IMI 331770		*Lupinus angustifolius*	Manildra, NSW	1, DAR 64010
	H 223	WA 1809		*Brassica rapa*	Cowaramup, WA	4
aphanidermatum	H 200	DAR 60714		*Capsicum annum*	Stuart's Point, NSW	1
	H 201	DAR 61304		*Cucumis sativus*	Sydney, NSW	1
butleri	H 202	DAR 35082		*Lycopersicon esculentum*	Kanwal, NSW	1
coloratum	H 218	DAR 64014		Soil	Young, NSW	1
debaryanum	H 209	404		NA	NA	3
	H 215	DAR 55029		*Triticum aestivum*	Horsham, VIC	1
irregulare	H 203	DAR 34697		Soil	Richmond, NSW	1
	H 204	400		NA	NA	3
	H 207	DAR 54950		*T. aestium*	Cowra, NSW	1
mamillatum	H 210	403		NA	NA	3
	H 214	IMI 254251		*Trifolium subterraneum*	Boxwood, VIC	3, DAR 63997
middletonii	H 213	IMI 293951		*Glycine max*	Casino, NSW	1, DAR 51509
myriotylum	H 205	DAR 48995		*Solanum tuberosum*	ARS Inst., Yanco, NSW	1
periplocum	H 206	DAR 50436		*Stenotaphrum secundatum*	Bourke, NSW	1
rostratum	H 211	IMI 331761		*Triticum aestivum*	Young, NSW	1, DAR 63928
species	H 208	DAR 34722A		NA	NA	NA
spinosum	H 220	DAR 43419		*Telopea* sp.	Camden, NSW	1
	H 221	WA 1354		*Pyrus* sp.	South Perth, WA	4
splendens	H 219	DAR 55026		*Hordeum vulgare*	Keith, SA	1
ultimum var. *sporangiiferum*	H 216	DAR 37968		*Macadamia integrifolia*	Lindendale, NSW	1
var. *ultimum*	H 217	DAR 35790a		*Brassica rapa*	Narromine, NSW	1
vanterpoolii	H 222	WA 1546		*Poa* sp.	Perth, WA	4
Saprolegnia						
diclina	H 301	1376A		NA	SA	14
ferax	H 302	1494		NA	SA	14
parasitica complex	H 303	1372		NA	SA	14
Fusarium avenaceum	H 401	C 5976		*Diathus caryophyllus*	Dog Hill, WA	4
Rhizoctonia spp.	H 400	C 6608		*Petroselinum* sp.	Landsdale, WA	4
Schizophyllum spp.	H 403	NA		Soil	Wilson's Promontory, VIC	15
Verticillium dahliae	H 402	C5433		*Persea americana*	NA	4

specificities. Based on their labeling patterns in the IFA (19–21), these MAbs were classified into the groups Zt, Zg, Cpa, Lpv, Gvv, Cpw, and ZCp.

IFA. Zoospores were fixed in 4% paraformaldehyde in 50 mM piperazine-*N,N′*-bis-(2-ethanesulfonic acid)(PIPES) buffer (pH 7.0). Aliquots (14 μl) of zoospores were applied to the wells of a multiwell microscope slide and air-dried at 37 C for 45 min. After a PBS rinse, 14 μl of the selected hybridoma supernatants was added and incubated at 37 C for 45 min. Purified nonimmune mouse antibody at 10 μg/ml was used as the negative control. Slides were washed twice in PBS, and 14 μl of FITC-conjugated sheep $F(ab')_2$ antimouse immunoglobulin (diluted 1:30 in PBS with 1% BSA added. The slides were incubated at 37 C for 45 min and then washed twice in PBS, rinsed in distilled water, and coverslips were mounted in a glycerol-based mounting medium containing 0.1% paraphenylenediamine. A Zeiss Axioplan microscope equipped with filters appropriate for FITC fluorescence was used to examine the slides. Zoospores showing fluorescence levels (rated visually) above the nonimmune mouse immunoglobulin (NIM) negative control were scored as positive (Table 2). The majority of isolates were screened twice. Isolates giving initially ambiguous results were screened three or four times.

ELISA. A standard indirect ELISA (7) was employed. Ninety-six-well microtiter plates were coated with 50 μl of crude mycelial extract containing 5–50 μg/ml of protein. The plates were incubated for 1 hr and then washed three times in PBS containing 0.05% Tween 20. All wells were blocked with 5% skim milk in PBS for 1 hr. Samples (50 μl) of the selected MAbs (hybridoma supernatants) were then incubated in the wells for 1 hr and washed as described above. Horseradish-peroxidase-conjugated sheep $F(ab')_2$ antimouse immunoglobulin diluted 1:2,000 in PBS-Tween was added to the wells, incubated for 1 hr, and washed as above. The substrate, 2,2′-azino-*bis*-(3-ethylbenzthiazoline sulfonic acid), was then incubated for 20 min. Purified NIM antibody at 10 μg/ml was used as the negative control. Plates were read at 405 nm, and wells were scored as positive if the reading was greater than twice the mean of NIM readings for all isolates on the plate. Wells were scored as negative if the reading was lower than this value (Table 3). Based on the ELISA readings for the known positives, a cutoff value of twice the mean NIM reading was established to ensure that no false positives would be read. All isolates were screened using two wells per isolate per plate, and all screens were conducted twice. Known positive (*P. cinnamomi* isolate H 1000) and negative (NIM) controls were included in all screens.

RESULTS

Immunofluorescent assay. Due to the difficulty of obtaining zoospores from some isolates, only those isolates listed in Table 2 were screened. Of the 24 MAbs tested, Zt-1, Zt-2, Cpa-4, Cpa-5, Cpa-6, Cpa-7, Cpa-10, Cpa-11, Lpv-2, Lpv-5, and Gvv were found to label the zoospores and/or cysts of all isolates of *P. cinnamomi* and no other species (Table 2). Zt-1, Zt-2, and Gvv MAbs usually labeled *P. cinnamomi* cells weakly. Zoospores and cysts labeled with Cpa-4, Cpa-5, Cpa-6, Cpa-7, Cpa-10, Cpa-11, Lpv-2, and Lpv-5 MAbs were always strongly fluorescent. Although Zg-2, Zg-3, Zg-4, and Cpa-12 labeled only *P. cinnamomi* isolates, they did not label

Table 2. Reaction of selected monoclonal antibodies with aldehyde-fixed zoospores and cysts in the immunofluorescence assay[a]

Species	Isolate	Zt-1	Zt-2	Zg-1	Zg-2	Zg-3	Zg-4	Cpa-3	Cpa-4	Cpa-5	Cpa-6	Cpa-7	Cpa-8	Cpa-9	Cpa-10	Cpa-11	Cpa-12	Lpv-1	Lpv-2	Lpv-3	Lpv-4	Lpv-5	Gvv	Cpw-4	ZCp-2	Tub[b]	NIM[c]
Phytophthora																											
boehmeriae	H 1026	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−
cactorum	H 1016	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−
	H 1039	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−
cambivora	H 1045	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−
	H 1063	−	−	−	−	NT	−	−	−	−	−	−	NT	−	−	−	NT	−	−	−	NT	NT	NT	+	+	+	−
cinnamomi	H 1001	+	NT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1003	+	NT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1004	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1005	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1006	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1008	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1009	+	NT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1010	+	NT	+	+	NT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1011	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1012	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1060	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1064	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1065	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1066	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1067	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1069	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1070	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1072	+	+	+	NT	NT	NT	+	+	+	+	+	NT	+	+	+	−	+	+	+	+	+	+	+	+	+	−
	H 1073	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1074	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1075	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	H 1076	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
citricola	H 1049	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−
	H 1056	−	−	NT	−	−	−	−	NT	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−
citrophthora	H 1018	−	−	−	−	NT	NT	−	−	NT	NT	NT	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−
cryptogea	H 1050	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−
	H 1051	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−
	H 1062	−	−	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	+	+	−
erythroseptica	H 1019	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	−	−	−	−	−	−	−	−	−	NT	NT	+	+	+	−
	H 1020	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−
meadii	H 1031	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−
megasperma	H 1043	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−
meg. var. *sojae*	H 1052	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	NT	−	−	−	−	−	NT	+	+	+	−
nicotianae	H 1041	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+	−	−	+	+	+	−
	H 1042	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+	−	−	+	+	+	−
nicotianae var. *nic.*	H 1032	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	+	+	+	−
	H 1033	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+	−	−	+	+	+	−
palmivora	H 1022	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−
species	H 1002	−	−	−	−	−	−	+	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	+	+	+	−
syringae	H 1055	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	NT	NT	NT	NT	NT	NT	+	+	+	−
vignae	H 1034	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−
Pythium																											
acanthicum	H 212	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	NT	−	+	−
aphanidermatum	H 200	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−
	H 201	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−
butleri	H 202	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
coloratum	H 218	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
mamillatum	H 214	−	NT	NT	−	−	−	NT	−	NT	−	−	NT	−	−	NT	−	−	−	−	−	−	−	−	−	+	−
middletonii	H 213	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	NT	−	−	−	−	−	−	NT	−	+	−
myriotylum	H 205	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
periplocum	H 206	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
species	H 207	−	NT	NT	−	−	−	NT	−	NT	−	−	NT	−	−	NT	−	−	−	−	−	−	−	−	−	+	−
Saprolegnia																											
diclina	H 301	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
ferax	H 302	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
parasitica complex	H 303		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−

[a] Positive reaction (+), negative reaction (−), not tested (NT).
[b] Anti-β-tubulin (Amersham Int., Australia).
[c] Nonimmune mouse immunoglobulin.

all of the *P. cinnamomi* isolates screened. MAbs Zg-1, Cpa-3, Cpa-8, Cpa-9, Cpa-12, Lpv-1, Lpv-3, and Lpv-4 labeled some of the other *Phytophthora* isolates screened. MAb ZCp-2 labeled all the *Phytophthora* isolates screened in the IFA but did not react with isolates of any other genus. Cpw-4 labeled all the *Phytophthora* species but also reacted with *Pythium aphanidermatum* (Edson) Fitzp. No MAbs were positive for the *Saprolegnia* isolates screened. In addition, there was no correlation to mating type, host, or location of isolation for any of the MAbs tested.

ELISA. Preliminary screening of the 24 MAbs against crude mycelial extracts of *P. cinnamomi* (isolate H1000) resulted in only Cpa-3, Cpa-4, Cpa-6, Cpa-7, Cpa-10, Lpv-1, Lpv-2, Lpv-3, Lpv-4, and Lpv-5 MAbs reacting positively. Thus only these MAbs were used when screening the other fungal isolates. MAbs Lpv-2 and Lpv-3 reacted with all the *P. cinnamomi* isolates but no other species (Table 3). All Cpa and Lpv-5 MAbs were negative for some of the *P. cinnamomi* isolates tested, whereas Lpv-1 and Lpv-4 were positive for some of the *P. nicotianae* isolates tested. No MAbs were found to be specific for the genus *Phytophthora* when tested in the ELISA.

DISCUSSION

Extensive screening of a wide variety of fungal isolates has identified the specificities of MAbs that were raised against aldehyde-fixed zoospores and cysts of *P. cinnamomi* isolate H1000 (6BR). In the IFA, 11 MAbs were found to be species-specific, and one MAb was genus-specific. In the ELISA, two MAbs were species-specific and none was genus-specific. The species-specific antibodies promise to be useful in diagnostic assays and commercial kits for the rapid and reliable identification of *P. cinnamomi.*

The antibodies we have characterized show much greater specificity than any others so far produced against a *Phytophthora* species. Although many of the antisera or MAbs produced by others have proved useful for monitoring the presence of *Phytophthora* species in water, soil, or infected plant material (1,2,4,25,28,31,33,36), none of these is species-specific, and cross-reactivity at both species and genus levels (1,2,5,16, 24,26–31,33,36,38) imposes a limitation on their potential value. It would appear that despite successes in obtaining species-specific antibodies to *Ophiostoma* (11), *Pseudocercosporella* (8), and *Penicillium islandicum* Sopp (10), the generation of species-specific antibodies to fungi is far from a routine procedure.

There are likely to be a number of reasons for the difficulties in obtaining species-specific antibodies for fungi. For a start it is still true, especially for *Phytophthora*, that the majority of studies have generated polyclonal antisera rather than MAbs to the target fungus. While a MAb recognizes a single antigenic epitope, a polyclonal antiserum, even after affinity purification or preadsorption, will recognize a range of epitopes on the antigen. This will increase the chances of cross-reactivity. The character of the antigenic preparation

Table 3. Reaction of Cpa and Lpv antibodies with crude extracts from fungal mycelia in the enzyme-linked immunosorbent assay[a]

Species	Isolate	Cpa-3	Cpa-4	Cpa-6	Cpa-7	Cpa-10	Lpv-1	Lpv-2	Lpv-3	Lpv-4	Lpv-5
Phytophthora											
batamanensis	H 1014	−	−	−	−	−	−	−	−	−	−
	H 1015	−	−	−	−	−	−	−	−	−	−
boehmeriae	H 1026	−	−	−	−	−	−	−	−	−	−
cactorum	H 1016	−	−	−	−	−	−	−	−	−	−
	H 1039	−	−	−	−	−	−	−	−	−	−
cambivora	H 1045	−	−	−	−	−	−	−	−	−	−
	H 1153	−	−	−	−	−	−	−	−	−	−
cinnamomi	H 1000	+	+	+	+	+	+	+	+	+	+
	H 1001	+	+	+	+	+	+	+	+	+	+
	H 1003	+	+	+	+	+	+	+	+	+	+
	H 1004	+	+	+	+	+	+	+	+	+	+
	H 1005	+	+	+	+	+	+	+	+	+	−
	H 1006	+	+	+	+	+	+	+	+	+	+
	H 1007	+	+	+	+	+	+	+	+	+	+
	H 1008	+	+	+	+	+	+	+	+	+	+
	H 1009	+	+	+	+	+	+	+	+	+	+
	H 1010	+	+	+	+	+	+	+	+	+	+
	H 1011	+	+	+	+	+	+	+	+	+	+
	H 1012	+	+	+	+	+	+	+	+	+	+
	H 1060	−	−	−	−	−	+	+	+	+	−
	H 1064	+	+	+	+	+	+	+	+	+	+
	H 1065	+	+	+	+	+	+	+	+	+	+
	H 1066	+	+	+	+	+	+	+	+	+	+
	H 1067	+	+	+	+	+	+	+	+	+	+
	H 1068	−	−	−	−	−	+	+	+	+	−
	H 1069	+	+	+	+	+	+	+	+	+	+
	H 1070	+	+	+	+	+	+	+	+	+	+
	H 1071	+	+	+	+	+	+	+	+	+	+
	H 1072	+	+	+	+	+	+	+	+	+	−
	H 1073	+	+	+	+	+	+	+	+	+	+
	H 1074	+	+	+	+	+	+	+	+	+	+
	H 1077	+	+	+	+	+	+	+	+	+	+
	H 1078	−	−	−	−	−	+	+	+	+	+
	H 1079	−	−	−	+	−	+	+	+	+	+
	H 1080	+	+	+	+	+	+	+	+	+	+
	H 1081	+	+	+	+	+	+	+	+	+	+
	H 1082	+	+	−	+	+	+	+	+	+	+
	H 1084	+	+	+	+	+	+	+	+	+	+
	H 1085	+	+	+	+	+	+	+	+	+	+
	H 1086	−	−	−	−	−	+	+	+	+	+
	H 1087	+	+	−	+	+	+	+	+	+	+
	H 1092	+	+	+	+	+	+	+	+	+	+
	H 1094	−	−	−	+	−	+	+	+	+	+
	H 1095	−	−	−	−	−	+	+	+	+	−
	H 1096	−	−	−	+	−	+	+	+	+	+
	H 1113	+	+	+	+	+	+	+	+	+	+
	H 1114	+	+	+	+	+	+	+	+	+	+
	H 1115	+	+	+	+	+	+	+	+	+	+
	H 1116	+	+	+	+	+	+	+	+	+	+
	H 1117	+	+	+	+	+	+	+	+	+	−
	H 1118	+	+	+	+	+	+	+	+	+	−
	H 1119	+	+	+	+	+	+	+	+	+	+
citricola	H 1017	−	−	−	−	−	−	−	−	−	−
	H 1046	−	−	−	−	−	−	−	−	−	−
	H 1049	−	−	−	−	−	−	−	−	−	−
	H 1056	−	−	−	−	−	−	−	−	−	−
	H 1131	−	−	−	−	−	−	−	−	−	−
	H 1132	−	−	−	−	−	−	−	−	−	−
	H 1133	−	−	−	−	−	−	−	−	−	−
	H 1134	−	−	−	−	−	−	−	−	−	−
	H 1135	−	−	−	−	−	−	−	−	−	−
	H 1136	−	−	−	−	−	−	−	−	−	−
	H 1137	−	−	−	−	−	−	−	−	−	−
	H 1157	−	−	−	−	−	−	−	−	−	−
citrophthora	H 1018	−	−	−	−	−	−	−	−	−	−
cryptogea	H 1050	−	−	−	−	−	−	−	−	−	−
	H 1051	−	−	−	−	−	−	−	−	−	−
	H 1120	−	−	−	−	−	−	−	−	−	−
	H 1121	−	−	−	−	−	−	−	−	−	−
	H 1122	−	−	−	−	−	−	−	−	−	−

(*continued on next page*)

[a]Positive reaction (+), negative reaction (−).

Table 3. (*continued from preceding page*)

Species	Isolate	Cpa-3	Cpa-4	Cpa-6	Cpa-7	Cpa-10	Lpv-1	Lpv-2	Lpv-3	Lpv-4	Lpv-5
	H 1123	–	–	–	–	–	–	–	–	–	–
	H 1124	–	–	–	–	–	–	–	–	–	–
	H 1125	–	–	–	–	–	–	–	–	–	–
	H 1126	–	–	–	–	–	–	–	–	–	–
dreschleri	H 1025	–	–	–	–	–	–	–	–	–	–
	H 1127	–	–	–	–	–	–	–	–	–	–
	H 1128	–	–	–	–	–	–	–	–	–	–
	H 1129	–	–	–	–	–	–	–	–	–	–
	H 1130	–	–	–	–	–	–	–	–	–	–
erythroseptica	H 1019	–	–	–	–	–	–	–	–	–	–
	H 1020	–	–	–	–	–	–	–	–	–	–
	H 1101	–	–	–	–	–	–	–	–	–	–
	H 1162	–	–	–	–	–	–	–	–	–	–
heveae	H 1030	–	–	–	–	–	–	–	–	–	–
meadii	H 1031	–	–	–	–	–	–	–	–	–	–
megasperma	H 1035	–	–	–	–	–	–	–	–	–	–
	H 1040	–	–	–	–	–	–	–	–	–	–
	H 1043	–	–	–	–	–	–	–	–	–	–
	H 1163	–	–	–	–	–	–	–	–	–	–
	H 1164	–	–	–	–	–	–	–	–	–	–
megasperma var. *megasperma*	H 1021	–	–	–	–	–	–	–	–	–	–
	H 1053	–	–	–	–	–	–	–	–	–	–
	H 1054	–	–	–	–	–	–	–	–	–	–
	H 1138	–	–	–	–	–	–	–	–	–	–
	H 1139	–	–	–	–	–	–	–	–	–	–
	H 1140	–	–	–	–	–	–	–	–	–	–
var. *sojae*	H 1052	–	–	–	–	–	–	–	–	–	–
	H 1057	–	–	–	–	–	–	–	–	–	–
	H 1058	–	–	–	–	–	–	–	–	–	–
	H 1059	–	–	–	–	–	–	–	–	–	–
	H 1141	–	–	–	–	–	–	–	–	–	–
	H 1142	–	–	–	–	–	–	–	–	–	–
	H 1143	–	–	–	–	–	–	–	–	–	–
	H 1144	–	–	–	–	–	–	–	–	–	–
nicotianae	H 1041	–	–	–	–	–	–	–	–	–	–
	H 1042	–	–	–	–	–	+	–	–	+	–
	H 1105	–	–	–	–	–	–	–	–	–	–
	H 1106	–	–	–	–	–	–	–	–	–	–
	H 1107	–	–	–	–	–	–	–	–	–	–
	H 1108	–	–	–	–	–	–	–	–	–	–
nicotianae var. *nicotianae*	H 1032	–	–	–	–	–	–	–	–	–	–
	H 1097	–	–	–	–	–	–	–	–	–	–
	H 1098	–	–	–	–	–	–	–	–	–	–
	H 1100	–	–	–	–	–	–	–	–	–	–
	H 1102	–	–	–	–	–	+	–	–	–	–
	H 1103	–	–	–	–	–	+	–	–	–	–
	H 1109	–	–	–	–	–	–	–	–	–	–
	H 1100	–	–	–	–	–	–	–	–	–	–
	H 1111	–	–	–	–	–	+	–	–	–	–
	H 1112	–	–	–	–	–	–	–	–	–	–
	H 1145	–	–	–	–	–	–	–	–	–	–
	H 1146	–	–	–	–	–	+	–	–	–	–
	H 1147	–	–	–	–	–	–	–	–	–	–
	H 1148	–	–	–	–	–	–	–	–	–	–
	H 1149	–	–	–	–	–	–	–	–	–	–
var. *parasitica*	H 1033	–	–	–	–	–	+	–	–	+	–
	H 1099	–	–	–	–	–	–	–	–	–	–
palmivora	H 1022	–	–	–	–	–	–	–	–	–	–
	H 1044	–	–	–	–	–	–	–	–	–	–
	H 1104	–	–	–	–	–	–	–	–	–	–
polymorphica	H 1023	–	–	–	–	–	–	–	–	–	–
sojae	H 1169	–	–	–	–	–	–	–	–	–	–
	H 1170	–	–	–	–	–	–	–	–	–	–
species	H 1002	–	–	–	–	–	–	–	–	–	–
	H 1024	–	–	–	–	–	–	–	–	–	–
	H 1089	–	–	–	–	–	–	–	–	–	–

(*continued on next page*)

Table 3. (*continued from preceding page*)

Species	Isolate	Cpa-3	Cpa-4	Cpa-6	Cpa-7	Cpa-10	Lpv-1	Lpv-2	Lpv-3	Lpv-4	Lpv-5
	H 1091	–	–	–	–	–	–	–	–	–	–
	H 1168	–	–	–	–	–	–	–	–	–	–
syringae	H 1055	–	–	–	–	–	–	–	–	–	–
vignae	H 1034	–	–	–	–	–	–	–	–	–	–
Pythium											
acanthicum	H 212	–	–	–	–	–	–	–	–	–	–
	H 223	–	–	–	–	–	–	–	–	–	–
aphanidermatum	H 200	–	–	–	–	–	–	–	–	–	–
	H 201	–	–	–	–	–	–	–	–	–	–
butleri	H 202	–	–	–	–	–	–	–	–	–	–
coloratum	H 218	–	–	–	–	–	–	–	–	–	–
debaryanum	H 209	–	–	–	–	–	–	–	–	–	–
	H 215	–	–	–	–	–	–	–	–	–	–
irregulare	H 203	–	–	–	–	–	–	–	–	–	–
	H 204	–	–	–	–	–	–	–	–	–	–
	H 207	–	–	–	–	–	–	–	–	–	–
mamillatum	H 210	–	–	–	–	–	–	–	–	–	–
	H 214	–	–	–	–	–	–	–	–	–	–
middletonii	H 213	–	–	–	–	–	–	–	–	–	–
myriotylum	H 205	–	–	–	–	–	–	–	–	–	–
periplocum	H 206	–	–	–	–	–	–	–	–	–	–
rostratum	H 211	–	–	–	–	–	–	–	–	–	–
species	H 208	–	–	–	–	–	–	–	–	–	–
spinosum	H 220	–	–	–	–	–	–	–	–	–	–
	H 221	–	–	–	–	–	–	–	–	–	–
splendens	H 219	–	–	–	–	–	–	–	–	–	–
ultimum											
var. *sporangiferum*	H 216	–	–	–	–	–	–	–	–	–	–
var. *ultimum*	H 217	–	–	–	–	–	–	–	–	–	–
vanterpoolii	H 222	–	–	–	–	–	–	–	–	–	–
Saprolegnia											
diclina	H 301	–	–	–	–	–	–	–	–	–	–
ferax	H 302	–	–	–	–	–	–	–	–	–	–
parasitica	H 303	–	–	–	–	–	–	–	–	–	–
Fusarium acanthicum	H 401	–	–	–	–	–	–	–	–	–	–
Rhizoctonia spp.	H 400	–	–	–	–	–	–	–	–	–	–
Schizophyllum commune	H 403	–	–	–	–	–	–	–	–	–	–
Verticillium dahliae	H 402	–	–	–	–	–	–	–	–	–	–

used for immunizations and screening will also affect the specificity of the resultant antibodies. Results obtained to date indicate that carbohydrates and glycoconjugates secreted or associated with plant cell walls may be highly antigenic (3) and possess epitopes common to a range of organisms (23,32,34). Thus, even when MAbs are produced, it is likely that problems with cross-reactivity will still arise when preparations such as mycelial homogenates, cell wall fractions, and culture filtrates (9) are used. On the other hand, three studies that have used wall-less spores as the antigenic preparation have produced species-specific and subspecies-specific antibodies (13,21,22). It would thus appear that antigens associated with the plasma membrane or with the contents of particular vesicles within the cell may possess greater specificity than antigens associated with cell walls and secretions. Consistent with this is the observation that in the IFA, Cpw-4, which targets the cyst wall, lacks species-specificity, reacting with all *Phytophthora* species and *Pythium aphanidermatum*.

The diagnostic antibodies that gave the strongest signals both in the IFA and ELISA were those belonging to the Lpv and Cpa groups, which target the contents of large peripheral vesicles and dorsal vesicles in the zoospores, respectively (14). The Cpa antigen is secreted during zoospore encystment and forms a coating over most of the surface of the cysts (14). Large peripheral vesicles do not undergo exocytosis. Their contents serve as a store of protein that is degraded during early germ tube growth (15). Both vesicles, however, reappear when nutrients or other factors become limiting to germling or mycelial growth (J. D. W. Dearnaley and A. R. Hardham, *unpublished*). In agreement with this observation, preliminary screening of crude mycelial extracts taken from 5-day cultures of *P. cinnamomi* resulted in strongly positive reactions with many of the Cpa and Lpv antibodies. Two of these (Lpv-2 and Lpv-3) were found to be species-specific in the ELISA, reacting with all the *P. cinnamomi* isolates screened and giving negative results with all other fungal isolates. Other antigens studied in the IFA—Zt, Zg, ZCp, and Cpw—were not detected in the mycelial extracts using ELISA. In some cases this may be due to the absence of the antigen in mycelia. However, Zt and Zg antigens are present (M. Cope, *personal communication*) and the reasons for their lack of detection are not yet known.

Many of the antibodies in the Lpv and Cpa groups display different reactivities when screened either in the IFA or ELISA. For example, in the IFA results, Lpv-2 and Lpv-5 react with different fungal isolates than do Lpv-1, Lpv-3, and Lpv-4 (Table 2). In the ELISA, Lpv-2 and Lpv-3 react differently to Lpv-1, Lpv-4, and Lpv-5. Lpv-1, Lpv-4, and Lpv-5 react differently to each other. The reactions of the Lpv MAbs were always strong in both IFA and ELISA, and the differences in patterns of reactivity shown by individual antibodies within each assay type are repeatable and thus appear to be real. These differences may reflect the recognition of distinct epitopes on the antigens.

The ability to identify fungi at the genus level is in many situations a desirable and useful feature to have in a diagnostic kit. Our results indicate that ZCp-2 could be used for the detection of *Phytophthora* species when used in the IFA. This MAb, however, detects its antigen only in zoospore and cyst preparations (21), thus limiting its usefulness to some degree. In many situations, there are distinct advantages in identifying a fungal pathogen, such as a species of *Phytophthora*, to the species level. Our results show that we have species-specific MAbs that can detect their antigens in zoospores and cysts using immunofluorescence microscopy and others that can detect their antigen in mycelial preparations using ELISA. In addition, the Lpv antigen has been found in mycelia growing in infected roots (B. K. Gabor, *unpublished*) suggesting that it may be possible to utilize this MAb in a species-specific test for the detection of *P. cinnamomi* in infected plant tissues.

Pathogen identification by IFA or ELISA would require little knowledge of the taxonomy of *Phytophthora* or of *P. cinnamomi*. Large numbers of samples could be routinely screened with ease and modest expense. The development of these rapid diagnostic assays that are specific for *P. cinnamomi* will greatly reduce the time, expense, and expertise currently required to identify this fungus in culture.

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