Monoclonal Antibodies-Based Immunofluorescence Test for Detection of Conidia of *Botrytis cinerea* on Cut Flowers

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Supported by grants from the Ministry of Agriculture, Nature Management and Fisheries, and the Association of Dutch Flower Auctions through the Commodity Board for Ornamentals.
We thank R. Pomp, J. Zilverentant, G. Schut, and I. de Jong for technical assistance and F. M. Dewey, Department of Plant Sciences, University of Oxford, for critically reading the manuscript.
Accepted for publication 23 December 1993.

ABSTRACT

Salinas, J., and Schots, A. 1994. Monoclonal antibodies-based immunofluorescence test for detection of conidia of *Botrytis cinerea* on cut flowers. Phytopathology 84:351-356.

Whole conidia, their extracellular material, and a putative cutin esterase isolated from conidia of *Botrytis cinerea* were used as antigens to raise monoclonal antibodies (MAbs) for the detection of conidia of *B. cinerea*. Using immunofluorescence, three selected MAbs recognized conidia of 43 isolates of *B. cinerea* from hosts representing six countries. The percentage of conidia that fluoresced ranged from 50 to 100%. Intensity of fluorescence was related more to the MAb than to the *Botrytis* isolate tested. MAbs showed no reaction with healthy gerbera flowers or to spores produced by other common airborne fungi and bacteria. Cross-reaction with conidia of four other species of *Botrytis* occurred, but their fluorescence patterns differed from those of conidia of *B. cinerea*.

Additional keywords: *Botrytis aclada, Botrytis elliptica, Botrytis squamosa, Botrytis tulipae*.

Botrytis cinerea Pers.:Fr. is a widespread pathogen producing infections on more than 200 hosts (7). Diseases caused by the fungus result in considerable losses to crops grown in the field and in greenhouses and during storage. Most of these crops are economically important, such as vegetables, fruits, ornamental flowers, bulbs, and forest-tree seedlings.

The fungus has become an important quality limiting factor in the production and export of cut flowers in the Netherlands. Conidia produced by the fungus spread easily through the air. After landing on flowers, conidia remain dormant until free water is available for germination. After germination, flowers become infected within a few hours (14). Especially during autumn and spring, lesions caused by the fungus can lead to serious economic losses (1). Symptoms can be visible in greenhouses during the growth of flowers but also can develop later. During storage, transport, and shipment of flowers, changes in temperature often occur, leading to condensation and subsequent infection when conidia of *B. cinerea* are present (14).

A rapid serological test, preferably based on specific monoclonal antibodies (MAbs), enables the detection of conidia of *B. cinerea* on flowers, fruits, and vegetables at all stages of production. Traditional procedures for isolating and identifying *B. cinerea* are laborious and time-consuming (9). Dewey et al (5) recently showed that MAbs can be very useful for detecting fungi by immunoassays (5).

In this paper, we report the selection of three MAbs directed against conidia of *B. cinerea* that have the potential to be used in a routine test for detecting conidia of this pathogen on cut flowers. The MAbs have been tested for cross-reactions with a number of *Botrytis* species, a large number of *B. cinerea* isolates, other airborne fungi, and some bacteria.

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MATERIALS AND METHODS

Fungal and bacterial cultures. *B. cinerea* isolates used in this study were obtained from plants infected in fields in The Netherlands, the United Kingdom, Israel, Spain, Italy, and Greece (Table 1). The isolates were kept as sporulating cultures on X-medium at 4 C in the dark (14). Production of conidia was carried out on tomato agar as described previously (13). Tomato agar was prepared by adding 300 g of ground tomato leaves to 1,000 ml of distilled water. The mixture was kept at 50 C during 2 h, after which 20 g of agar was added. Sterilization took place by autoclaving for 20 min at 121 C. Conidia were harvested dry from cultures with a suction pump and stored dry in flasks at −20 C until use.

B. aclada isolates 001 and 002 were obtained from the Bulb Research Centre collection (LBO; Lisse, The Netherlands); isolates 006, 007, and 008 were supplied by N. J. Fokkema (Research Institute for Plant Protection [IPO-DLO], Wageningen, The Netherlands). A *B. elliptica* isolate and *B. tulipae* isolate were obtained from the LBO collection. Conidia of these three *Botrytis* spp. isolates were produced on X-medium and sampled as above. A *B. squamosa* isolate was obtained from the IPO-DLO collection. Production of conidia was carried out on sterile onion leaves, and the conidia were sampled dry as described above.

Penicillium olsonii, Cladosporium herbarum, Aspergillus ochraceus, Pholiota sp., *Torula herbarum, Humicola grisea, Zygosporium masonii, Verticillium psalliotae, Trichoderma harzianum, Xanthomonas maltophilia, and Methylobacterium* sp. were isolated during May 1992 from the surface of greenhouse-grown gerbera flowers. These fungi and bacteria were provided by A. Kerssies (Research Station for Floriculture, Aalsmeer, The Netherlands); the fungi were identified by the Central Bureau for Fungal Cultures (CBS; Baarn, The Netherlands), and the bacteria were identified by J. D. Janse of the Plant Protection Service (Wageningen, The Netherlands).

Airborne fungi isolated from greenhouses. Petri dishes containing a selective medium for detection of *B. cinerea* (9) were mounted in spore traps and placed in two greenhouses containing gerbera plants for 8 h (9). After incubation for 12–20 days under a 24-h light regime, petri dishes containing different airborne fungi (*Penicillium, Aspergillus, Alternaria, Cladosporium, Trichoderma,* and *Mucor* spp.), but no *B. cinerea*, were selected. Spores were collected from these plates with a suction pump and stored at −20 C. The spore mixtures were used for screening hybridoma

TABLE 1. Geographic origin, year of isolation, and reaction of *Botrytis cinerea* isolates with monoclonal antibodies (MAbs) 4H10, 9E11, and 14E5 in an immunofluorescence assay[a]

				Reaction with MAb[b]		
Isolate	Host	Year	Origin	4H10	9E11	14E5
Bc-1	*Phaseolus vulgaris*	1970	The Netherlands	nd	+	+/−
Bc-7	*Lycopersicum esculentum*	1970	The Netherlands	+	+/−	+
Bc-8	*Taxus baccata*	1985	The Netherlands	nd	+/−	+
Bc-9	*Dianthus cariophyllus*	1985	The Neterhlands	nd	+	+/−
Bc-10	*Cyclamen graecum*	1985	The Netherlands	nd	+/−	+/−
Bc-12	*Gerbera jamesonii*	1986	The Netherlands	nd	+/−	−/+
Bc-13	*G. jamesonii*	1986	The Netherlands	nd	nd	+/−
Bc-14	*G. jamesonii*	1986	The Netherlands	nd	+/−	+/−
Bc-15	*G. jamesonii*	1986	The Netherlands	+	nd	+
Bc-16	*G. jamesonii*	1986	The Netherlands	nd	+	+
Bc-17	*G. jamesonii*	1986	The Netherlands	+	+	+
Bc-18	*G. jamesonii*	1986	The Netherlands	+	+	+
Bc-20	*Rosa* sp.	1990	The Netherlands	nd	+	+/−
Bc-21	*Rosa* sp.	1990	The Netherlands	nd	+	−/+
Bc-23	*Rosa* sp.	1990	The Netherlands	nd	+	+/−
Bc-24	*Rosa* sp.	1990	The Netherlands	nd	+	+/−
Bc-25	*Rosa* sp.	1990	The Netherlands	+/−	+	nd
Bc-26	*Rosa* sp.	1990	The Netherlands	nd	+/−	nd
Bc-27	*Rosa* sp.	1989	The Netherlands	nd	+	nd
Bc-28	*Rosa* sp.	1989	The Netherlands	nd	+	nd
Bc-29	*Gerbera* sp.	1991	The Netherlands	+/−	++	nd
Bc-31	*Rosa* sp.	1988	The Netherlands	+	+	+/−
Bc-33	*Rosa* sp.	1988	The Netherlands	+	+	+/−
Bc-40	*Rosa* sp.	1989	The Netherlands	+	+	+
Bc-42	*Rosa* sp.	1989	The Netherlands	+	+	+/−
Bc-45	Unknown	...	...	+/−	+/−	+/−
Bc-46	*Rosa* sp.	1992	Israel	+	+	+/−
Bc-47	*Rosa* sp.	1992	The Netherlands	+	+	+/−
Bc-48	*Rosa* sp.	1992	Israel	+	+	+/−
Bc-49	*Rosa* sp.	1992	Israel	+	+	+
Bc-50	*Rosa* sp.	1992	Israel	+/−	+	+/−
Bc-51	Unknown	...	The Netherlands	++	+	+
Bc-52	Unknown	...	The Netherlands	++	+	+
Bc-53	Ascospore culture	...	Italy	+/−	+	+/−
Bc-54	Unknown	...	The Netherlands	++	++	+/−
Bc-55	Ascospore culture	...	Italy	++	+/−	++
Bc-57	*L. esculentum*	1992	Greece	+	+	+
Bc-58	*Tulipa* sp.	1992	The Netherlands	++	+	+
Bc-59	*Rosa* sp.	1992	The Netherlands	+	+	+/−
Bc-60	*Fragaria* sp.	...	United Kingdom	+	+/−	+/−
Bc-61	*Fragaria* sp.	...	United Kingdom	+	−/+	+/−
Bc-62	*Fragaria* sp.	...	United Kingdom	++	+	+/−
Bc-66	*Capsicum annuum*	1986	Spain	+	+	+

[a] Conidia of sporulating cultures were harvested and incubated with the indicated MAbs. After a washing step, the reaction was visualized with goat-anti-mouse fluorescein isothiocyanate (FITC)-labeled antibodies and evaluated under a UV microscope.
[b] −/+ = weak fluorescence with about 50% of the conidia; +/− = weak fluorescence with 80% of the conidia; + = fluorescence with more than 90% of the conidia; ++ = strong fluorescence with 100% of the conidia, nd = not done.

supernatants. Because the composition of the populations of airborne fungi may change between seasons, airborne fungi were collected during April and November 1991 and January 1992.

Antigen preparation and immunization. A mixture of conidia from 10 *B. cinerea* isolates (Bc-7, Bc-8, Bc-9, Bc-10, Bc-12, Bc-13, Bc-14, Bc-15, Bc-18, and Bc-25) was used for antigen preparation. Three antigen preparations were used for immunization: whole conidia, extracellular material, and a putative cutin esterase.

A mixture of whole conidia was washed eight times with 20 mM piperazine-HCl, pH 6.0, to ensure that most of the extracellular material was removed. Mice were injected with about 5×10^6 conidia per 0.2 ml of phosphate-buffered saline (PBS), four times at 4-wk intervals. Freund's incomplete adjuvant (FIA; Sigma Chemical Co., St. Louis, MO) was used for the first three immunizations and was omitted for the last immunization.

The supernatant from the wash steps that contained most of the extracellular material, was concentrated on an Amicon cell system YM10 ultrafilter membrane (Amicon Corporation, Scientific Systems Division, Danvers, MA) until a protein concentration of 1 mg/ml was reached. Mice were immunized with extracellular material containing 100 μg of extracellular proteins, four times at 4-wk intervals. For the first immunization, Freund's complete adjuvant (FCA; Sigma) was used; for the second and third immunizations, FIA was used; and adjuvant was omitted for the last immunization.

The cutin esterase was isolated from the extracellular material. Mice were injected with 30 μg of cutin esterase in PBS, four times at 4-wk intervals. For the first immunization, FCA was used; for the second and third immunizations, FIA was used; and no adjuvant was used for the last immunization.

Purification of a putative cutin esterase. The enzyme was purified according to Salinas (13). Briefly, proteins were concentrated on a Amicon YM10 ultrafilter membrane until a protein concentration of 5 mg/ml was achieved, followed by gel filtration on a Sephacryl S-300 column (2.2 × 100 cm) (Pharmacia LKB Biotechnology, Uppsala, Sweden), and equilibrated with 10 mM piperazine-HCl buffer, pH 6.0. After elution, esterase active fractions were pooled and concentrated with the Amicon system. The pooled fractions were applied to a DEAE Sepharose CL-6B column (1.6 × 10 cm) equilibrated with 20 mM piperazine buffer, pH 6.8. After elution with a linear gradient from 0 to 1 M NaCl, fractions with esterase activity were pooled and concentrated. The final purification step took place with a chromatofocusing PBE 94 (Pharmacia) column (1 × 20 cm) equilibrated with 20 mM piperazine-HCl buffer, pH 6.0. Proteins were eluted from the column with 200 ml of polybuffer 74, pH 4.0 (Pharmacia), followed by a linear gradient of 0–1 M NaCl. In each step, the effluent from the column was monitored for absorption at 280 nm. Esterase activity was measured with paranitrophenyl butyrate as substrate (15).

Production of MAbs. MAbs were produced as described by Schots et al (17); 3 days after the last immunization the mice splenocytes were fused with SP 2/0-Ag14 myeloma cells using polyethylene glycol 4000. The cell suspension was plated out in 96-well microtiter plates with selective medium containing the equivalent of 10^4 splenocytes at 100 μl per well. The cell-culture supernatants were screened 12–17 days after the cell fusion for the presence of specific antibodies by immunofluorescence or by both enzyme-linked immunosorbent assay (ELISA) and immunofluorescence. The hybridomas that secreted antibodies that reacted with *B. cinerea*, but not with the airborne microorganisms, were cloned in microtiter plates by limiting dilution (one cell per well) until stability. Stable clones were expanded in spinner flasks for large-scale production of MAbs. The cell lines were preserved by freezing them in fetal calf serum/dimethyl sulfoxide (92/8 v/v) and were maintained in liquid nitrogen.

ELISA. Hybridoma cell lines producing MAbs against cutin esterase were screened by ELISA (12). Briefly, microtiter plates were coated with 20 ng of purified cutin esterase per well and blocked with bovine serum albumin (BSA) to avoid nonspecific reactions. Hybridoma-culture supernatants were added, followed by a goat–anti-mouse alkaline phosphatase conjugate. The reaction was visualized with paranitrophenyl phosphate and measured in an ELISA reader at 405 nm. The microtiter plates were washed under running tap water after each incubation. In a similar assay, the reactivity of selected MAbs with extracts from healthy gerbera flowers was tested, using microtiter plates coated with these extracts. Gerbera-flower extracts were obtained by grinding five flowers in 100 ml of ice-cold PBS; after filtration through cheesecloth, flowers extracts were diluted 1:1 (v/v) in coating buffer.

Immunofluorescence. Millipore multi screen-HV 96-well filtration plates (Millipore Corp., Bedford, MA), pore size 0.45 μm, were blocked with 5% donor horse serum in PBS/0.1% Tween 20 (PBST) for 30 min at 37 C and washed four times with PBST. A 100-μl aliquot of either *B. cinerea* conidia or spores or cells of other microorganisms at 5×10^6/ml in PBST containing 0.1% BSA and 100 μl of hybridoma-culture supernatant were added to each well. Plates were incubated for 1 h at 37 C and washed four times with PBST. Subsequently, 50 μl of a goat–anti-mouse IgG(H+L)-fluorescein isothiocyanate (FITC) conjugate (Sigma), diluted 1:100 in PBST containing 0.1% BSA, was added to each well and incubated for 1 h at 37 C. Plates were washed twice with PBST and once with ultrapure water. Conidia or cells were resuspended in 15 μl of antiquench solution (8); 2 μl from each well was taken and placed on a 24-well multitest slide (Nutacon, Cel-line Associates Inc., Newfield, NJ). The reaction of the conidia or cells was determined with a fluorescence microscope.

Isotype determination. The subclass of the immunoglobulins produced by the hybridomas was determined with an ELISA as described by Schots et al (17). Briefly, each hybridoma producing antibodies (Table 2) was tested in ELISA with rat–anti-mouse IgA, IgM, IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, IgG, and Kappa. The reaction of the antibodies with rat–anti-mouse isotype antibodies was visualized with a rat–anti-mouse immunoglobulin alkaline

TABLE 2. Immunofluorescence reaction of a mixture of *Botrytis cinerea* isolates and other airborne fungi with nine monoclonal antibodies raised against conidia of *Botrytis cinerea*

Antibody	Antigen[a]	Isotype	*B. cinerea* positive %	*Oidium* sp.[b]	Airborne fungi[c] April 1991	November 1991	January 1992
2G11	E.m.	IgM	65	+[d]	–[d]	–	nd[d]
4A3	E.m.	IgM	60	–	–	–	nd
4H10	Conidia	IgM	90	–	–	–	–
7B9	E.m.	IgM	60	+	–	–	nd
9A12	E.m.	IgM	60	–	–	–	nd
9C7	E.m.	IgM	70	+	–	–	nd
9E11	E.m.	IgG_1	85	–	–	–	–
10G1	E.m.	IgM	90	–	–	+	nd
14E5	Cutin esterase	IgG_1	80	–	–	–	–

[a] E.m. = extracellular material isolated from conidia of *B. cinerea*.
[b] *Oidium* sp. isolated from gerbera flowers.
[c] A mixture of spores from commonly occurring airborne fungi (*Penicillium*, *Aspergillus*, *Alternaria*, *Cladosporium*, *Trichoderma*, and *Mucor* spp.) collected in gerbera greenhouses during different seasons.
[d] + = positive reaction observed; – = no reaction observed; nd = not done.

phosphatase conjugate.

Protein determination. Protein concentrations of the extracellular proteins and cutin esterase were determined according to Bradford (3), using BSA as a standard.

RESULTS

Selection of hybridoma cell lines for detection of conidia of *B. cinerea*. Hybridoma-fusion experiments carried out with splenocytes from mice immunized with whole conidia, extracellular material, or cutin esterase resulted in nine hybridomas that produced MAbs that reacted, in the immunofluorescence assay, against *Botrytis* sp. (Figs. 1 and 2). Hybridomas positive for cutin esterase were selected by ELISA and immunofluorescence (Tables 2 and 3). Hybridomas producing antibodies against whole conidia or extracellular material were selected by the immunofluorescence test (Table 2). During the first rounds of screening, most of the cell lines that produced antibodies against *B. cinerea* were discarded because the antibodies cross-reacted with the spores of airborne fungi (data not shown). In later tests, MAb 10G1 cross-reacted with airborne fungi isolated during November 1991 (Table 2). MAbs 2G11, 7B9, and 9C7 cross-reacted with *Oidium* sp. (Table 2). A total of five hybridoma cell lines producing *Botrytis*-specific MAbs was obtained. MAb 4H10 was obtained with conidia; MAbs 4A3, 9A12, and 9E11 were obtained with extracellular material; and the MAb 14E5 was obtained with cutin esterase (Table 2).

Of the five hybridoma cell lines specific for conidia of *B. cinerea*, cell lines 4H10, 9E11, and 14E5 were characterized in more detail. Supernatants from these three cell lines did not cross-react with extracts of healthy gerbera flowers (Table 3) or spores from nine fungi and cells from two bacteria isolated from the flower surface. These three MAbs reacted with conidia of all 43 *B. cinerea* isolates tested (Table 1). However, differences in fluorescence intensity were observed on the conidia among the different *B. cinerea* isolates. Figure 1 shows a strong fluorescence reaction. The percentage of conidia showing fluorescence ranged from 50 to 99% depending on isolate and MAb (Table 4).

Cross-reaction with conidia of other *Botrytis* species. MAb 9E11 cross-reacted with *B. aclada* and *B. tulipae* but not with *B. squamosa* and *B. elliptica*. MAb 14E5 did not cross-react with *B. elliptica* but reacted weakly with *B. aclada* and *B. tulipae*. MAb 4H10 reacted with *B. elliptica, B. tulipae, B. squamosa,* and weakly with two *B. aclada* isolates (Table 5). The reaction of the MAbs with these four *Botrytis* species in immunofluorescence was typified by fluorescent patches on the conidial surface (Fig. 2). This in contrast to the reaction with conidia of *B. cinerea* that showed even fluorescence of the whole conidium (Fig. 1).

TABLE 3. Reaction of monoclonal antibodies 4H10, 9E11, and 14E5 against a putative cutin esterase and extracts of gerbera flowers in enzyme-linked immunosorbent assay (ELISA)

Antiserum	ELISA (A_{405nm}) Putative cutin esterase	Extracts of gerbera flowers
4H10	nd[a]	0.055
9E11	nd	0.060
14E5	1.900	0.058
Preimmune mouse serum	0.056	0.061

[a]Not done.

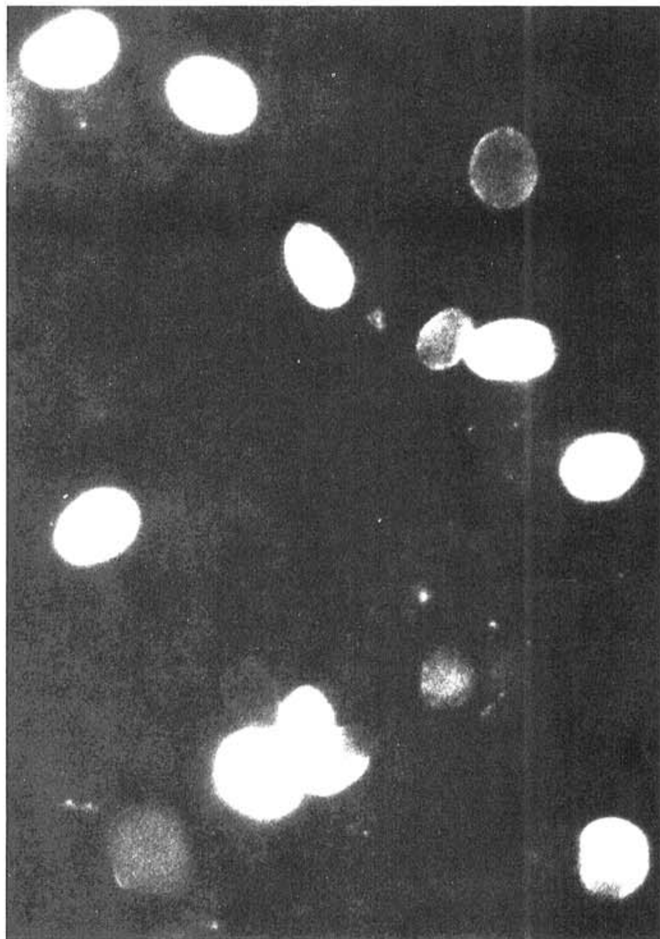

Fig. 1. Immunoreaction of monoclonal antibody 9E11 with conidia of *Botrytis cinerea*. The immunoreaction was visualized with a goat-anti-mouse fluorescein isothiocyanate conjugate.

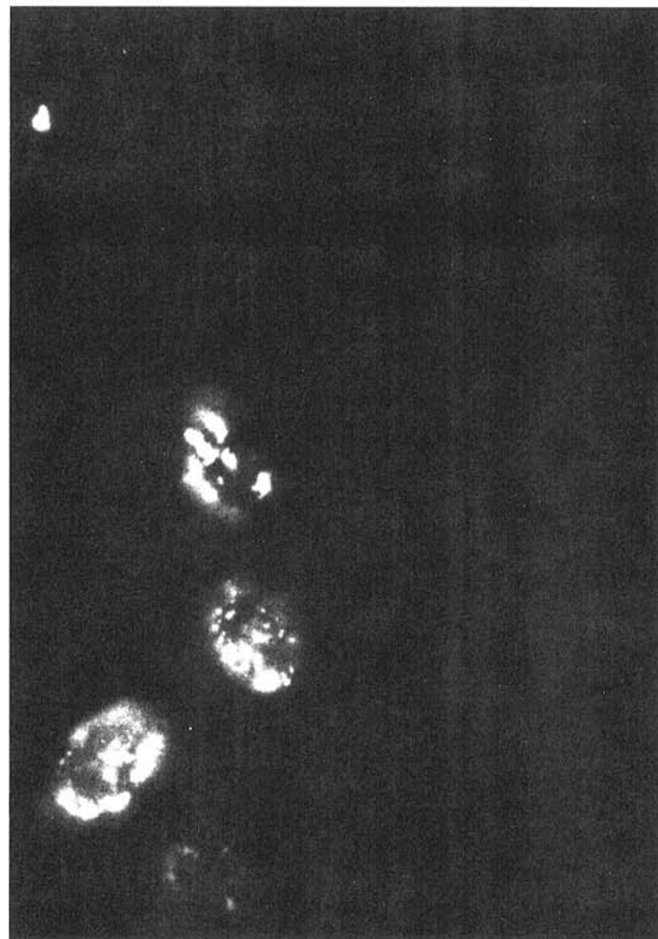

Fig. 2. Fluorescence pattern of conidia of *Botrytis squamosa* after immunoreaction with monoclonal antibody 4H10 followed by goat-anti-mouse fluorescein isothiocyanate conjugate.

TABLE 4. Percent conidia of 10 isolates of *Botrytis cinerea* that reacted in immunofluorescence with different monoclonal antibodies (MAbs) produced against *B. cinerea*[a]

MAb	Isolate (% conidia)									
	Bc-7	Bc-8	Bc-13	Bc-15	Bc-16	Bc-17	Bc-21	Bc-25	Bc-27	Bc-29
2G11	99	73	50	50	90	89	80	80	80	85
4A3	82	70	99	94	94	90	70	75	80	99
4H10	99	67	83	70	99	99	70	80	95	80
7B9	79	75	75	79	99	73	99	90	92	91
9A12	91	50	80	56	86	92	80	78	57	58
9C7	93	42	54	99	99	99	99	99	90	99
9E11	83	99	74	99	99	96	89	86	99	99
10G1	99	79	93	99	91	75	99	90	99	99

[a] MAbs 2G11, 4A3, 7B9, 9A12, 9C7, 9E11, and 10G1 were raised from mice immunized with extracellular material obtained from a mixture of 10 *B. cinerea* isolates, whereas whole conidia were used as antigen to obtain MAb 4H10.

TABLE 5. Cross-reactivity of three monoclonal antibodies (MAbs) with different species of *Botrytis* determined by immunofluorescence

Botrytis sp.	MAbs[a]		
	9E11	4H10	14E5
B. aclada 001	+/−	−	+/−
B. aclada 002	−	+/−	−
B. aclada 006	+	+/−	nd
B. aclada 007	+	−	nd
B. aclada 008	+	−	nd
B. elliptica	−	+	−
B. tulipae	+	+	+/−
B. squamosa	−	+	nd

[a] − = no immunofluorescence; +/− = weak fluorescence with more than 80% of the conidia; + = strong fluorescence with more than 90% of the conidia; nd = not done.

DISCUSSION

Three MAbs produced by hybridoma cell lines 4H10, 9E11, and 14E5 were selected for their specificity in reacting with conidia of *B. cinerea* isolates collected from different countries and hosts. The results presented in this paper show that whole conidia, extracellular proteins, and cutin esterase can be used as antigens to raise specific MAbs for detection of conidia of *B. cinerea*. We chose this strategy because the complex nature of fungi makes it difficult to obtain specific antibodies for diagnostic purposes. Whole cells or fungal components have been used to raise antibodies for detection of fungal pathogens with varying results (2,4,6,16,18). However, it can be concluded that no general strategy can be developed for antigen selection, because it depends too much on the species being detected.

Purified molecules have been used to raise monoclonal and polyclonal antibodies for research in different plant sciences (5). However, the knowledge of the molecular character of antigens used to raise antibodies for detection of fungal plant pathogens is limited. MacDonald et al (10) fractionated the fungal surface washes used as antigen by gel filtration. They reported that MAbs specific to four fungi bound to the low molecular weight fractions, whereas nonspecific MAbs bound only to the high molecular weight fractions. The molecular characterization of the antigens recognized by the three MAbs described here has not been carried out. Nevertheless, MAb 14E5 raised against a putative cutin esterase, a 100-kDa protein, reacted specifically with conidia of *Botrytis*. Thus, we have shown that a single protein of higher molecular weight can be used as an antigen for development of MAbs specific to conidia of *Botrytis*. Western blot studies have to be performed to detect possible cross-reactions with low molecular weight proteins. Based on the results of MacDonald et al (10), Bossi and Dewey (2) used low molecular weight components ($<$30 kDa) from surface washes of *B. cinerea* as antigens. However, Western blot studies indicated that the specific MAbs obtained with these antigens recognize high molecular weight components.

Studies on cross-reactions were carried out with conidia from other species of *Botrytis*. The fact that all three antibodies recognized equally well conidia of *B. tulipae* implies a high immunological similarity between *B. tulipae* and *B. cinerea*. A similar situation was described by Bossi and Dewey (2) between *B. cinerea* and *B. fabae*. Cross-reactions also were observed with our MAbs between *B. aclada, B. elliptica,* and *B. squamosa*, indicating common epitopes. At present, we do not know the chemical composition of these epitopes. Common epitopes also were found by Cousin et al (4) who raised antibodies reacting with four species of *Botrytis* using antigens isolated from *B. tulipae*.

Different results were observed with regard to the recognition of *B. aclada* by our MAbs and those MAbs raised by Bossi and Dewey (2). The antigen fraction used by Bossi and Dewey (2) is more similar to the antigen used to raise MAb 9E11 (extracellular material) than to MAb 4H10 (whole conidia), whereas cross-reaction patterns of their MAbs to *B. aclada* are more like those of MAb 4H10 than those of MAb 9E11. The reaction pattern of MAb 4H10 with conidia from *B. squamosa* differed considerably from that observed with *B. cinerea*; only patches on the surface of the conidia fluoresced (Fig. 2), and not all MAbs reacted with this species of *Botrytis*.

The fungal and bacterial flora associated with gerbera flowers varies with the seasons. Indeed, we found one MAb (10G1; Table 2) that cross-reacted with fungi isolated during autumn but not with any isolated during spring. Cross-reaction of MAbs 4H10, 9E11, and 14E5 with spores of airborne fungi other than *Botrytis* present in greenhouses seems very unlikely because the screening of the MAbs was conducted with spores collected during different years and seasons. The three MAbs did not react with spores of nine fungi and cells of two bacteria isolated from the surface of gerbera flowers during May 1992, which corroborates the specificity of the three MAbs.

Most of the prerequisites for the development of a routine test have been met. The MAbs do not react with other fungi and bacteria generally present in greenhouses. Also, no reaction with extracts from gerbera flowers was observed. The final prerequisite is that MAbs should react with a large number of isolates to avoid false negatives. The three MAbs have considerable diagnostic potential because a positive reaction was observed with all *B. cinerea* isolates tested. However, not all spores reacted with the MAbs (Table 4). A possible explanation may be changes in the cell wall or the disappearance of the extracellular material from dead or dying conidia.

A routine test based on MAbs can replace tests based on selective media (9) or polyclonal antisera. Polyclonal antisera are not very specific or sensitive (11). The latter is often caused by cross-absorption carried out to improve the specificity, which results in a dramatic decrease in sensitivity. Disadvantages of selective media include time and the fact that most are not completely selective. Some knowledge of fungal taxonomy is still required. The advantages of a test based on MAbs include speed and specificity. The test can be performed within a few hours, and the reaction pattern of the MAb used, concerning specificity and sensitivity, is known and should not vary, because the antibody is available forever.

The test for detection of the presence of *B. cinerea* conidia on gerbera flowers will be based on an immunofluorescence assay.

The advantages of this assay are its sensitivity and specificity. In one trial, even a single conidium isolated from roses was detected. A total of 85% of the *B. cinerea* conidia on artificially infected flowers can be washed from the ray florets (data not shown) and are detected in the test. Some preliminary experiments, using gerbera flowers and roses obtained from a flower auction, have shown that the test can be implemented in routine practice. Trials to confirm this are presently in progress. It is anticipated that this test also can be used with other flowers sensitive to *B. cinerea* infections.

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