Antifungal Activity of Natural Compounds against Thiabendazole-Resistant *Fusarium sambucinum* Strains

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Several natural compounds that had been previously identified as effective potato sprout inhibitors were examined for antifungal activity against three thiabendazole (TBZ)-resistant strains and a TBZ-sensitive (wild-type) strain of *Fusarium sambucinum*. Salicylaldehyde was the most inhibitory compound of those tested when applied as a volatile and completely inhibited fungal growth at headspace gas levels of 20 μg/mL or lower. Cinnamaldehyde, salicylaldehyde, and thymol prevented all growth when incorporated in media at 0.1% (v/v) levels. TBZ-resistant strains were only partially inhibited by TBZ concentrations that completely inhibited the wild-type strain. Treatment of tubers with compounds after wounding and inoculation with fungal spores was generally ineffective in suppressing dry rot, possibly due to lack of direct contact between the fungi and the compounds.

INTRODUCTION

Potato tuber dry rot caused by several *Fusarium* species is one of the most economically important diseases of stored potatoes worldwide (Boyd, 1972). The *Fusarium* species that are the major causal agents of dry rot in storage are *F. sambucinum* (also known as *F. sulphureum*) and *F. coeruleum* (O'Brien and Rich, 1976). In cold storage such as occurs in North America, *F. sambucinum* is the predominant species (O'Brien and Rich, 1976). Crop losses due to dry rot average about 6% of total stored tubers, but losses up to 25% have been reported (Chelkowski, 1989). *Fusarium* spp. infect tubers only through wounds associated with harvesting and handling. The rapid and thorough formation of suberin and wound periderm appears to be an important resistance mechanism (O'Brien and Leach, 1983). Control of dry rot has generally been accomplished by a combination of procedures, which include avoiding damaging tubers during harvest, promoting tuber suberization (skin-set) after harvest, and applying fungicides (O'Brien and Rich, 1976).

The fungicide thiabendazole (TBZ) is currently the only postharvest fungicide registered for dry rot control on potatoes (Powelson et al., 1993). Recently, tolerance to TBZ has been found in many *F. sambucinum* strains across the United States, Canada, and Europe (Tivoli et al., 1986; Powelson et al., 1993). This resistance has been found to be due to a genetic mutation inherited either as a single gene or as closely linked genes, and resistant strains have comparable biological fitness to sensitive strains (Desjardins et al., 1993). Resistance appears to have arisen independently several times via selection from indigenous TBZ-sensitive populations (Desjardins et al., 1993). Therefore, the need exists for additional fungicides to be used in an integrated management program for dry rot control.

Two classes of naturally occurring compounds were found to inhibit post dormant potato tubers from sprouting and also to inhibit several different fungal species (Vaughn and Spencer, 1991, 1993). Prevention of sprouting is extremely important, as undesirable changes that occur include weight loss, susceptibility to bruising, production of toxic glycoalkaloids, and accumulation of reducing sugars (causing browning of fried potato products via the Maillard reaction). The only compound presently registered as a sprout inhibitor for application to potatoes in storage in the United States is isopropyl *N*-(3-chlorophenyl)carbamate (CIPC). Recently, CIPC has been the object of debate over possible residue problems (Barton, 1992; Mondy et al., 1992). While CIPC is effective as a sprout inhibitor, it has not been reported as having effective antifungal activity in commercial storage against *F. sambucinum*. In fact, application of CIPC before suberization and wound healing are finished was found to increase the incidence and severity of *Fusarium* dry rot (Cunningham, 1953).

The objective of this study was to investigate the activity of several naturally occurring compounds, previously identified as potato tuber sprout inhibitors, for antifungal activity against TBZ-sensitive and TBZ-resistant strains of *F. sambucinum*.

MATERIALS AND METHODS

Chemicals. All compounds used in this study (benzaldehyde, cineole, cinnamaldehyde, CIPC, menthol, salicylaldehyde, thiabendazole, and thymol) were obtained from Aldrich and used without further purification.

Fungal Cultures. All cultures of *F. sambucinum* were obtained from Dr. Anne Desjardins, USDA/ARS, Peoria, IL. Numbers with the prefix R are deposit numbers for strains at the Fusarium Research Center, The Pennsylvania State University, State College, PA (Desjardins et al., 1993). Four TBZ-resistant strains isolated from potatoes grown in either Idaho (R-9240) or North Dakota (R-9239, R-9272, R-9275) and a wild-type TBZ-sensitive strain from Germany (R-6380) were examined. All strains were maintained on V-8 juice agar (ATCC Medium 343) plates at 25 °C.

Plant Material. *Solanum tuberosum* L. cv. Russet Burbank certified seed tubers were used throughout this study and were stored at 4 °C for 2–4 months until used in tests. This cultivar had been shown previously to be susceptible to *F. sambucinum* (Corsini and Pavek, 1986). All tubers weighed between 150 and 250 g and were free of any evident defects and diseases.

Fungal Bioassays. Resistance of fungal cultures to volatilized compounds was bioassayed using a double-jar system. This consisted of 75-mL jars containing 10 mL of sterilized V-8 juice medium. These jars were then placed onto 5.5-cm filter paper disks (Whatman No. 1) inside 275-mL jars onto which the test compounds were applied. The jars were made airtight with aluminum foil cap liners. After addition of all components, the total gas headspace in the system was 250 mL. Bioassays were initiated by placing 7-mm plugs of agar cut from actively growing margins of 7–10-day-old cultures and placed mycelial surface down on the surface of the agar in the 75-mL jars. Compounds were then pipetted onto the filter paper disks and the jars sealed

Table 1. Antifungal Activity of Vapor-Phase Compounds against *F. sambucinum* Cultures

	MIC[a] (μg/mL) against fungal strain				
	R-6380	R-9239	R-9240	R-9272	R-9275
	10 °C Treatments				
benzaldehyde	40	40	40	100	100
cineole	100	400	100	100	100
cinnamaldehyde	>400	>400	>400	>400	>400
menthol	>400	>400	>400	>400	>400
salicylaldehyde	4	4	4	4	4
thymol	400	>400	400	>400	>400
	20 °C Treatments				
benzaldehyde	40	100	40	100	100
cineole	400	400	100	400	400
cinnamaldehyde	>400	>400	>400	>400	>400
menthol	>400	>400	>400	>400	>400
salicylaldehyde	4	20	4	4	4
thymol	>400	>400	>400	>400	>400

[a] Minimum inhibition concentration (MIC) is defined as the lowest level with no visible growth.

and placed in the dark for 7 days at 10 or 20 °C. The minimum inhibition concentration (MIC) was determined as the lowest level with no visible growth.

Sprout inhibitors were examined as media components at 0.01%, 0.1%, and 1.0% (v/v) by adding stocks of each compound to cooled sterilized V-8 medium. Thiabendazole (10 and 50 μg/mL) was added to media prior to autoclaving. The growth of each strain was measured using 100 mm × 15 mm plastic Petri plates containing 15 mL of medium. Plates were inoculated with 7-mm plugs of mycelia from actively growing cultures and incubated at 10 °C in the dark for 14 days.

Treatment of Inoculated Tubers. The purpose of this study was to determine the activity of sprout inhibitors on inoculated potato tubers exposed in a manner similar to that encountered by potatoes stored in commercial bins. Potato tubers were surface disinfected with 10% chlorine bleach (0.525% sodium hypochlorite) for 15 min and rinsed in sterile distilled water (SDW). Four holes (2 mm wide, 10 mm deep), located equidistant on each tuber midway between the stem and bud ends, were punched with a flame-sterilized nail held at a right angle by a hemostatic forceps which regulated the depth of the hole. Immediately after wounding, 100 μL of a macroconidial suspension containing approximately 10^5 macroconidia/mL of either the wild-type strain (R-6380) or a TBZ-resistant strain (R-9239) was injected into each hole. Uninoculated (water) controls were injected with 100 μL of SDW. Suspensions were prepared by gently scraping 3-week-old fungal cultures with a glass rod after each culture was flooded with SDW and the spore concentration was adjusted with SDW. Tubers were kept for 2 h at 25 °C and then treated either by (a) placing tubers in closed 9.2-L flasks containing 5 g of each compound (this was theoretically sufficient to create saturated headspace gas concentrations for all compounds) for 48 h or (b) dipping tubers for 30 s in either 1% (v/v) solutions of the naturally occurring compounds or 50 μg/mL TBZ, each solution containing 0.05% Tween 20 as an emulsifier (controls contained only Tween 20), and allowing any excess solution to drain off. After treatment, tubers were placed in paper bags in a growth chamber kept at 10 °C and 95% relative humidity. Each treatment consisted of two replicates of 10 tubers each. After storage for 14 days, tubers were cut to fully expose the wound sites and rated on a scale similar to that used by Platt (1992): 0 = no invasion of tuber tissue by the fungus (wound site similar to water controls); 1 = 1–4-mm invasion from the edge of the wound by the fungus; 2 = 4–10-mm invasion; and 3 = >10-mm invasion for each tuber inoculation site.

RESULTS AND DISCUSSION

Antifungal Activity of Volatilized Compounds. The results obtained from exposing cultures of the five *F. sambucinum* strains to the sprout suppressants in the vapor phase are shown in Table 1. Compounds were examined at both 10 and 20 °C because of the possibility of application either (a) when the tubers are initially placed in storage, when they are kept at 20 °C to allow the tubers to suberize, or (b) later during extended storage when they are kept at 10 °C. In general, all five strains responded similarly to each of the compounds. The higher storage temperature had a negative effect on toxicity; in several cases compounds were less inhibitory at 20 °C than at 10 °C. Potato tubers are currently treated with TBZ during the loading of tubers into storage bins and treated with CIPC by fogging it into the ventilation systems of the storage bins as an aerosol. CIPC concentrations of 20 ppm or greater in the peel are required to inhibit sprouting (Corsini et al., 1979). It is probable that any compound developed for commercial use will be applied in a similar manner. We have previously shown that exposure to a saturated atmosphere of salicylaldehyde for 24 h resulted in complete suppression of sprouting (Vaughn and Spencer, 1993). This concentration of salicylaldehyde is much greater than that required to prevent fungal growth in these tests. Although the absolute concentrations of salicylaldehyde or other compounds that would be obtained in commercial bins are unknown, they are probably higher than the MICs found in this study.

Table 2. Growth of *F. sambucinum* Cultures by Compounds Incorporated in Culture Media

	mycelial growth[a] (% of control) of fungal strain				
treatment	R-6380	R-9239	R-9240	R-9272	R-9275
benzaldehyde					
0.01%	91	81	86	83	97
0.10%	78	62	68	64	72
1.00%	0	0	0	0	0
cineole					
0.01%	94	93	102	99	103
0.10%	92	92	82	91	108
1.00%	84	83	91	99	93
cinnamaldehyde					
0.01%	66	72	74	75	70
0.10%	0	0	0	0	0
1.00%	0	0	0	0	0
menthol					
0.01%	91	82	86	92	95
0.10%	7	18	8	38	32
1.00%	0	0	0	0	0
salicylaldehyde					
0.01%	78	69	77	76	85
0.10%	19	24	17	50	30
1.00%	0	0	0	0	0
thymol					
0.01%	11	15	0	22	8
0.10%	0	0	0	0	0
1.00%	0	0	0	0	0
thiabendazole					
10 μg/mL	0	62	81	68	72
50 μg/mL	0	32	44	32	39
LSD (α = 0.05)	6	8	12	11	8

[a] Growth was measured as colony diameters taken from representative points on the growing margins.

Compounds Incorporated in Media. Because TBZ is currently applied as an emulsified postharvest spray (at a rate of approximately 13 mg of TBZ/kg of potatoes) to tubers as they are being loaded into storage bins, the sprout suppressants were tested against fungal strains as emulsions in V-8 agar medium. All of the natural compounds except cineole completely inhibited growth of the five strains at 1.00% concentrations (Table 2). However, only cinnamaldehyde and thymol were completely inhibitory at 0.10% levels, with thymol additionally completely suppressing growth of strain R-9240 at 0.01%. Cinnamaldehyde and salicylaldehyde completely inhibited sprouting when tubers were dipped in 1% emulsions for 10 s (Vaughn and Spencer, 1993). TBZ completely inhibited

Table 3. Decay of Potato Tubers Inoculated with *F. sambucinum* Strains and Treated with Volatilized Compounds

	tuber decay rating[a] with fungal strain	
treatment	R-6380	R-9239
control	3.0ab[b]	3.3a
benzaldehyde	3.4a	3.1a
cineole	2.9ab	2.5b
cinnamaldehyde	2.5bc	3.4a
menthol	2.3c	3.0ab
salicylaldehyde	2.9ab	3.3a
thymol	2.5bc	2.8ab

[a] Tuber inoculation sites rated from 0 to 4 for degree of decay. [b] Ratings followed by the same letter are not significantly different ($P = 0.05$).

Table 4. Decay of Potato Tubers Inoculated with *F. sambucinum* Strains and Treated with Emulsified Compounds

	tuber decay rating[a] with fungal strain	
treatment	R-6380	R-9239
control	3.1a[b]	2.9b
1% benzaldehyde	2.8ab	3.6a
1% cineole	2.5abc	3.1b
1% cinnamaldehyde	2.4bc	3.3ab
1% menthol	1.8c	3.3ab
1% salicylaldehyde	2.8ab	3.5a
1% thymol	2.3bc	3.1b
50 μg/mL TBZ	2.4bc	3.2ab

[a] Tuber inoculation sites rates from 0 to 4 for degree of decay. [b] Ratings followed by the same letter are not significantly different ($P = 0.05$).

the wild-type strain R-6380 at both 10 and 50 μg/mL, while the resistant strains were only partially suppressed, even though the higher TBZ level represents about 500 times the level required to inhibit R-6380. Indeed, suppression is similar to results reported for effective TBZ dosage for 50% inhibition of radial growth (ED_{50}) for these strains (Desjardins et al., 1993).

Treatment of Inoculated Tubers. Treatment of wounded/inoculated tubers with sprout suppressants as vapor-phase volatiles produced only a modest reduction in tuber decay for two of the treatments (Table 3). Menthol reduced decay in tubers inoculated with R-6380, while cineole did likewise for tubers inoculated with R-9239. No significant differences between the controls and the other treatments were found with either strain.

Analyses of inoculated tubers treated with emulsified compounds demonstrated significant reduction in decay for several of the R-6380-inoculated treatments (Table 4). Cinnamaldehyde, menthol, thymol, and TBZ reduced decay compared to controls, with menthol being particularly effective. However, none of the treatments decreased decay by R-9239; in fact, several of the treatments (benzaldehyde, salicylaldehyde) had higher levels of rot than the controls. The relative lack of control by any of the treatments may be primarily due to lack of direct contact between the compounds and the fungi. Several methods of wounding and inoculating tubers were tried, and if tubers were wounded so that large surfaces of the tubers were exposed, even the controls developed little or no dry rot (results not reported), presumably due to washing off of the inocula during treatments. Although most of the other research dealing with artificially inoculated tubers used syringe injection into small (2 mm or less) wounds (Boyd, 1952; Corsini and Pavek, 1986; O'Brien and Leach, 1983; Platt, 1992), this type of wound may not represent reality. The antifungal activity of these compounds when applied to field-wounded tubers may be much higher due to increased contact between compound and pathogen.

Several of the tested compounds have been previously examined for antifungal activities. Benzaldehyde inhibited *Aspergillus niger*, *Mucor mucedo*, and *Penicillium chrysogenum* when added directly to nutrient media at 250–500 μg/mL (Kang et al., 1992). In the present study *F. sambucinum* strains were more tolerant to benzaldehyde, as all five strains were only partially inhibited at 0.1% (approximately 1000 μg/mL). Thymol concentrations of 500 μg/mL completely inhibited the growth of isolated cultures of *Aspergillus parasiticus* (Buchanan and Shepherd, 1981). Spices containing thymol as a major constituent were shown to decrease the growth and production of mycotoxins in *Aspergillus flavus*, *Aspergillus ochraceus*, and *Aspergillus versicolor* (Hitokoto et al., 1980). Thymol completely suppressed mycelial growth of *A. flavus* and *A. parasiticus* at 1.0 mM (Thompson, 1990). Cinnamaldehyde, however, was only mildly antifungal against several *Rhizopus* species (Thompson, 1989). While the use of isolated cultures is useful in determining threshold toxicity levels, it does not necessarily reflect levels that will come in contact with internal fungal propagules. As was previously discussed, it is apparent from our results using inoculated potato tubers that under conditions where the pathogen had deeply penetrated the tubers, insufficient levels of the compounds came into contact with the fungi.

Beyond the need for additional measures to control sprouting and dry rot to prevent losses, both CIPC and TBZ are detectable as residues in both cooked and uncooked potatoes (Friar and Reynolds, 1991; Mondy et al., 1992). Although the majority of both chemicals are present in the peel (and thus may be removed by peeling), the recent increase in consumption of unpeeled potatoes or of peel products such as fried potato skins suggests that residues of both compounds may be of concern. Research concerning long-term human consumption of CIPC and TBZ (as well as other pesticides) has not and probably cannot be conducted, so the use of compounds of known safety already present in the average diet would be highly desirable. Benzaldehyde, salicylaldehyde, and substituted benzoic acids are naturally occurring constituents present in very low levels in both uncooked and cooked potato tubers (Coleman et al., 1981; Mazza and Pietrzak, 1990). In fact, benzaldehyde was among the five volatile impact compounds contributing to baked potato odor/flavor (Coleman et al., 1981). Benzaldehyde and salicylaldehyde were previously found to be effective sprout inhibitors (Vaughn and Spencer, 1993) and were the most inhibitory to *F. sambucinum* strains in this study. Unlike thiabendazole, very little of which was destroyed during cooking at 100 °C (Friar and Reynolds, 1991), the majority of residual traces of added benzaldehyde and salicylaldehyde would be expected to be lost to volatilization during cooking, lessening any negative effects on sensory qualities (odor, taste). Additionally, both compounds are on the GRAS (generally recognized as safe) list, have low mammalian toxicities, and would likely receive positive consumer acceptance. However, they have not yet been approved for use on potatoes used for food or feed.

Conclusions. The present study indicates that the use of several naturally occurring compounds as post harvest potato sprout inhibitors, especially salicylaldehyde, could lessen the incidence of potato tuber dry rot caused by the fungus *F. sambucinum*. However, it appears that if the fungus has deeply penetrated the tubers prior to chemical

treatment, none of the treatments are effective in preventing dry rot.

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Registry No. Supplied by the Author: Benzaldehyde, 100-52-7; cineole, 470-82-6; cinnamaldehyde, 14371-10-9; CIPC, 101-21-3; menthol, 2216-51-5; salicylaldehyde, 90-02-8; thiabendazole, 148-79-8; thymol, 89-83-8.

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