A New Selective Medium for the Recovery and Enumeration of *Monilinia fructicola*, *M. fructigena*, and *M. laxa* from Stone Fruits

Achour Amiri, Imre J. Holb, and Guido Schnabel

First and third authors: Department of Entomology, Soils, and Plant Sciences, Clemson University, Clemson, SC 29634; second author: University of Debrecen, Centre for Agricultural Sciences and Engineering P.O. Box 36, H-4015 Debrecen, Hungary & Plant Protection Institute, Hungarian Academy of Sciences, P.O. Box 102, H-1525 Budapest, Hungary.
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ABSTRACT

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Isolation of *Monilinia* spp. from stone and pome fruit surfaces is difficult due to the presence of several fast-growing fungal species such as *Rhizopus*, *Alternaria*, and *Penicillium* spp. Therefore, a new selective medium (acidified potato dextrose agar [pH 3.6] amended with fosetyl-aluminum [fosetyl-AL] at 500 µg/ml) (APDA-F500) was developed for the recovery of *Monilinia* propagules. The antifungal agents fosetyl-Al, dichloran, ammonium molybdate, and 2-deoxy-D-glucose (2-dD-glucose) were tested in potato dextrose agar (PDA) for their selective activity against *Monilinia fructicola* and seven common fungal contaminants of peach, including *Alternaria alternata*, *Aspergillus niger*, *Colletotrichum acutatum*, *Gilbertella persicaria*, *Penicillium expansum*, *Phomopsis amygdali*, and *Rhizopus stolonifer*. Dichloran, ammonium molybdate, and 2-dD-glucose inhibited spore germination and mycelial growth of all test fungi, including *M. fructicola*, at comparable levels. Fosetyl-Al added to PDA (PDA-F) at 500 or 1,000 µg/ml did not inhibit germination of any of the fungi but had a strong effect on mycelial growth of six of eight test fungi at 1,000 µg/ml, with the exceptions being *R. stolonifer* and *M. fructicola*. Germination and mycelial growth of *M. fructicola* were least affected on APDA-F500 compared with the other test fungi. On APDA-F500 at pH 3.2 and 3.6, germination of *M. fructicola* was not inhibited but mycelial growth was reduced by 54.2 and 24.2%, respectively. In all, 17 *M. fructicola*, 6 *M. fructigena*, and 6 *M. laxa* isolates collected from different geographic locations and diverse hosts were evaluated for their germination and mycelial growth on APDA-F500 (at pH 3.6). Germination was not inhibited for any isolate and relative mycelial growth was 45.8 to 83.3%. Field-grown peach fruit from South Carolina and Hungary and plum fruit from Hungary were used to test the selectivity of APDA-F500 for the recovery of three *Monilinia* spp. compared with PDA-F500 and *Monilinia* selective medium (MSM) previously developed for *Monilinia* spp. detection. Percent recovery of *M. fructicola* from South Carolinian peach fruit was highest on APDA-F500 (0, 17, and 69% in June, July, and August, respectively) compared with PDA-F500 (0, 3.5, and 50%, respectively) and MSM (0, 0, and 6.8%, respectively). Moreover, APDA-F500 selectively recovered *M. fructigena* and *M. laxa* propagules from the surfaces of Hungarian peach and plum fruit. Our results indicate that APDA-F500 is a useful medium for selective isolation and enumeration of the three most common *Monilinia* spp. attacking stone fruits worldwide.

Monilinia spp. are widespread fungal plant pathogens that cause extensive damage on members of the Rosaceae family, including important agricultural crops such as pome and stone fruits. *Monilinia fructicola*, *M. fructigena*, and *M. laxa* are the most common species within this genus (26). *M. fructicola* is most prevalent in North and South America (7,35,40,45) and has recently emerged in several European countries (9,13,48). In Spain and Italy, the two major countries producing stone fruits in Europe, *M. laxa* and *M. fructigena* are the most important cause of postharvest brown rot in peach and nectarine (36,54). *Monilinia* spp. commonly infect blossoms, twigs, and fruit, causing blossom blight, twig blight, and fruit brown rot, respectively. Conidia of the fungus overwinter in fruit mummies or cankerous lesions (10,35) serve as a primary inoculum source and cause blossom blight in the spring. Under unfavorable climatic conditions, infections remain latent in immature fruit until conditions become favorable for disease development later in the season (8,16,21).

The recovery and isolation of plant pathogens enables scientists to study the genotypic and phenotypic diversity and the dynamics of pathogen populations (4). However, the presence of bacterial, yeast, and several other fungal contaminants can make such efforts difficult. In one investigation dedicated to study the diversity of *Monilinia* spp. and the fungal microflora of stone fruit mummies in California orchards, more than 20 fungal genera were isolated (27). *Penicillium*, *Cladosporium*, *Mucor*, and *Rhizopus* spp. were most frequently isolated, while the recovery of *Monilinia* spp. was <4%. In respect to *M. fructicola*, the recovery of isolates can be especially challenging in dry years when isolations have to be performed on asymptomatic fruit. This is well documented by a study conducted by Emery et al. (16), where the incidence of latent infections of *M. fructicola* on commercially grown peach fruit varied between 0.4 and 22%.

The use of a selective medium can greatly facilitate isolation attempts. Selective media have been developed for the isolation of fungal plant pathogens such as *Botrytis* spp. (14,32), *Fusarium* spp. (53), *Alternaria* spp. (28), and others. The only medium available for isolation of *Monilinia* spp. (*Monilinia* selective medium [MSM]) was developed >30 years ago by Phillips and Harvey (49). MSM consists of peach juice, streptomycin, and neomycin to limit the development of bacteria and pentachloronitrobenzene (PCNB) at 1,000 µg/ml to limit the growth of other fungal contaminants of peach fruit. The MSM medium has not been particularly selective for *M. fructicola* (49) and requires a 5- to 7-day incubation period (49). One reason for the weak selectivity of MSM might be related to the limited antifungal activity of PCNB, as reported for *Penicillium* spp. (19), *Fusarium*

Corresponding author: G. Schnabel; E-mail address: schnabe@clemson.edu

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spp. (43,53), and *Epicoccum* spp. (55). Furthermore, PCNB is undesirable as a constituent of media because of its carcinogenicity (17,42).

The development of an efficient *Monilinia* spp.-selective medium is useful to study aspects of the ecology and biodiversity of *Monilinia* spp., which may ultimately lead to improved disease management practices. The goal of this study was to develop an efficient selective medium for *Monilinia* spp. Specific objectives were to (i) screen for active ingredients that selectively support germination and mycelial growth of *M. fructicola, M. laxa*, and *M. fructigena*; (ii) investigate their inhibitory activity against several potential fungal peach contaminants; and (iii) determine the ability of the newly developed medium for *Monilinia* spp. recovery from asymptomatic stone fruits.

MATERIALS AND METHODS

Fungal isolates and inoculum production. Forty-three isolates of fungal opportunists or pathogens of peach fruit, including *Alternaria alternata*, *Aspergillus niger*, *Colletotrichum acutatum, Gilbertella persicaria*, *M. fructicola*, *M. fructigena*, *M. laxa*, *Phomopsis amygdali*, *Penicillium expansum*, and *Rhizopus stolonifer* were used in this study (Table 1). Isolates were stored at –80°C on silica gel (grade 40; Sigma-Aldrich, St. Louis) and new cultures were grown from this stock on potato dextrose agar (PDA) medium (EMD Chemicals Inc., Gibbstown, NJ) for each experiment. Conidia of *Monilinia* spp. and *Alternaria alternata* isolates were produced on V8 agar and acidified PDA (APDA; pH 4.2), respectively. Conidia from the remaining isolates were obtained on PDA medium incubated at 22°C for 3 to 4 days, with the exception of *C. acutatum* and *Phomopsis amygdali* isolates, which had to be stimulated for sporulation by keeping cultures in the dark for 7 days before exposure to a 12-h photoperiod for 10 to 15 days. For each experimental run, conidia were freshly harvested and resuspended in 10 ml of sterile distilled water containing 0.05% Tween 80. Conidia were filtered through cheesecloth and the final concentration was determined using a hemacytometer.

Fungicides and media recipes. Four active ingredients (a.i), including dichloran (Botran 75WP; Gowan Company, Yuma, AZ), ammonium molybdate tetrahydrate (ammonium molybdate; EMD Chemical Inc.), 2-desoxy-D-glucose (2-dD-glucose) (grade II, 98%; Sigma-Aldrich), and fosetyl-Aluminum (fosetyl-Al) (Aliette WDG; Bayer Crop Science, Research Triangle Park, NC), were evaluated for their effect on germination and mycelial growth of the test fungi. These active ingredients were chosen because antifungal effects against common peach pathogens were reported previously. For example, dichloran was shown to inhibit *R. sto-*

TABLE 1. Characteristics of fungal isolates used in this study

Fungal species	Isolate name	Geographic origin (region, country)	Host	Year of isolation
Monilinia fructicola	Mf.Pdt11	South Carolina, United States	Peach	2007
	Mf.DMap4	South Carolina, United States	Peach	2008
	Mf.Egpc5	South Carolina, United States	Peach	2007
	Mf.Egpc12	South Carolina, United States	Peach	2007
	Mf.Bmpc9[a]	Georgia, United States	Peach	2006
	Mf.Bpc1	Georgia, United States	Peach	2007
	Mf.Bpc8	Georgia, United States	Peach	2007
	Mf.SLf8	Georgia, United States	Peach	2008
	139[b]	California, United States	Peach	2005
	NJ07	New Jersey, United States	Peach	2006
	NJ08	New Jersey, United States	Peach	2006
	NJ012	New Jersey, United States	Nectarine	2006
	NJ015	New Jersey, United States	Peach	2006
	2F	New York, United States	Cherry	2007
	6c	New York, United States	Cherry	2007
	10B	New York, United States	Cherry	2007
	13B	New York, United States	Cherry	2007
Monilinia fructigena	MFG.E4	Szabolcs, Hungary	Apple	2002
	MFG.D10	Hajdú, Hungary	Plum	2001
	MFG.BP7	Pest, Hungary	Apple	2001
	MFG.ME16	Borsod, Hungary	Apple	2001
	MFG.MA11	Szabolcs, Hungary	Apple	2001
	MFG.DP24	Hajdú, Hungary	Apple	2002
Monilinia laxa	ML.E14	Szabolcs, Hungary	Cherry	2000
	ML.D5	Hajdú, Hungary	Plum	2002
	ML.BP5	Pest, Hungary	Cherry	2001
	ML.ME6	Borsod, Hungary	Peach	2001
	ML.MA21	Szabolcs, Hungary	Cherry	2001
	ML.DP7	Hajdú, Hungary	Plum	2002
Alternaria alternata	Aa.by1	Georgia, United States	Peach	2006
	Aa.sc1	South Carolina, United States	Peach	2006
Colletotrichum acutatum	Ca.cca1	Georgia, United States	Peach	2006
	Ca.ca2	South Carolina, United States	Peach	2006
Phomopsis amygdali	Pa.by1	Georgia, United States	Peach	2006
	Pa.eg1	South Carolina, United States	Peach	2006
Rhizopus stolonifer	Rs.by2	Georgia, United States	Peach	1996
	Rs.by5	Georgia, United States	Peach	1996
Gilbertella persicaria	Gp.pch1	South Carolina, United States	Peach	2006
	Gp.mmy1	South Carolina, United States	Nectarine	2006
Penicillium expansum	Pe.exp1	South Carolina, United States	Apple	2006
	Pe.exp2	South Carolina, United States	Pear	2006
Aspergillus niger	An.cr1	South Carolina, United States	Peach	2006
	An.cro1	South Carolina, United States	Peach	2007

[a] Amiri et al. (3).
[b] Ma et al. (39).

lonifer at 2.5 µg/ml and *Aspergillus* spp. and *Penicillium* spp. at 12.5 µg/ml (33); 2-dD-glucose was shown to reduce mycelial growth of *R. stolonifer* and *Penicillium expansum* at 1 µg/ml (15); ammonium molybdate at 10 mM significantly reduced the incidence of *Penicillium expansum* and *R. stolonifer* in apple fruit (44); and fosetyl-Al was shown to be active against a range of fungi at concentrations ranging from 100 to >2,000 µg/ml (18). All active ingredients were incorporated into autoclaved and cooled (50°C) PDA used as basal medium after preliminary tests using several media (data not shown). Dichloran was added to PDA at final concentrations of 5, 15, and 25 µg/ml; ammonium molybdate at 10^3, 5×10^3, and 10^4 µg/ml; fosetyl-Al at 500, 750, and 1,000 µg/ml; and 2-dD-glucose at 1, 2, and 5 µg/ml. The inhibitory effect of these four active ingredients incorporated into PDA was compared with MSM (49). The MSM medium consisted of 2% agar (Acros Organics, Morris Plains, NJ) and 5% peach juice obtained by mixing yellow cling canned peach halves (Great Value brand; Wal-Mart, Bentonville, AR) for 1 min in a blender. The mixture was autoclaved for 20 min and cooled to 50°C before adding PCNB (99%) at 1,000 µg/ml pentachloronitrobenzene (Sigma-Aldrich) and streptomycin sulphate (Biosciences, Inc., La Jolla, CA) at 1,000 µg/ml. Fungicide-amended PDA and MSM media were poured into polystyrene petri dishes (90 mm in diameter) and dishes were stored at 4°C for up to 7 days.

Germination of *M. fructicola* and other test fungi on MSM and fungicide-amended PDA. A 100-µl spore suspension (10^5 conidia/ml) volume from each isolate was spread evenly onto solidified media in petri dishes using a sterilized glass rod. Dishes were sealed with Parafilm (Pechiney Plastic Packaging, Menasha, WI) and incubated at 22°C, and percent germination was determined after 24 and 72 h. To calculate germination frequencies, each petri dish was divided into three sectors of comparable sizes with a marker and 100 spores per sector were examined under a microscope. A conidium was considered germinated when it had produced a germ tube two times longer than its diameter. Results were expressed as percent conidial inhibition relative to the control PDA. Three petri dish replicates were assessed for each treatment and the entire experiment was conducted twice.

Mycelial growth and sporulation of *M. fructicola* and other test fungi on MSM and fungicide-amended PDA. Plates containing the media were inoculated with mycelial plugs (5 mm in diameter) taken from the advancing margins of 2- to 3-day-old *Penicillium expansum*, *R. stolonifer*, and *G. persicaria* colonies; 4- to 7-day-old *M. fructicola, Alternaria alternata,* and *Aspergillus niger* colonies; and 7- to 10-day-old *C. acutatum* and *Phomopsis amygdali* colonies. Five petri dishes were inoculated for each isolate–fungicide concentration combination. Dishes were sealed with parafilm and incubated at 22°C in the dark, and radial mycelial growth was measured after 48 and 96 h. Percent growth inhibition on fungicide-amended PDA and MSM was calculated relative to the unamended PDA control. The entire experiment was conducted twice. Sporulation of each fungus was evaluated using the same petri dishes used for mycelial growth test. The percent sporulation of each isolate was scored using a scale from 0 to 4, where 0 = no sporulation, 1 = 1 to 25, 2 = 26 to 50, 3 = 51 to 75%, and 4 = >75% sporulation expressed relative to the sporulation on the control dishes. Three petri dishes were assessed for each treatment and the entire experiment was conducted twice.

Effect of pH on the efficacy of fosetyl-Al on germination and mycelial growth of tested fungi. The effect of pH on fosetyl-Al activity against conidial germination and mycelial growth was investigated. Autoclaved PDA was cooled to 50°C; acidified to pH 2.8, 3.2, 3.6, 4.0, or 5.2 using lactic acid (Sigma-Aldrich); and amended with fosetyl-Al at 500 µg/ml, resulting in a medium designated APDA-F500. Nonacidified but fosetyl-Al-amended PDA was designated PDA-F500. Fosetyl-Al was tested at 500 µg/ml based upon results from mycelial growth and germination inhibition. The pH of the medium was verified using a pH meter (Acumet Basic AB15; Fisher Scientific, Fair Lawn, NJ). Conidial germination and mycelial growth tests were conducted as described above and all experiments were conducted twice.

Germination and mycelial growth of *Monilinia* spp. from different geographic origins and hosts on APDA-F500. In total, 29 isolates (17 *M. fructicola*, 6 *M. fructigena*, and 6 *M. laxa*) were tested for germination and mycelial growth on APDA-F500. These isolates were collected from five states within the United States, including Georgia, South Carolina, New York, New Jersey, and California, and four counties in Hungary, including Hajdú, Szabolcs, Borsod, and Pest (Table 1). Isolates from South Carolina, Georgia, New Jersey, and California were collected from peach fruit, except for isolate NJ012 from New Jersey. Isolates from New York were obtained from cherry fruit. *M. fructigena* isolates were collected from apple (Hajdú, Szabolcs, Borsod, and Pest) and plum (Hajdú) fruits, while *M. laxa* isolates were collected from plum (Hajdú), cherry (Szabolcs and Pest), and peach (Borsod) fruits. Two isolates from each species were tested for germination: *M. fructicola* (isolates Mf.Bmpc9 and 139), *M. fructigena* (isolates MFG.E4 and MFG.D10), and *M. laxa* (isolates ML.E14 and ML.D5). All 29 isolates were tested for mycelial growth on APDA-F500. Germination and mycelial growth were evaluated on APDA-F500 (pH 3.6) as described above. Three and five petri dish replicates were assessed for germination and mycelial growth, respectively, and the entire experiment was conducted twice.

Recovery of *Monilinia* propagules from peach and plum fruit surfaces. PDA-F500, APDA-F500, and MSM media were tested for their ability to recover *M. fructicola* propagules from freshly harvested peach fruit (cv. Redskin). On 6 June, 9 July, and 13 August 2007, 10 asymptomatic fruit of similar size (≈4, 7, and 9 cm in diameter, respectively) were harvested at each evaluation time from the Musser Fruit Research Center in Seneca, SC. In addition, the ability of APDA-F500 medium to recover *M. laxa* and *M. fructigena* propagules from peach and plum fruit grown in Hungary was evaluated. Twenty asymptomatic plum (cv. Cacanska rodna) and 20 peach (cv. Cresthaven) fruits were harvested in the vicinity of Debrecen, Hungary on 16 and 29 August 2008, respectively. The fruit surface microflora was washed off within 48 h of harvest with 100 ml of sterile water as described previously (2). A 100-µl aliquot of each fruit wash was spread over PDA-F500, APDA-F500 (pH 3.6), and MSM from peach fruit collected in South Carolina. Fruit washes from peach and plum fruit collected in Hungary were spread on APDA-F500. Three replicate petri dishes were inoculated for each fruit. Colonies of *M. fructicola* and other microorganisms were counted on each petri dish after 4 days of incubation at 22°C using a digital colony counter (Bel-Art Products, Pequannock, NJ). A conidial suspension of *M. fructicola* (isolate Mf.Bmpc9) at 10^4 conidia/ml was spread on each media and used as a standard for *M. fructicola* identification. The washed fruit were peeled and the peels were arranged in a square format used to calculate the fruit surface. Colony counts were converted to conidia per square centimeter of peach or plum surface. Five colonies resembling *M. fructicola*, *M. fructigena*, or *M. laxa* and other most-observed fungal propagules growing on each media were randomly selected for identification. Colonies from one dish of each medium were transferred to new PDA dishes, grown for 5 to 7 days, and identified by amplifying and sequencing the ribosomal internal transcribed spacer (ITS) regions ITS 1 and ITS 2 as described previously (31).

Statistical analysis. All laboratory experiments were conducted twice and, because results from the two experimental runs were similar (variance between the two runs ≤0.05), data were

combined for analysis. Based on the five replicates from two experimental runs, data from conidial germination, sporulation, and mycelial growth inhibition experiments were expressed as percent relative to the control (PDA). Means and 95% confidence intervals (CIs) were computed for statistical evaluation (11). No formal hypothesis test was carried out due to violation of underlying assumptions because several values were close to 100 or 0. Number of colony counts of each microorganism on each medium

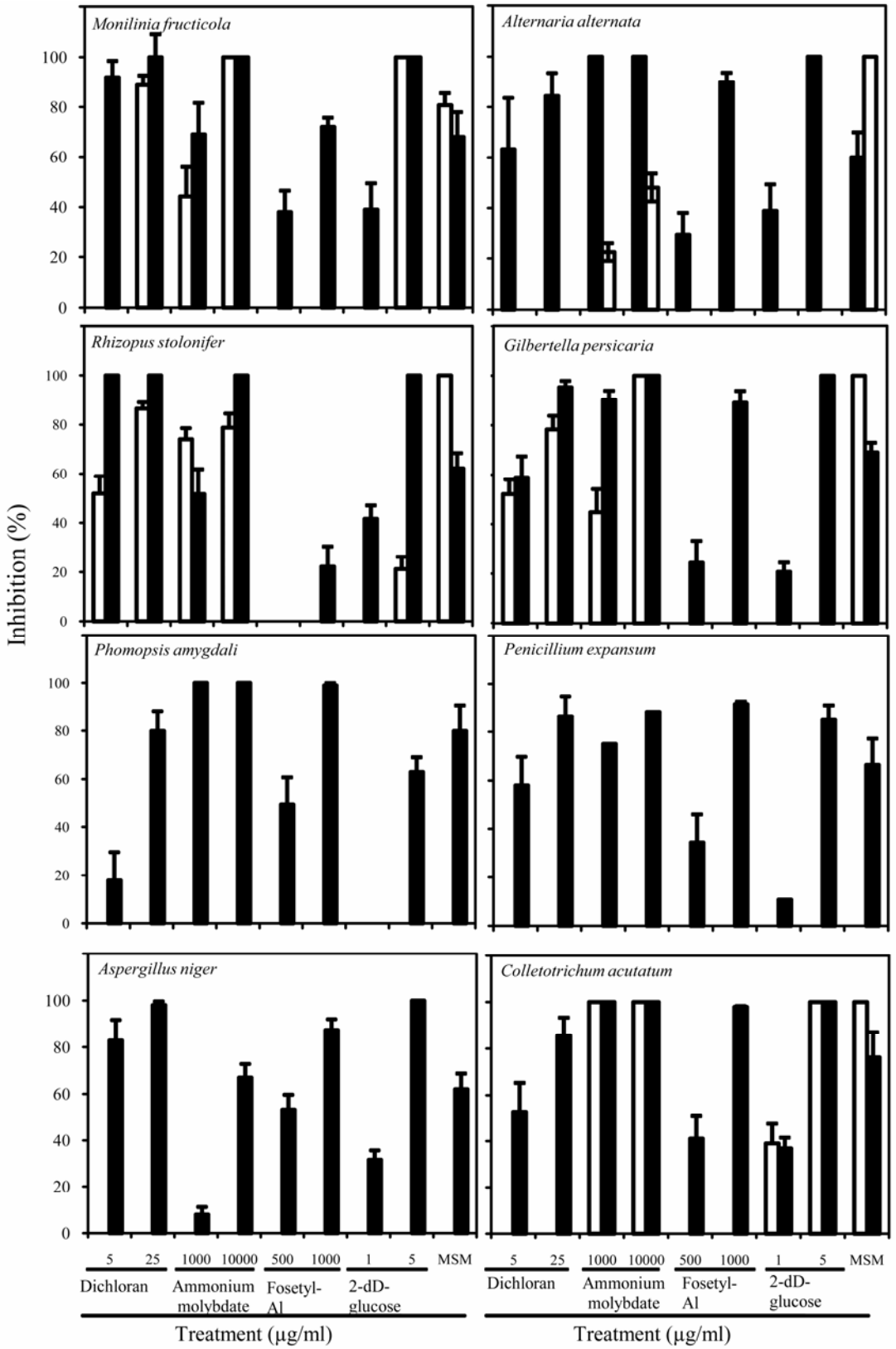

Fig. 1. Conidial germination (open bars) and mycelial growth (closed bars) inhibition of *Monilinia fructicola* and seven other fungal contaminants of peach fruit grown on *Monilinia* selective medium (MSM) and potato dextrose agar (PDA) amended with various active ingredients relative to unamended PDA after 72 h for germination and 96 h for mycelial growth at 22°C. Data are the means (two isolates per species) of 18 and 10 replicates per media and active ingredient concentration across two experimental runs for spore germination and mycelial growth, respectively. Vertical bars indicate the standard deviations.

was transformed to the number of colonies per square centimeter of fruit surface. Data from recovery tests of *Monilinia* spp. from peach and plum surfaces were examined using analysis of variance after the assumption of a normal distribution of errors was verified. Means were compared using the Newman-Keuls test. Analysis was performed using STATISTICA program (version 6.0; Statsoft, Inc.).

RESULTS

Germination and mycelial growth of test fungi on MSM and fungicide-amended PDA. All fungi tested germinated without inhibition on PDA after 24 h. No or weak germination of *C. acutatum*, *Phomopsis amygdali*, *Alternaria alternata*, and *M. fructicola* was observed after 24 h of incubation regardless of the fungicide treatment (data not shown). Dichloran and ammonium molybdate had no germicidal effect on *P. amygdali*, *Aspergillus niger, Penicillium expansum*, and *Alternaria alternata* (dichloran only) after 72 h of incubation at 22°C (Fig. 1). These two compounds, applied at 25 and 10,000 µg/ml, respectively, inhibited germination of *M. fructicola* (89 and 100%), *R. stolonifer* (86.5 and 78.7%), *G. persicaria* (72.8 and 100%), and *C. acutatum* (ammonium molybdate only, 100%). All fungi germinated without inhibition on PDA amended with fosetyl-Al at 500 or 1,000 µg/ml. Germination of *M. fructicola* and *C. acutatum* was completely inhibited by 2-dD-glucose at 5 µg/ml but no effect was observed on the germination of the other fungi. *Phomopsis amygdali*, *Aspergillus niger*, and *Penicillium expansum* germinated (100%) on MSM medium, whereas conidial germination of *M. fructicola* was 80.5% inhibited relative to nonamended PDA (Fig. 1).

In general, mycelial growth of isolates grown on ammonium molybdate-amended PDA and MSM was sparse and lacked uniformity. Growth inhibition data were also collected 48 h after inoculation but mycelial development of some test fungi was insufficient for data collection (data not shown). Dichloran, ammonium molybdate, and 2-dD-glucose added to PDA at the higher rate inhibited strongly mycelial growth of all eight fungal species. The average percent inhibition relative to nonamended PDA was 62.9 to 100% (Fig. 1). Dichloran inhibited mycelial growth of all test fungi at the lower rate (5 µg/ml) by >50%, with the exception of *Phomopsis amygdali* (17.8%). Mycelial growth inhibition of all fungi tested was 52 to 100% at the lower rate (1,000 µg/ml) of ammonium molybdate, with the exception of *A. niger* (8% inhibition). Dichloran and 2-dD-glucose completely inhibited *M. fructicola* mycelial growth at 5 µg/ml. Among the four active ingredients tested, fosetyl-Al at 1,000 µg/ml inhibited *R. stolonifer* and *M. fructicola* mycelium the least (72 and 22%, respectively). The inhibition of mycelial growth of the eight fungi tested on fosetyl-Al-amended PDA at 500, 750, and 1,000 µg/ml was 0.0 to 53.1, 10.8 to 91.1 (data not shown), and 22.2 to 99.0%, respectively (Fig. 1). Mycelial growth of all eight test fungi was inhibited at a similar level on MSM medium, and the average percent inhibition ranged from 60% in *Alternaria alternata*, *R. stolonifer*, and *Aspergillus niger* to 67.6% in *M. fructicola* and 76.2% in *C. acutatum* (Fig. 1).

Sporulation of test fungi on MSM and fungicide-amended PDA. Overall, sporulation was reduced on MSM and on PDA amended with the higher concentration of all four active ingredients, with the exception of *Phomopsis amygdali* and *Aspergillus niger* on 2-dD-glucose-amended PDA (Table 2). In contrast to all other fungi tested, sporulation of *M. fructicola* was readily

TABLE 2. Relative sporulation of *Monilinia fructicola* and seven other fungal species on *Monilinia* selective medium (MSM) and on potato dextrose agar amended with different active ingredients

	Relative sporulation[a]								
	Active ingredient (µg/ml)								
	Dichloran		Ammonium molybdate		Fosetyl-aluminum		2-deoxy-D-glucose		
Fungal species	5	15	10^3	10^4	500	750	1	5	MSM
Monilinia fructicola	2	0	1	0	4[b]	3	2	0	0
Alternaria alternata	2	1	0	0	2	0	3	2	0
Rhizopus stolonifer	0	0	1	0	4	2	2	0	0
Gilbertella persicaria	0	0	2	0	4	1	0	0	0
Phomopsis amygdali	3	2	2	0	2	1	4	4[b]	2
Penicillium expansum	4	2	4	2	3	1	1	0	2
Aspergillus niger	3	0	4[b]	1	3	1	4	4[b]	1
Colletotrichum acutatum	0	0	0	0	2	0	3	0	0

[a] Sporulation relative to potato dextrose agar (PDA). Data are the mean sporulation level of 12 replicates (from two isolates per species) per media and active ingredient concentration across two experimental runs after 4 days of incubation at 22°C. Sporulation levels 0, 1, 2, 3, and 4 indicate no sporulation, ≤25, 26 to 50, 51 to 75, and >76% sporulation, respectively, relative to the PDA control.
[b] Sporulation heavier than control.

TABLE 3. Effect of pH on the germination and mycelial growth of *Monilinia fructicola* and seven other fungal contaminants of peach fruit on acidified potato dextrose agar amended with fosetyl-aluminum (PDA-F)[a]

	Inhibition of conidial germination (%)					Inhibition of mycelial growth (%)				
		PDA-F at pH					PDA-F at pH			
Fungal species	APDA[b]	2.8	3.2	3.6	4.0	APDA[b]	2.8	3.2	3.6	4.0
Monilinia fructicola	0	50.5 ± 8.1	0	0	0	18.1 ± 6.6	84.2 ± 5.8	54.2 ± 8.2	24.2 ± 4.3	0
Alternaria alternata	0	100	100	100	23.1 ± 1.2	21.2 ± 3.9	100	96.3 ± 1.7	73.5 ± 13.2	13.1 ± 3.3
Rhizopus stolonifer	6.6 ± 1.9	100	100	50.5 ± 10.1	16.2 ± 2.3	29 ± 4.7	96.3 ± 3.7	91.9 ± 4.6	39.6 ± 5.9	8.8 ± 1.1
Gilbertella persicaria	0	100	100	55.7 ± 8.4	0	36.3 ± 5.7	100	84.0 ± 8.4	43.9 ± 4.9	3.8 ± 0.7
Phomopsis amygdali	62.6 ± 7.3	100	100	100	62.6 ± 9.3	59.4 ± 10.8	100	100	88.3 ± 5.8	39.6 ± 5.3
Penicillium expansum	12.6 ± 1.7	100	72.3 ± 5.2	65.8 ± 7.3	12.6 ± 1.7	14.2 ± 6.8	100	98.3 ± 1.7	78.3 ± 6.9	19.8 ± 1.4
Aspergillus niger	32.6 ± 5.8	100	100	75.2 ± 8.4	0	33.5 ± 9.6	100	100	34.0 ± 4.4	0
Colletotrichum acutatum	4.6 ± 1.7	100	100	100	24.6 ± 6.2	7.6 ± 2.8	100	87.6 ± 7.7	72.1 ± 3.3	32.3 ± 5.5

[a] Data are means ±95% confidence intervals of 10 replicates (from two isolates per species) per media and pH value across two experimental runs.
[b] Acidified PDA (APDA) at pH 3.6.

observed on PDA amended with fosetyl-Al at 750 µg/ml (Table 2). All other active ingredients completely inhibited sporulation of *M. fructicola* at the higher rates, including on MSM.

Effect of pH on the efficacy of fosetyl-Al. All fungi tested were strongly inhibited (>90%) on unamended APDA medium at pH <3.2 (data not shown). At pH 5.2, all fungi, with the exception of *Phomopsis amygdali*, grew >50%. Consequently, only inhibition data from APDA at pH 3.6 are shown (Table 3). Unamended APDA medium at pH 3.6 inhibited conidial germination of *C. acutatum*, *R. stolonifer*, *Penicillium expansum*, *Aspergillus niger*, and *Phomopsis amygdali* by 4.6, 6.6, 12.6, 32.6, and 62.6%, respectively, whereas no inhibition of *M. fructicola*, *Alternaria alternata*, and *G. persicaria* was observed on APDA (Table 3). The fungi tested were differentially inhibited on APDA-F500. A strong inhibition (>50%) of conidial germination of all fungi was observed on APDA-F500 medium at pH 3.6, except *M. fructicola* (0%) (Table 3); complete germination inhibition was observed for *Alternaria alternata, Phomopsis amygdali*, and *C. acutatum*. At pH 3.2, germination of all tested fungi was completely inhibited, with the exception of *Penicillium expansum* and *M. fructicola* (72.3 and 0% inhibition, respectively). *M. fructicola* was the only fungus able to germinate (50.5% inhibition) on APDA-F500 at pH 2.8. Even though germination of *R. stolonifer* and *Penicillium expansum* was not entirely inhibited

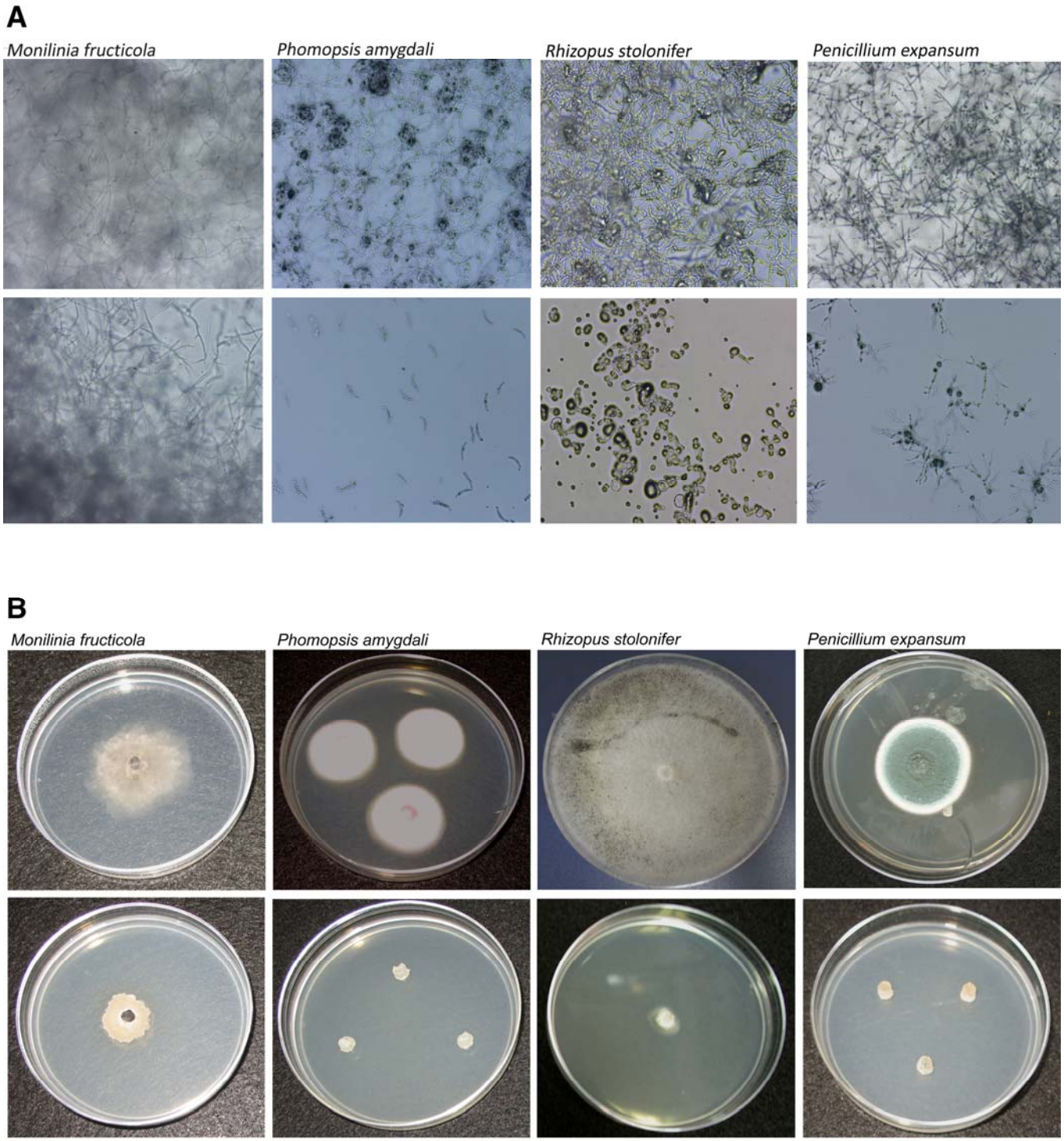

Fig. 2. Germination (**A**) and hyphal growth (**B**) of *Monilinia fructicola*, *Phomopsis amygdali*, *Rhizopus stolonifer,* and *Penicillium expansum* on potato dextrose agar (PDA) (first row) and acidified PDA amended with fosetyl-aluminum at 500 µg/ml (APDA-F500) at pH 3.6 (second row) after 72 h of incubation at 22°C.

on APDA-F500 at pH 3.6, the germ tube length was substantially reduced for *R. stolonifer* and, to a lesser degree, for *Penicillium expansum* compared with *M. fructicola* (Fig. 2A).

The average growth inhibition on unamended APDA at pH 3.6 varied between 7.6% for *C. acutatum* and 59.7% for *Phomopsis amygdali* (Table 3). Mycelial growth of the fungi tested was reduced by lowering the pH of the PDA-F500 medium. At pH 2.8, mycelial growth of most fungi was completely inhibited, with the exception of *M. fructicola* (84.2% inhibition) and *R. stolonifer* (96.3% inhibition). Germ tube elongation of *M. fructicola* was less affected at pH 3.6 compared with the other fungi tested (Fig. 2B).

Intraspecific variation in germination and mycelial growth of *Monilinia* spp. on APDA-F500. Germination of the three *Monilinia* spp. was not inhibited (100% germination) on APDA-F500 at pH 3.6 after 72 h (Fig. 3). Relative mycelial growth of *M. fructicola, M. fructigena*, and *M. laxa* isolates on APDA-F500 was 45.8 to 83.3, 58.8 to 80.4, and 53.3 to 79.8%, respectively, after 96 h of incubation (Table 4).

Recovery of *Monilinia* propagules from peach and plum fruit surfaces. No *M. fructicola* propagules were recovered on any of the media at the early (June) sampling date (Table 5). The recovery of *M. fructicola* was significantly higher ($P = 0.03$) on PDA-F500 and APDA-F500 compared with MSM at the July and August sampling dates. However, the number of contaminants (i.e., bacteria, yeast, and other fungi) was eight to nine times greater on PDA-F500 and MSM at the June and July sampling dates and two to three times higher at the August sampling date compared with APDA-F500 (Table 5). *M. fructigena* and *M. laxa* propagules were collected in August from peach and plum fruit collected in Hungary. The recovery of *M. fructigena* and *M. laxa* on APDA-F500 from peach was 64.3 and 4.2% and from plum was 56.2 and 3%, respectively (Table 6).

Among fruit surface contaminants, *Cladosporium* spp. were recovered most frequently on APDA-F500 in June while *Aureobasidium* spp. and other nonidentified yeast were recovered at higher rates later in the growing season. Propagules of *Alternaria* spp. were recovered on PDA-F500 and MSM but not on APDA-F500 during the three sampling periods. There was a significant ($P = 0.017$) reduction in recovery of bacteria on APDA-F500 compared with PDA-F500 and on MSM, despite the high concentration of streptomycin (1,000 µg/ml) in the later medium (Table 5). The slow growth of *M. fructicola* propagules on MSM made its distinction from other contaminants more difficult compared with APDA-F500 and PDA-F500 (Fig. 4). Other common fungi isolated from peach surfaces included *R. stolonifer* and *Geotrichum candidum* (on PDA-F500 only), *Fusarium equiseti*, *Ustilago esculenta*, *Epicoccum nigrum*, *Rhizosphaera macrospora*, and the yeast *Issatchenkia orientalis*.

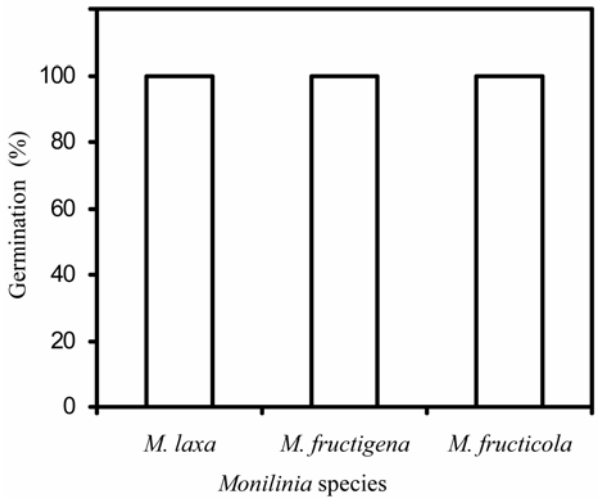

Fig. 3. Germination of three *Monilinia* spp. on acidified potato dextrose agar amended with fosetyl-aluminum at 500 µg/ml relative to unamended potato dextrose agar after 72 h. Data are the means of 36 replicates (two isolates per species × three plate × three countings per plate) across two experimental runs.

DISCUSSION

The recovery of *Monilinia* spp. from plants is an important step for studying their biology and ecology, but isolation attempts can be challenging. Bacteria and yeasts are generally faster growing, omnipresent on fruit surfaces, and tend to develop suppressive or antagonistic activities against fungi. Additionally, other fast-growing fungi can overgrow the target fungus during isolation and culturing. MSM was previously developed for the recovery of *Monilinia* spp. from stone fruits (49) but, in this study, MSM was suppressive to mycelial growth of *M. fructicola*. MSM also did not prevent germination of three of the eight common peach fungal contaminants tested in this study, including *Penicillium expansum*, *Phomopsis amygdali*, and *Aspergillus niger*. The weak selectivity shown in this study is in agreement with an earlier report stating that growth of *Monilinia* spp. on MSM can be obscured by bacteria, yeast, and other fungi, especially when free water is present on the medium surface when plating the sample (49). With the new selective medium APDA-F500 developed in this study, the recovery of *Monilinia* spp. from stone fruit is much improved. Many potential fungal contaminants were suppressed while *Monilinia* colonies were still developing.

We examined four antifungal agents, including 2-dD-glucose, ammonium molybdate, dichloran, and fosetyl-Al. These agents

TABLE 4. Mycelial growth of *Monilinia* spp. isolates from different geographic origins and hosts on acidified potato dextrose agar amended with fosetyl-Al at 500 µg/ml

Species, isolate	Geographic origin	Relative growth (%)[a]
Monilinia fructicola		
Mf.Pdt11	South Carolina, United States	49.7 ± 9.8
Mf.DMap4	South Carolina, United States	63.3 ± 7.5
Mf.Egpc5	South Carolina, United States	48.1 ± 7.0
Mf.Egpc12	South Carolina, United States	56.6 ± 13.5
Mf.Bmpc9	Georgia, United States	75.6 ± 13.6
Mf.Bpc1	Georgia, United States	58.1 ± 13.7
Mf.Bpc8	Georgia, United States	52.4 ± 13.8
Mf.SLf1	Georgia, United States	83.3 ± 13.5
139	California, United States	69.1 ± 11.6
NJ07	New Jersey, United States	81.8 ± 9.7
NJ08	New Jersey, United States	52.2 ± 6.1
NJ012	New Jersey, United States	57.7 ± 6.8
NJ015	New Jersey, United States	46.5 ± 3.9
2F	New York, United States	45.8 ± 5.7
6c	New York, United States	76.6 ± 13.5
10B	New York, United States	48.6 ± 10.1
13B	New York, United States	53.9 ± 12.7
M. fructigena		
MFG.E4	Szabolcs, Hungary	78.8 ± 2.5
MFG.D10	Hajdú, Hungary	77.4 ± 2.2
MFG.BP7	Pest, Hungary	58.8 ± 6.4
MFG.ME16	Borsod, Hungary	65.6 ± 10.6
MFG.MA11	Szabolcs, Hungary	63.2 ± 5.4
MFG.DP24	Hajdú, Hungary	80.4 ± 6.8
M. laxa		
ML.E14	Szabolcs, Hungary	71.7 ± 3.1
ML.D5	Hajdú, Hungary	73.5 ± 3.2
ML.BP5	Pest, Hungary	54.6 ± 11.3
ML.ME6	Borsod, Hungary	69.0 ± 5.9
ML.MA21	Szabolcs, Hungary	53.3 ± 9.4
ML.DP7	Hajdú, Hungary	79.8 ± 4.8

[a] Relative growth relative to unamended potato dextrose agar after 96 h of incubation at 22°C. Data are the mean of 10 replicate petri dishes across two experimental runs.

TABLE 5. Recovery of *Monilinia fructicola* and other microorganisms from peach surfaces on acidified potato dextrose agar amended with fosetyl-Al at 500 µg/ml (APDA-F500) (pH 3.6), potato dextrose agar amended with fosetyl-Al at 500 µg/ml (PDA-F500), and *Monilinia* selective medium (MSM)

	Mean number of colonies per square centimeter of fruit surface ± SD[a]								
	6 June			9 July			13 August		
Organism	APDA-F500	PDA-F500	MSM	APDA-F500	PDA-F500	MSM	APDA-F500	PDA-F500	MSM
Monilinia fructicola	0	0	0	5 ± 0.8	8 ± 1.2	0	76 ± 6.8	99 ± 9.8	8 ± 0.4
Aureobasidium spp.	7 ± 1.2	19 ± 1.6	13 ± 1.4	8 ± 1.1	7 ± 1.2	8 ± 1.4	7 ± 1.9	2 ± 0.2	2 ± 0.8
Cladosporium spp.	12 ± 2.3	103 ± 7.1	109 ± 14.7	2 ± 0.9	4 ± 0.9	23 ± 0.8	2 ± 0.8	2 ± 0.8	2 ± 0.6
Alternaria spp.	0	19 ± 2.5	12 ± 2.0	0	1 ± 0.3	9 ± 0.2	0	3 ± 0.3	12 ± 1.2
Bacteria	3 ± 0.8	72 ± 9.0	27 ± 4.2	1 ± 0.4	185 ± 8.5	34 ± 4.5	3 ± 0.4	35 ± 8.1	17 ± 1.9
Yeast	5 ± 1.2	12 ± 2.3	93 ± 14.3	12 ± 2.9	24 ± 3.2	156 ± 8.3	20 ± 2.1	31 ± 3.3	71 ± 1.9
Other	2 ± 0.4	7 ± 1.4	1 ± 0.2	2 ± 0.4	1 ± 0.5	0	3 ± 0.4	26 ± 3.2	4 ± 0.9
Total contaminants	29 ± 1.2	232 ± 4.3	254 ± 6.1	25 ± 1.1	222 ± 2.4	230 ± 2.4	35 ± 1.1	99 ± 2.7	108 ± 1.1
M. fructicola (%)	0	0	0	16.6	3.5	0	69.1	50.0	6.8

[a] Data are means ± standard deviations (SD) of nine replicates per fruit (10 fruit/period) and medium. Peach fruit were washed in 100 ml of distilled water with 0.05% Tween 80 and a 100-µl aliquot of each fruit-wash was spread over media. Propagules of *Monilinia* and other microorganisms were counted on each dish after 4 days of incubation at 22°C using a digital colony counter and expressed in number of colonies per square centimeter of fruit surface.

TABLE 6. Recovery of *Monilinia fructigena*, *M. laxa*, and other microorganisms from peach and plum surfaces on acidified potato dextrose agar amended with fosetyl-aluminum at 500 µg/ml (APDA-F500) (pH 3.6)

	Mean number of colonies/cm^2 fruit surface ± SD[a]	
Organism	Peach	Plum
Monilinia fructigena	116.5 ± 22	53.7 ± 8.1
Monilinia laxa	7.5 ± 2.3	2.9 ± 2.2
Aureobasidium spp.	10.2 ± 3.3	2.0 ± 1.4
Cladosporium spp.	11.3 ± 2.6	8.5 ± 2.9
Alternaria spp.	0	0
Bacteria	9.3 ± 2.9	7.2 ± 3.4
Yeast	16.9 ± 5.2	14.4 ± 4.1
Other	9.1 ± 2.9	6.9 ± 2.2
Total contaminants	31.5 ± 6.3	40.8 ± 6.6
M. fructigena (%)	64.3	56.2
M. laxa (%)	4.2	3.0

[a] Data are means ± standard deviations (SD) of nine replicate plantings per fruit. In total, 20 plum and 20 peach fruit were sampled on 16 and 29 August 2008, respectively.

were chosen because of their suppressive activity against some common peach fruit pathogens and contaminants. The sugar analog 2-dD-glucose was reported to reduce the mycelial growth of *Rhizopus stolonifer*, *Botrytis cinerea*, and *Penicillium expansum* at 1 mg/ml without completely inhibiting *M. fructicola* (15). In this study, both germination and mycelial growth of *M. fructicola* were completely inhibited at a much lower concentration (5 µg/ml), whereas germination of most other fungi tested was not inhibited. Similarly, ammonium molybdate showed activity against *Penicillium expansum* (37,44,46) and *R. stolonifer* (44) but also inhibited germination and mycelial growth of *M. fructicola* in the present study. Additionally, ammonium molybdate at 5 mM enhanced the biocontrol activity of yeasts against *M. fructicola* on sweet cherry (50). Dichloran (2,6-dichloro-4-nitroaniline) formulated as Botran 75WP is a protectant fungicide, recommended for pre- and postharvest Rhizopus rot control in stone, berry, and grape fruit. Dichloran was reported to inhibit *R. stolonifer* and *Aspergillus* spp. at 2.5 µg/ml and *Penicillium* spp. at 12.5 µg/ml when added to a minimum medium (33). We confirmed the activity of dichloran against *R. stolonifer* but also noticed an inhibitory effect against *M. fructicola* at low concentrations. Dichloran was less active against mycelial growth of *Alternaria alternata*, *Phomopsis amygdali*, and *Penicillium expansum* and did not affect conidial germination of these organisms. Among other potential fungal growth suppressors evaluated, rose bengal, ammonium sulfate, sodium moybdate dehydrate, and ziram were either suppressive of *Monilinia* spp. growth or with no effect against the other fungal contaminants (data not shown).

Compared with the three other active ingredients investigated, fosetyl-Al was the most suitable selective agent for the development of a new *Monilinia* spp.-selective medium. The fungicide fosetyl-Al amended to fenaminousulf medium at 250 µg/ml was previously recommended for selective isolation of *Rhizoctonia solani* from soil (20). In this study, fosetyl-Al alone failed to inhibit germination and mycelial growth of any of the fungi tested but reduced sporulation at the higher concentration (>750 µg/ml), with the exception of *M. fructicola*. The acidification of fosetyl-Al-amended PDA to pH 3.2 to 3.6 resulted in germination and mycelial growth inhibition of all of the seven test fungi but *M. fructicola* in vitro. Germination of *Monilinia* spp. isolates originating from different geographic locations was not inhibited on APDA-F500, and little variation in mycelial growth inhibition was observed. Therefore, APDA-F500 selectively supports growth of different genotypes of the three most common *Monilinia* spp., assuming that isolates from different locations are genetically diverse. At pH 3.2 and 3.6, APDA-F500 limited the growth of contaminants effectively while supporting *M. fructicola* development during isolation. The *Monilinia* spp.-selective properties of APDA-F500 were confirmed on stone fruits from Hungary, documenting the successful recovery of *M. fructigena* and *M. laxa* under different environmental conditions. Acidification of PDA helped suppress bacterial growth, which is consistent with earlier reports (1,27,34), and also inhibited key fungal contaminants at pH 3.2.

Fosetyl-Al belongs to the chemical group known as phosphonates and its activity has been reported against several fungi, including a range of *Phytophthora* spp. (12,47). Numerous studies on the mode of action of phosphonates have identified alteration of fungal metabolism (5,52) and changes in cell morphology (29,30). It was also suggested that a perturbation of phosphorous metabolism in fungi may affect their sensitivity to phosphonates (23,51). Lowering the pH increased the fungicidal activity of fosetyl-Al. It is possible that the change of the H^+ gradient in the acidified medium may have affected the fungal membrane permeability for fosetyl-Al of most tested fungi but to a lesser degree for *Monilinia* spp. This would be consistent with findings by Barchietto et al. (6) showing that, at a stable pH, the uptake of fosetyl-Al can be different from one fungus to another. For example, the activity of fosetyl-Al against *Phytophthora nicotianae* was shown to be higher at pH values of 4 to 7 (22), whereas its activity was not pH related against *P. palmivora* (41).

The new APDA-F500 medium is suitable for inoculum density determination on stone fruit surfaces, which may aid in the prediction of preharvest brown rot disease pressure. The inhibition of bacteria and selective reduction of fungal growth resulted in small, easily distinguishable, and countable *Monilinia* colonies. Propagule counts on APDA-F500 revealed a progressive buildup

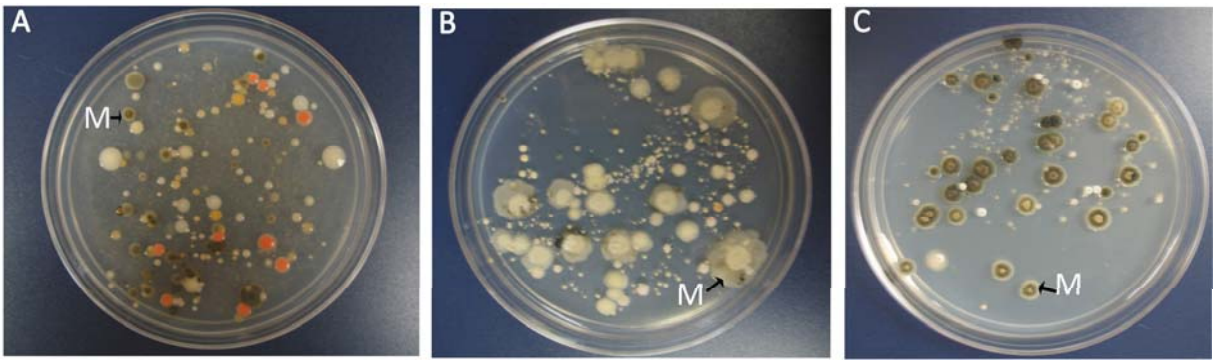

Fig. 4. Growth and colony morphology of *Monilinia fructicola* and other organisms washed off from peach fruit (cv. Contender) on **A,** *Monilinia* spp.-selective medium (MSM) ; **B,** potato dextrose agar (PDA) amended with fosetyl-aluminum (fosetyl-AL) at 500 µg/ml; and **C,** acidified PDA amended with fosetyl-Al at 500 µg/ml, pH 3.6, after 4 days of incubation at 22°C. M indicates *M. fructicola* propagules.

of *M. fructicola* on peach fruit from South Carolina as summer progressed, which is consistent with earlier studies (16,38,49). The highest density of *M. fructicola* propagules on peach fruit was found at the August sampling date. A comparable density of *M. fructigena* propagules was found on peach and plum fruit collected in August in Hungary. The low density of *M. laxa* population on fruit surfaces from Hungary may be explained by the epidemiology of this species. *M. laxa* causes primarily blossom blight and is a minor fruit rot pathogen in Hungary on plum and peach (24). On the other hand, *M. fructigena* has not been reported to cause blossom infections in Central Europe. This species occurs primarily on ripening fruit and can cause significant fruit brown rot, especially on fruit with severe insect damage (25).

In summary, APDA-F500 was found to be a suitable medium for selective isolation of *Monilinia* spp. from stone fruit. This medium suppressed bacteria and inhibited or limited the growth of fungi and other organisms commonly encountered on stone fruit. APDA-F500 also yielded comparable recovery rates of *Monilinia* spp. of stone fruit under different environmental conditions. APDA-F500 will facilitate *Monilinia* spp. recovery from stone fruits and will be a useful tool for the study of the biology, epidemiology, and quantification of *Monilinia* propagules as part of disease management efforts.

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LITERATURE CITED

1. American Public Health Association. 1978. Standard Methods for the Examination of Dairy Products, 14th ed. American Public Health Association Inc. Washington, DC.
2. Amiri, A., and Bompeix, G. 2005. Diversity and population dynamics of *Penicillium* spp. on apples in pre- and postharvest environments: Consequences for decay development. Plant Pathol. 54:74-81.
3. Amiri. A., Scherm, H., Brannen, P. M, and Schnabel, G. 2008. Laboratory evaluation of three rapid, agar-based assays to assess fungicide sensitivity in *Monilinia fructicola*. Plant Dis. 92:415-420.
4. Andrews, J. H., Kinkel, L. L., Berbee, F. M., and Nordheim, E. V. 1987. Fungi leaves and the theory of island biogeography. Microbiol. Ecol. 14:277-290.
5. Barchietto, T., Saindrenan, P., and Bompeix, G. 1988. Characterization of phosphonate uptake in two *Phytophthora* spp. and its inhibition by phosphate. Arch. Microbiol. 151:54-58.
6. Barchietto, T., Saindrenan, P., and Bompeix, G. 1992. Physiological responses of *Phytophthora citrophtora* to a subinhibitory concentration of phosphonate. Pestic. Chem. Physiol. 42:151-166.
7. Batra, L. R. 1991. World species of *Monilinia* (fungi): their ecology, biosystematics and control. Mycologia Memoir No. 16. J. Cramer, Berlin.
8. Biggs, A. R., and Northover, J. 1988. Early and late-season susceptibility of peach fruits to *Monilinia fructicola*. Plant Dis. 72:1070-1074.
9. Bosshard, E., Hilber-Bodmer, M., Schärer, H. J., Bünter, M., and Duffy, B. 2006. First report of quarantine brown rot pathogen *Monilinia fructicola* on imported stone fruits in Switzerland. Plant Dis. 90:1554.
10. Byrde, R. J. W., and Willets. H. J. 1977. The Brown Rot Fungi of Fruit: Their Biology and Control. Pergamon Press, Oxford.
11. Cohen, G. 1994. The earth is round ($P < 0.05$). Am. Psychol. 49:997-1003.
12. Darakis, G. A., Bourbos, V. A., and Skoudridakis, M. T. 1997. Phosphonate transport in *Phytophthora capsici*. Plant Pathol. 46:762-772.
13. Duchoslavová, J., Širucková, I., Zapletalová, E., Navrátil, M., and Šafárová, D. 2007. First report of brown rot caused by *Monilinia fructicola* on various stone and pome fruits in the Czech Republic. Plant Dis. 91:907.
14. Edwards, S. G., and Seddon, B. 2001. Selective media for the specific isolation and enumeration of *Botrytis cinerea* conidia. Lett. Appl. Microbiol. 32:63-66.
15. El Ghaout, A., Wilson, C. L., and Wisniewski, M. 1995. Sugar analogs as potential fungicides for postharvest pathogens of apple and peach. Plant Dis. 79:254-258.
16. Emery, K. M., Michailides, T. J., and Scherm, H. 2000. Incidence of latent infection of immature peach fruit by *Monilinia fructicola* and relationship to brown rot in Georgia. Plant Dis. 84:853-857.
17. Fairchild, E. J., Lewis, R. S., and Taken, R. L. 1977. Registry of Toxic Effects of Chemical Substances. U. S. Department of Health, Education and Welfare, Cincinnati.
18. Fenn, M. E., and Coffey, M. D. 1984. Studies on the in vitro and in vivo antifungal activity of fosetyl-Al and phosphorous acid. Phytopathology 74:606-611.
19. Frisvad, J. C. 1981. A selective and indicative medium for groups of *Penicillium viridicatum* producing different mycotoxins in cereal. J. Appl. Bacteriol. 54:409-416.
20. Gangopadhyay, S., and Grover, R. K. 1985. A selective medium for isolating *Rhizoctonia solani* from soil. Ann. Appl. Biol. 106:405-412.
21. Gell, I., De Cal, A., Torres, R., Usall, J., and Melgarejo, P. 2008. Relationship between the incidence of latent infections caused by *Monilinia* spp. and the incidence of brown rot of peach fruit: factors affecting latent infection. Eur. J. Plant Pathol. 121:487-498.
22. Guest, D. I. 1984. The influence of cultural factors on the direct antifungal activities of fosetyl-Al, propamocarb, metalaxyl, SN 75196 and dowco 444. Phytopathol. Z. 111:155-164.
23. Guest, D. I., and Grant, B. R. 1991. The complex action of phosphonates as antifungal agents. Biol. Rev. 66:159-187.
24. Holb, I. J. 2003. Analyses of temporal dynamics of brown rot development on fruit in organic apple production. Int. J. Hortic. Sci. 9:97-100.
25. Holb, I. J., and Scherm, H. 2008. Quantitative relationships between different injury factors and development of brown rot caused by *Monilinia fructigena* in integrated and organic apple orchards. Phytopathology 98:79-86.

26. Honey, E. E. 1928. The monilioid species *Sclerotinia*. Mycologia 20:127-157.
27. Hong, C. X., Michailides, T. J., and Holtz, B. A. 2000. Mycoflora of stone fruit mummies in California orchards. Plant Dis. 84:417-422.
28. Hong, S. G., and Pryor, B. M. 2004. Development of selective media for the isolation and enumeration of *Alternaria* species from soil and plant debris. Can. J. Microbiol. 50:461-468.
29. Jiang, Y. 1990. Alternations in the host-parasite interface between *Phytophthora infestans* and tomato leaf cells after treatment with fosetyl-Al. Plant Sci. 71:267-276.
30. Jiang, Y., and Grossman, F. 1992. Subcellular alternations in *Phytophthora infestans* infecting tomato leaves treated with fosetyl-Al. Pestic. Biochem. Physiol. 44:226-238.
31. Kwasna, H., Ward, E., and Bateman, G. L. 2006. Phylogenetic relationship among zygomycetes from soil base on ITS1/2 rDNA sequences. Mycol. Res. 110:501-510.
32. Kerssies, A. 1990. A selective medium for *Botrytis cinerea* to be used in a spore-trap. Neth. J. Plant Pathol. 96:247-250.
33. King, A., Hocking, A. D., and Pitt, I. J. 1979. Dichloran-rose bengal medium for enumeration and isolation of molds from foods. Appl. Environ. Mircobiol. 37:959-964.
34. Koburger, J. A. 1976. Yeasts and molds. Pages 225-229 in: Compendium of Methods for the Microbiological Examination of Foods. M. L. Speck, ed. American Public Health Association Inc., Washington, DC.
35. Landgraf, F. A., and Zehr, E. 1982. Inoculum sources of *Monilinia fructicola* in South Carolina peach orchards. Phytopathology 72:185-190.
36. Larena, I., Torres, R., De Cal, A., Liñan, M., Melgarejo, P., and Domenichini, P. 2005. Biological control of postharvest brown rot (*Monilinia* spp.) of peaches by field applications of *Epicoccum nigrum*. Biol. Control 32:305-310.
37. Lima, G., Spina, A. M., Castoria, R., De Curtis, F., and De Cicco, V. 2005. Integration of biocontrol agents and food-grade additives for enhancing protection of stored apples from *Penicillium expansum*. J. Food Prot. 68:2100-21006.
38. Luo, Y., Michailides, T. J., Morgan, D. P., Krueger, W. H., and Buchner, R. P. 2005. Inoculum dynamics, fruit infection, and development of brown rot in prune orchards in California. Phytopathology 95:1132-1136.
39. Ma, Z., Yoshimura, M. A., and Michailides, T. J. 2003. Identification and characterization of benzimidazole resistance in *Monilinia fructicola* from stone fruit orchards in California. Appl. Environ. Microbiol. 29:7145-7152.
40. Malvárez, G., Rodriguez, A., Aguilar, C., Silveira, A. C., Silvera, E., Burgueño, J., and Mondino, P. 2004. *Monilinia fructicola*, the only *Monilinia* species currently causing brown rot of peach (*Prunus persica*) in Uruguay. Fitopatologia 39:126-132.
41. Martin, H., Grant, B. R., and Stehmann, C. 1998. Inhibition of organic pyrophosphate by phosphonate—a site of action in *Phytophthora* spp. Pestic. Biochem. Physiol. 61:65-77.
42. Matsumuto, M., Aiso, S., Senoh, H., Yamazaki, K., Arito, H., Nagano, K., Yamamoto, S., and Matsushima, T. 2006. Carcinogenicity and chronic toxicity of para-chloronitrobenzene in rats and mice by two-year feeding. J. Environ. Pathol. Toxicol. Oncol. 25:571-584.
43. Nash, S. M., and Snyder, W. C. 1962. Quantitative estimations by plate counts of propagules of bean root rot *Fusarium* in field soils. Phytopathology 52:567-572.
44. Nunes, C., Usall, J., Teixidó, N., de Eribe, X. O., and Viñas, I. 2001. Control of post-harvest decay of apples by pre-harvest and post-harvest application of ammonium molybdate. Pest Manage. Sci. 57:1093-1099.
45. Ogawa, J. M., and English, H. 1991. Diseases of Temperate Zone Tree Fruit and Nut Crops. Publ. 3345. University of California, Division of Agriculture and Natural Resources, Oakland.
46. Palou, L., Usall, J., Smilanick, J. L., Aguilar, M. J., and Viñas, I. 2002. Evaluation of food additives and low-toxicity compounds as alternative chemicals for the control of *Penicillium digitatum* and *Penicillium italicum* on citrus fruit. Pest Manage. Sci. 58:459-466.
47. Panicker, S., and Gangadharam, K. 1999. Controlling downy mildew of maize caused by *Peronosclerospora sorghi* by foliar sprays of phosphonic acid compounds. Crop Prot. 18:115-118.
48. Petróczy, M., and Palkovics, L. 2006. First report of brown rot caused by *Monilinia fructicola* on imported peach in Hungary. Plant Dis. 90:375.
49. Phillips, D. J., and Harvey, J. M. 1975. Selective medium for detection of inoculum of *Monilinia* spp. on stone fruits. Phytopathology 65:1233-1236.
50. Qin, G. Z., Tian, S. P., Xu, Y., Chan, Z. L., and Li, B. Q. 2006. Combination of antagonist yeasts with two food additives for control of brown rot caused by *Monilinia fructicola* on sweet cherry fruit. J. Appl. Microbiol. 100:508-515.
51. Saindrenan, P., Barchietto, T., and Bompeix, G. 1990. Effect of phosphonate on the elicitor activity of culture filtrates of *Phytophthora cryptogea*. Plant Sci. 67:245-251.
52. Smillie, R. H., Dunstan, R. H., Grant, B. R., Grifith, J. M., Iser, J., and Niere, J. O. 1990. The mode of action of antifungal agent phosphate. OEPP/EPPO Bull. 20:185-192.
53. Takehara, T., Kuniyasu, K., Mori, M., and Hagiwara, H. 2003. Use of nitrate-nonutilizing mutant and selective media to examine population dynamics of *Fusarium oxysporum* f. sp. *spinaciae* in soil. Phytopathology 93:1173-1181.
54. Tian, S. P., and Bertolini, P. 1999. Effect of temperature during conidial formation of *Monilinia laxa* on conidial size, germination and infection of stored nectarines. Phytopathol. Z. 147:635-641.
55. Vujanovic, V., Hamel, C., Jabaji-Hare, S., and St.-Arnaud, M. 2002. Development of a selective myclobutanil agar (MBA) medium for the isolation of *Fusarium* species from asparagus fields. Can. J. Microbiol. 48:841-847.