Characterization of Antifungal Volatile Compounds Evolved from Solarized Soil Amended with Cabbage Residues

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ABSTRACT

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The objectives of this study were to characterize volatile compounds evolved from solarized soil amended with cabbage residues and to assess the effect of these volatile compounds on soilborne fungi. Quantitative and qualitative differences in volatile compounds from heated and nonheated cabbage-amended soil were found. Heated cabbage-amended soil generated a wide range of volatile compounds, including alcohols, aldehydes, sulfides, and isothiocyanates. The levels of isothiocyanates and aldehydes generated in heated soil were significantly correlated with reduced propagule numbers of *Pythium ultimum* and *Sclerotium rolfsii* during exposure. Viability of *P. ultimum* and *S. rolfsii* was reduced in soil amended with dried, ground cabbage residues when the soil was heated in a controlled-environment system simulating a diurnal solarization temperature curve at a sublethal maximum of 38 C. The propagule numbers of the two fungi were reduced by more than 95% when they were exposed for 14 days to volatile compounds generated from heated cabbage-amended soil but were not reduced to this extent when exposed to compounds generated from nonheated cabbage-amended soil. Total microbial activity in heated cabbage-amended soil rapidly decreased during direct exposure to the heat treatment. The microbial activity of soil exposed only to volatile compounds from heated cabbage-amended soil increased, however, suggesting selective toxicity of the volatile compounds to soil microbiota, additional biocidal activity of compounds in the soil's liquid and/or solid phases, and contribution of microbial antagonism to pathogen control.

Additional keywords: biological control, *Brassica oleracea*, cruciferae, cultural control, fluorescein diacetate, soil heating.

Organic soil amendments are used routinely to aid in various aspects of crop production and can either increase or decrease incidence and severity of plant diseases (3,6,16,22). Amendment of soil with cruciferous residues can suppress certain soilborne pathogens and root diseases (16,22,23). Cruciferous plants are characterized by a high content of sulfur-containing compounds, which when exposed to enzymatic decomposition, can generate toxic volatile compounds (1,5,7,10).

Solarization is a method of disease control accomplished by sealing the soil surface with prefabricated or sprayable plastic film to trap solar radiation and accumulate heat. Soil temperatures can be raised to levels that are lethal to many plant pathogens, and other physical and biological changes occur that contribute to the biocidal effect (2,4,8,12,21,24,27,29). Under suitable climatic conditions, solarization can control a wide range of soilborne pests, including fungi, bacteria, weeds, nematodes, and insects. Solarization's mode of action is complex, involving direct thermal destruction of propagules, shifts in microbial populations and activity, and changes in the soil's physical and chemical properties. Because solarization is a passive, meteorologically dependent process, integration of other physical, chemical, and biological control methods is desirable for improvement of efficacy and predictability of pathogen control (22–24,28). The control of certain soil pathogens in solarized soil is improved with inorganic or organic amendments, including dried, ground cabbage residues (22–24,29). Little is known about the characteristics and changes in concentrations of volatile compounds in heated amended soil and the effect on pathogens and other microorganisms, however.

The objectives of this study were to identify volatile compounds generated in cabbage-amended and solarized soil, describe the evolution of the volatile compounds, and assess the effect of solarization of cabbage-amended soil on *Pythium ultimum* Trow and *Sclerotium rolfsii* Sacc.

MATERIALS AND METHODS

Soil and cabbage amendment. The soil used was Hanford fine sandy loam (46% sand, 45% silt, 9% clay; pH 7.4), which was naturally infested with *P. ultimum* (~50 cfu/g of soil, plated on selective agar to determine the level of infestation). It was taken from a field at the University of California Kearney Agricultural Center (central San Joaquin Valley). The soil was collected from the top 20 cm, air-dried, then stored in the shade at room temperature until it was used. In some experiments, sclerotia of *S. rolfsii* from 30-day-old cultures on Jhoam medium (9) also were used. The sclerotia were harvested from agar plates, air-dried for 21 days, and stored, desiccated, at room temperature until they were used. Then, ~150 sclerotia were mixed with 2 g of soil and placed in nylon mesh bags. The leaf and stem residues of green cabbage (*Brassica oleracea* L. var. *capitata* L.) were collected from a commercial cabbage field 1 wk after plants were harvested, air-dried for 2 wk, and ground in a Thomas-Wiley laboratory mill (Arthur H. Thomas, Philadelphia, PA) until they passed through a 20-mesh (0.85-mm openings) sieve. Dried, ground cabbage residues were then mixed with soil at desired concentrations. Water was added to produce a final moisture content of 10% (approximately field capacity), and the mixture was homogenized by hand for 5 min. Soil moistened with water served as the nonamended control.

Controlled-environment experiments. The amended soil was heated in a modified Wisconsin-type waterbath as previously described (31). The temperature in the tank was regulated by a 24-h appliance timer and was altered diurnally from a minimum of 29–32 C to a maximum of 45 C. The maximum temperature

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was held for 4 h daily. This heating course was similar to that typically found in the upper 10–20-cm layer during solarization in the San Joaquin Valley in the summer months. In some of the experiments, maximum temperature was adjusted to 38 C, which was sublethal to the target pathogens.

The amended soils were loaded into 2-L widemouthed mason jars with dome lids (Fig. 1). A hole was drilled in the lid, and a perforated polypropylene tube (7 cm long × 10 mm inside diameter) was inserted through it. The walls of the tube were sealed to the surrounding lid with silicone glue, and a rubber septum was placed inside the tube to provide a headspace. The perforated tube was partially buried in the soil when the lid was closed to enable us to sample gas from the soil atmosphere. Thus, the apparatus allowed gas to be sampled by the insertion of a syringe needle through the rubber septum to pull gas from the headspace. The sealed system prevented the escape of volatile compounds from the jar while the gas sample was taken. Polypropylene syringes (20-ml) were filled with 10 g of moistened soil and placed over the jars with a hypodermic needle (23-gauge) that was inserted through the rubber septum to allow gas movement between the two containers. The pistons of the syringes were retracted and secured to draw volatile compounds from the treated soil in the jars to the nontreated soil in the syringes. The jars and attached syringes were placed in the heating tank and incubated for 30 days. Nonheated treatments were prepared similarly and incubated at 25 C. The syringes on top of the jars were not affected by the heat and stayed at room temperature (23–25 C), as recorded by micrologger (CR21X; Campbell Scientific, Inc., Logan, UT). The soil samples with *P. ultimum* and sclerotia of *S. rolfsii* from the nylon mesh bags were taken both from the heated jars and from the syringes attached to the jars at predetermined time periods and assayed for survival of these fungi as described below. The syringe plunger was reworked at sampling dates to ensure diffusion of volatile compounds. The gas samples were also taken periodically by syringe from the jars and analyzed for composition and concentration of volatile compounds by gas chromatography as described below.

Solarization of cabbage-amended soil in field microplots. Amended and nonamended soil samples naturally infested with *P. ultimum* (~50 cfu/g of soil) were placed in rectangular polystyrene containers without lids (TriState Plastics, Dixon, KY; 30 cm long, 25 cm wide, 10 cm deep). Nylon bags with sclerotia of *S. rolfsii* (~150 sclerotia per bag mixed with 2 g of soil) were randomly placed in the bottom of each container (10 bags in each container). A perforated tube glued to a lid (as described in the section on the jar tests) was placed in the center of each box to provide headspace. The boxes were buried in the field with the tops at the soil-surface level in a randomized block design with three replicates each of solarized and nonsolarized treatments. Each replicate also contained both *P. ultimum* and *S. rolfsii* for use in fungal viability tests. The solarized plots were covered with 4-m × 4-m clear polyethylene film sheets; the nonsolarized amended and nonamended treatments were left uncovered. The soil temperatures at sample depth (10 cm) were continuously recorded by micrologger.

Microbial assays. Soil and sclerotia samples were periodically taken from the jars and the syringes from the controlled-environment experiments and from containers from the field microplots (three subsamples per replicate). Air-dried soil (0.2 g) was sprinkled on a selective agar medium and incubated at 25 C to enumerate propagules of *P. ultimum* (19). Thirty sclerotia of *S. rolfsii* were placed on potato dextrose agar (9) and incubated at 30 C. Colonies were counted after 48–96 h. The numbers of "total" fungi at the end of each solarization or controlled-environment experiment were determined as follows. Soil (2 g)

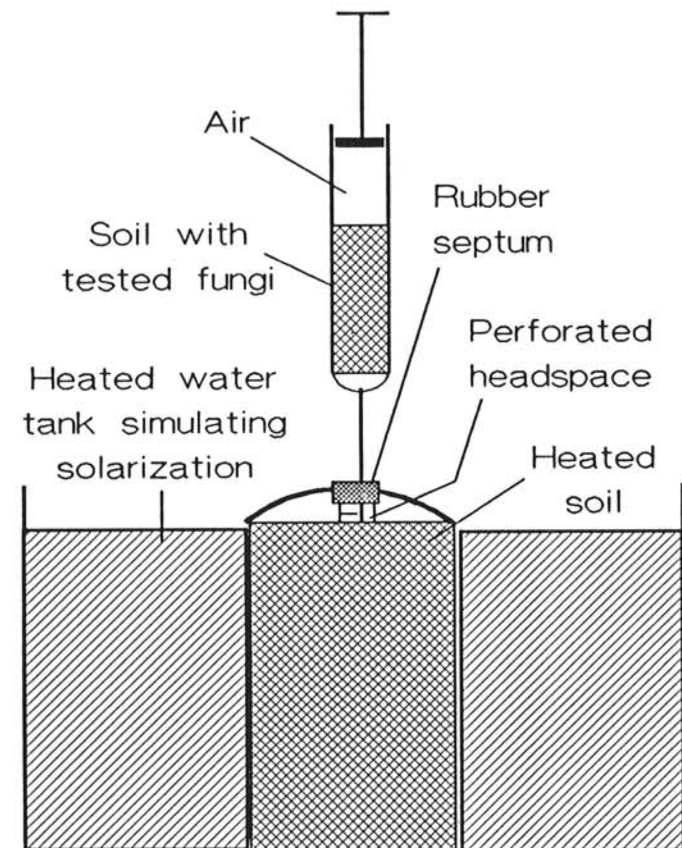

Fig. 1. System used for controlled-environment simulation of solarization to test the effects of heating on the evolution of volatile compounds and on pathogen control in cabbage-amended soil.

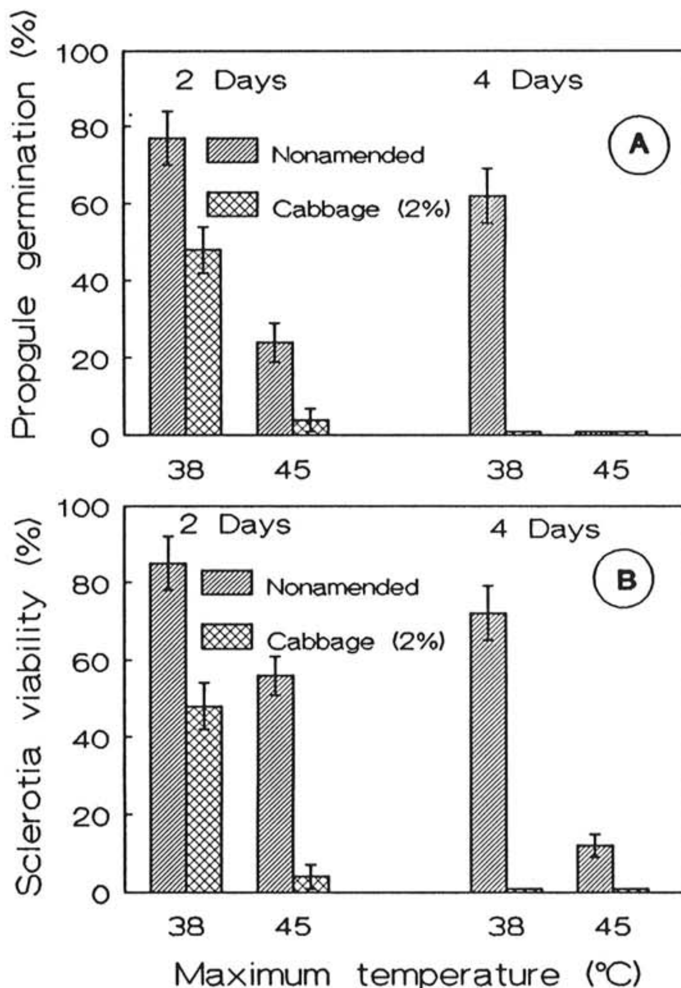

Fig. 2. Effect of duration of heating cabbage-amended soil (2%, w/w) in a solarization simulation system with different temperature maxima on **A,** propagule germination of *Pythium ultimum* and **B,** viability of sclerotia of *Sclerotium rolfsii* in soil. Standard errors are indicated.

from each replicate was suspended in water agar (0.1%) supplemented with 0.1 mg/L of $MgSO_4$ and serially diluted. A 0.2-ml aliquot of appropriate dilutions was spread on Martin Agar (9). The plates were incubated in the dark at 25 C, and colonies were counted after 7 days.

Sampling and analysis of volatile compounds. Gas samples were drawn from the headspace above the soil in the mason jars or in the field microplots through rubber septa into 3-ml gastight syringes. Volatile compounds in the experimental soils were identified by gas chromatography. One milliliter of each vapor sample was injected into a gas chromatograph (HP5895, Hewlett Packard, Avondale, PA) fitted with a flame ionization detector and an Ultra 1 fused silica capillary column (25 m long × 0.32 mm inside diameter) packed with cross-linked methyl silicone gum (0.52-mm film thickness; temperature of injection port and detector, 185 C; air flow rate, 200 ml/min; helium flow rate, 25 ml/min; hydrogen flow rate, 25 ml/min). The chromatograph oven temperature was programmed to an initial isothermal period of 5 min at 35 C, followed by a temperature increase to 150 C at a rate of 15 C/min, with the maximum temperature maintained for 8 min. The retention times of peaks appearing on the chromatograms were compared with those of the vapor phases of the reference compounds that were reported as possible products of cabbage decomposition (5,7,14).

Determination of microbial activity in soil. Hydrolysis of fluorescein diacetate (FDA) was used to estimate general microbial activity in the experimental soil (26,31). Samples were taken from amended or nonamended nonsolarized or solarized soil, and three 8-g subsamples of soil from each replicate were incubated with 50 ml of 60 mM sodium phosphate buffer (pH 7.6) for 30 min on a reciprocal shaker (150 rpm). Subsequently, 0.5 mg of FDA dissolved in 250 μl of acetone was added to each suspension, and the suspensions were further incubated on the reciprocal shaker for 1 h as described. Samples (4 ml) of each suspension were transferred to a centrifuge tube, and an equal volume of acetone was added to terminate the hydrolytic reaction. The tubes were then centrifuged for 10 min at 10,000 *g*, and the optical density of the supernatant was determined at 490 nm by a spectrophotometer (Spectronic 20D, Milton Roy, USA, Ivyland, PA). The amount of FDA hydrolyzed was calculated from a standard curve of fluorescein concentration, which was performed according to the procedure of Chen et al (3).

Statistical analyses. Each experiment was conducted at least three times. Data from replicate trials were pooled because variances among trials were homogeneous. Data were analyzed first by analysis of variance to test for possible interactions among the main effects. Treatment means were separated with Fisher's protected least significance difference test or a *t* test as indicated. Regression analysis was conducted to test for a possible relation between volatile compounds and pathogen control. The analysis of data also included repeated measurements analysis where indicated. All analyses were performed with SAS software (SAS Institute Inc., Cary, NC; release 6.04 for personal computer). Statistical significance was accepted at $P \leq 0.05$.

TABLE 1. Relative concentrations (mm^2) of volatile compounds evolved from soil amended with dried cabbage residues and heated in a solarization simulation system[x,y]

Chemical	Retention time (min)	Treatment	Duration of heating (days)[z]							
			1	3	7	10	14	17	21	24
Unidentified	1.49	Nonheated	447	429	150	251	56	0	0	130
		Heated	312*	20*	25	250	145*	1,535*	764*	165
Acetaldehyde	1.56	Nonheated	0	0	0	0	0	0	53	8
		Heated	20*	55*	25*	10	15*	15*	35	40*
Carbon dioxide	1.60	Nonheated	16,361*	2,007	1,025	1,967	3,559	5,124	2,983	1,177
		Heated	0	25*	1,700	2,200	3,500	8,950*	750*	820*
Methanol	1.64	Nonheated	0	0	0	0	29	514	0	0
		Heated	60*	30*	50*	0	0*	0*	210*	0
Methanethiol	1.70	Nonheated	0	822	537	776	207	190	262	330
		Heated	4,153*	4,800*	6,000*	8,500*	3,700*	1,400*	1,340*	551*
Ethanol	1.76	Nonheated	0	1,250	7,516	2,393	2,689	3,179	2,125	2,100
		Heated	700*	290*	250*	280*	460*	550*	600*	1,200*
Formaldehyde	1.96	Nonheated	0	0	0	0	0	0	0	0
		Heated	0	35*	30*	205*	250*	350*	680*	580*
Dimethyl sulfide	2.05	Nonheated	0	0	0	0	0	0	0	0
		Heated	0	250*	250*	300*	350*	200*	200*	170*
Unidentified	2.89	Nonheated	0	0	154	125	247	1,132	800	782
		Heated	0	0	0*	0*	0*	0*	0*	0*
Acetic acid	3.02	Nonheated	0	0	274	138	0	0	0	0
		Heated	0	0	0*	0*	0	0	0	0
Unidentified	3.82	Nonheated	0	0	0	0	12	32	50	193
		Heated	0	0	0	0	0	0*	0*	0*
Unidentified	4.65	Nonheated	0	0	0	0	0	0	0	0
		Heated	0	0	0	460*	800*	560*	350*	130*
Dimethyl sulfide	5.83	Nonheated	0	0	0	0	0	0	0	0
		Heated	22*	71*	53*	73*	80*	80*	296*	340*
Unidentified	6.67	Nonheated	0	0	0	0	0	0	0	0
		Heated	0	0	15*	40*	0	0	0	22
Allyl isothiocyanate	8.89	Nonheated	0	0	0	0	20	0	0	0
		Heated	0	10*	20*	30*	60	0	0	0
Unidentified	13.52	Nonheated	0	0	0	0	0	0	0	0
		Heated	0	0	20*	0	20	10	0	0
Phenyl isothiocyanate	13.82	Nonheated	0	0	0	0	20	0	0	0
		Heated	10*	15*	15*	20*	20	10	0	0

[x] Soil was amended with 2% (w/w) dried, ground cabbage residues and heated in sealed glass jars in a modified Wisconsin soil temperature tank for the indicated periods of time. Daily temperature fluctuation was similar to that during soil solarization, with a maximum temperature of 45 C for 4 h. Nonheated cabbage-amended soil was similarly prepared and incubated at room temperature. Identification and concentrations of volatile compounds were determined by gas chromatography.

[y] Relative volatile concentrations were determined by peak area triangulation of the detector response.

[z] Asterisks denote significant differences in concentrations between heated and nonheated amended soil, according to the *t* test ($P \leq 0.05$).

RESULTS

Effect of heating cabbage-amended soil on soilborne pathogens. Heating alone was effective in reducing numbers of *P. ultimum* and *S. rolfsii* propagules recovered on agar medium when the soil maximum temperature in the simulation tank was 45 C but not when the maximum temperature was 38 C (Fig. 2). Significant interaction between temperature and cabbage amendments was obtained. When maximal temperature was 38 C, *P. ultimum* and *S. rolfsii* numbers were significantly reduced in heated cabbage-

TABLE 2. Effect of soil temperature on relative concentrations (mm^2) of volatile compounds evolved from solarized cabbage-amended soil in field microplots[y,z]

Chemical	Retention time (min)	Length of solarization (days)	Detector response at soil temperature (C)					Mean
			30	33	37	42	45	
Acetaldehyde	1.56	3	0	0	33	43	50	24 a
		7	0	0	0	0	0	0 b
		14	0	0	0	0	0	0 b
		21	0	0	0	0	0	0 b
Mean			0 B	0 B	8 A	11 A	12 A	
Carbon dioxide	1.60	3	0	0	10	162	229	80 b
		7	35	40	133	250	513	194 a
		14	166	150	250	200	275	208 a
		21	0	0	0	0	0	0 c
Mean			50 D	48 D	98 C	153 B	254 A	
Methanethiol	1.70	3	28	25	171	133	152	102 a
		7	30	70	127	130	159	83 a
		14	20	20	20	265	225	110 a
		21	0	0	0	0	0	0 b
Mean			20 C	29 C	80 B	132 A	134 A	
Ethanol	1.76	3	25	30	70	83	115	65 b
		7	85	65	55	75	399	134 a
		14	90	100	100	150	290	146 a
		21	0	0	0	0	0	0 c
Mean			50 B	49 B	56 B	77 B	201 A	
Unidentified	1.80	3	0	0	0	84	95	36 b
		7	26	60	100	257	720	233 a
		14	0	0	0	0	0	0 c
		21	0	0	0	0	0	0 c
Mean			7 D	13 CD	25 C	85 B	204 A	
Formaldehyde	1.96	3	0	10	38	45	85	36 b
		7	27	23	48	120	150	74 a
		14	10	10	20	25	45	22 b
		21	0	0	0	0	0	0 c
Mean			9 D	11 D	27 C	48 B	70 A	
Dimethyl sulfide	2.05	3	127	161	320	593	620	364 b
		7	195	306	471	700	1,310	596 a
		14	70	80	186	330	480	229 b
		21	0	0	0	0	0	0 c
Mean			98 D	137 D	244 C	406 B	603 A	
Dimethyl disulfide	5.83	3	16	17	20	30	85	34 b
		7	61	40	50	70	177	80 a
		14	0	0	0	0	0	0 c
		21	0	0	0	0	0	0 c
Mean			19 B	14 B	18 B	25 B	66 A	
Unidentified	8.52	3	0	0	0	84	95	36 b
		7	0	0	0	78	33	22 b
		14	0	0	0	100	180	56 a
		21	0	0	0	0	0	0 c
Mean			0 B	0 B	0 B	66 A	77 A	
Unidentified	13.52	3	40	15	20	25	40	28 b
		7	10	57	60	55	80	52 a
		14	0	10	35	50	35	26 b
		21	0	0	15	20	25	12 b
Mean			13 C	21 BC	33 AB	38 A	45 A	
Phenyl isothiocyanate	13.82	3	0	0	0	10	15	5 b
		7	10	20	15	15	25	17 a
		14	0	10	10	15	20	11 ab
		21	0	0	10	10	15	7 b
Mean			3 C	6 BC	9 B	13 AB	19 A	

[y] Volatile samples were taken from solarized amended soil (2% cabbage residues) in microplots during July, 1992. Soil temperature was recorded at each sampling. Identification and concentrations of volatile compounds were determined by gas chromatography. Relative volatile concentrations were determined by peak area triangulation of the detector response.

[z] The experiment was conducted in a factorial design with repeated measurement analysis. There was no significant interaction found between temperature and sampling day, and since polynomial regression analysis of both time and soil temperature gave a lack-of-fit, mean separation is given for the main effect. Thus, within each compound, different uppercase letters denote differences with respect to soil temperatures, and different lowercase letters denote differences with respect to length of solarization ($P \leq 0.05$).

amended soil. The numbers of *P. ultimum* propagules at a maximum temperature of 38 C in cabbage-amended soil decreased 50 and 100% after 2 and 4 days, respectively, compared with 23 and 36% decreases in the corresponding nonamended and heated soil (Fig. 2A). The viability of sclerotia of *S. rolfsii* at a maximal temperature of 38 C in cabbage-amended soil was reduced 48 and 100% after 2 and 4 days, respectively, compared with 15 and 28% reduction in the corresponding nonamended heated soil (Fig. 2B).

Effect of heating on release of volatile compounds from cabbage-amended soil in controlled-environment experiments. Significant differences in volatile compounds from heated and nonheated cabbage-amended soil were recorded (Table 1). Volatile compounds identified from cabbage-amended soil consisted primarily of aldehydes, alcohols, and sulfur-containing compounds. Several peaks were not identified. In general, concentrations of volatile compounds were significantly higher from heated cabbage-amended soil than in the corresponding nonheated soil. This trend was evident during the first week of incubation but was more pronounced during the second and third weeks. Concentrations of some volatile compounds decreased sharply after 3 wk (Table 1). The principal volatile compounds detected in heated amended soil were aldehydes (formaldehyde and acetaldehyde) and sulfur compounds, including isothiocyanates. Some of these were not detected in nonheated cabbage-amended soil. In contrast, the headspace of nonheated cabbage-amended soil contained higher amounts of ethanol and acetic acid than heated cabbage-amended soil. The principal gas detected in nonamended heated or nonheated soil was CO_2, but there was no consistent difference in CO_2 concentrations between heated and nonheated soil. Volatile compounds detected in heated nonamended soil also included small amounts of ethanol and others that were unidentified (data not shown).

Effect of soil temperature on generation of volatile compounds from solarized cabbage-amended soil in the field. Samples of the soil atmosphere were taken at different hours of the day, and soil temperature was recorded at each sampling time. In general, evolution of volatile compounds increased directly with soil temperature (Table 2). This trend was evident in all assays during the first 2 wk of solarization. Vapor samples drawn from cabbage-amended and solarized soil contained compounds similar to those from the simulation experiments (Tables 1 and 2). However, some compounds, such as methanol and allyl isothiocyanate, which were detected in the controlled-environment trials, were not recorded in cabbage-amended and solarized soil. No volatile compounds were detected from unsealed amended or nonamended soil. In general, generation of volatile compounds increased during the first 2 wk, then decreased to undetectable levels after 3 wk. Propagules of *P. ultimum* and sclerotia of *S. rolfsii* were reduced to undetectable levels in solarized soil after 7 days. The viability of these fungi was not affected in the nonsolarized soil, either nonamended or amended with cabbage residues.

Effect of volatile compounds from heated cabbage-amended soil on soilborne pathogens in controlled-environment experiments. Soil samples were periodically removed from syringes connected to the headspace of mason jars containing cabbage-amended soil and placed on agar media to determine the viability of *P. ultimum* and *S. rolfsii* propagules. The number of viable *P. ultimum* and *S. rolfsii* propagules were reduced by 75 and 93%, respectively, after 10 days of exposure to volatiles from heated cabbage-amended soil (Fig. 3). No colony of either fungus was recovered after 15 days of exposure. Volatile compounds from nonheated cabbage-amended soil reduced the numbers of viable propagules of *P. ultimum* by 15% and of *S. rolfsii* by 12% after incubation for 4 wk in the simulation tank. Volatile compounds from nonamended soil that was either heated or nonheated had no effect on pathogen viability.

Significant inverse sigmoidal dosage-response curves were obtained between the evolution of isothiocyanates and aldehydes and the recovery of *P. ultimum* and *S. rolfsii* from nonheated

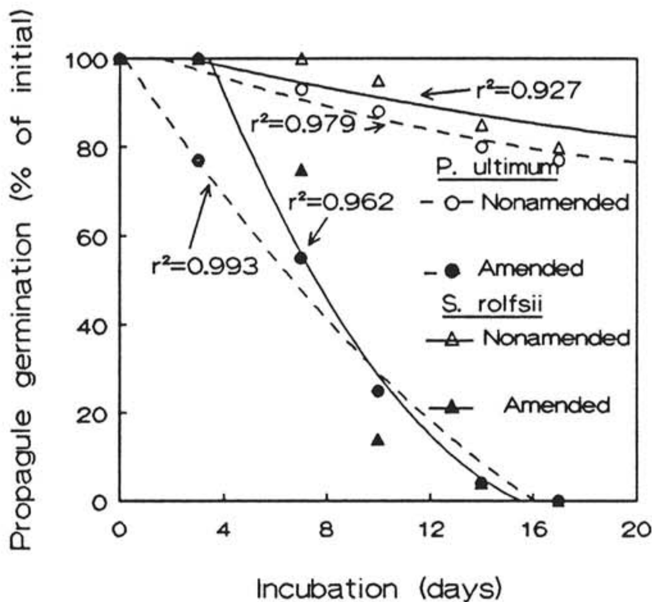

Fig. 3. Effect of volatile compounds from heated soil (45 C) nonamended or amended with dried cabbage residues (2%, w/w) on propagule germination of *Pythium ultimum* and *Sclerotium rolfsii* in a system described in Figure 1. Equations of the regression lines are: *P. ultimum* nonamended, $Y = 103.21 - 2.025x + 0.034x^2$; *P. ultimum* amended, $Y = 108.09 - 10.09x + 0.21x^2$; *S. rolfsii* nonamended, $Y = 103.78 - 1.59x + 0.024x^2$; *S. rolfsii* amended, $Y = 155.27 - 17.92x + 0.51x^2$.

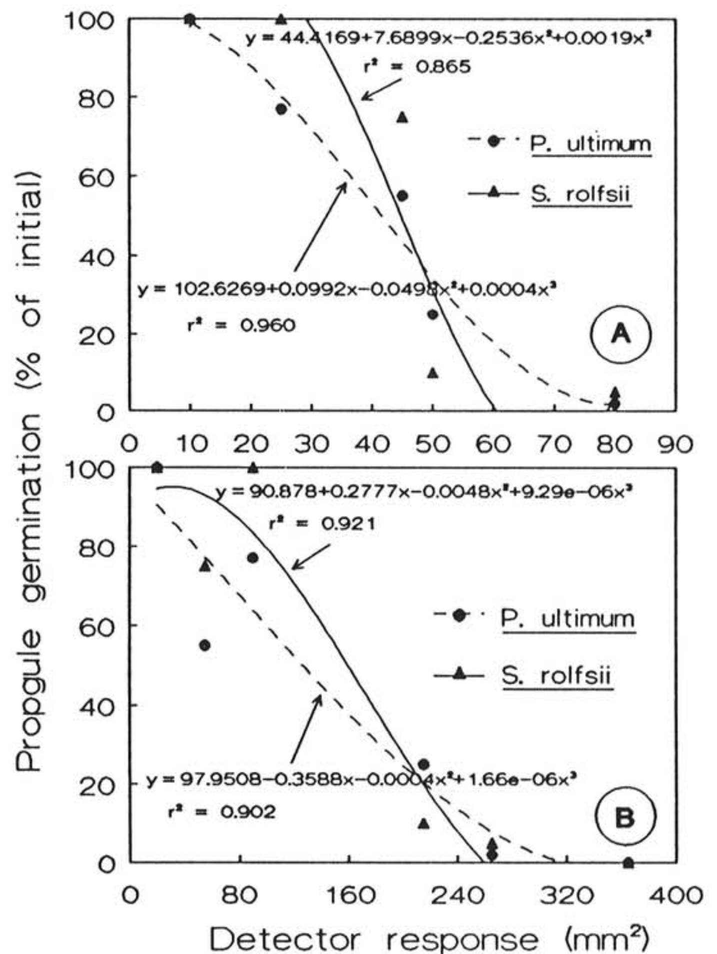

Fig. 4. Relationship between propagule germination of *Pythium ultimum* and *Sclerotium rolfsii* and gas chromatograph detector response (relative concentration) of **A,** isothiocyanates and **B,** aldehydes generated from heated cabbage-amended soil (2%, w/w). Survival of fungi in syringes affected only by volatile compounds was plotted against the relative volatile compound concentration expressed as the triangulated area of the gas chromatograph response.

soil exposed to volatile compounds in the controlled-environment experiments (Fig. 4). No correlation was found between the germination of pathogen propagules and other compounds detected in the soil atmosphere of heated cabbage-amended soil.

Relationship between the release of volatile compounds and microbial activity in cabbage-amended soil. The numbers of "total" fungi were decreased by 96% (from 7×10^4 to 3×10^3 cfu/g of dry soil) after the cabbage-amended soil was heated for 4 wk in the controlled-environment trials. The numbers of fungi in the heated nonamended soil were similarly reduced (6×10^3 cfu/g of dry soil after 4 wk). In field microplot experiments, the numbers of "total" fungi were reduced by 95% in cabbage-amended soil (from 8.6×10^4 to 4.3×10^3 cfu/g of dry soil) after 4 wk of solarization. The numbers of fungi in the solarized nonamended soil after 4 wk were 5×10^3 cfu/g of dry soil.

Microbial activity, expressed by the extent of FDA hydrolysis, was less in heated soil than in nonheated control soil (Table 3). A significant interaction between cabbage amendments and soil heating was obtained during the first 2 wk of incubation. In heated soil, reduction in microbial activity was faster in cabbage-amended soil than in nonamended soil, decreasing to 50% of initial activity after 7 days of heating. After 14 days, however, microbial activity in amended or nonamended heated soils was similar. Microbial activity increased by 115% in the cabbage-amended nonheated soil during the first week of incubation but decreased during the second week to the initial level. Microbial activity in soil in the syringes that were exposed only to volatile compounds from the various soil treatments was determined after 4 wk of heating. Activity in the soil that was exposed to volatile compounds from heated cabbage-amended soils was higher than that in the soils exposed to volatile compounds from nonamended soil (Table 3).

DISCUSSION

Heating cabbage-amended or nonamended soil at a maximal temperature of 45 C under controlled-environment conditions was very effective in reducing the viability of *P. ultimum* and *S. rolfsii* propagules, as has also been shown in other studies (21,24,28). Heating cabbage-amended soil was very effective in controlling *P. ultimum*, even at a maximum temperature of 38 C. Fungal propagules exposed to sublethal heat are weakened, which leads to reduced viability and susceptibility to other biological and physical control mechanisms (17). In this study, numbers of propagules of *P. ultimum* and *S. rolfsii* were reduced by more than 95% when the propagules were exposed to volatile compounds from heated cabbage-amended soil for 14 days. Similar results were obtained with heated compost-amended soil (*unpublished data*).

TABLE 3. Effect on microbial activity (micrograms of fluorescein diacetate hydrolyzed per gram of soil per minute) of duration of heating cabbage-amended soil (2%, w/w) in a solarization simulation system[x]

Treatment	Incubation (wk)[y]				
	1	2	3	4	4 (air)[z]
Nonheated					
Nonamended	0.32 Ba	0.19 Ba	0.19 Aa	0.15 Aa	0.18 Ba
Cabbage-amended	0.43 Aa	0.25 Aa	0.23 Aa	0.21 Aa	0.38 Aa
Heated					
Nonamended	0.19 Ab	0.08 Ab	0.08 Ab	0.09 Ab	0.23 Ba
Cabbage-amended	0.12 Bb	0.07 Ab	0.08 Ab	0.09 Ab	0.36 Aa

[x] Nonamended soil and amended soil (2% cabbage residue) were heated in a solarization simulation system (maximum temperature 45 C). Samples were taken from soil treatments at weekly intervals. Initial level of microbial activity in soil was 0.22 μg per gram of soil per minute, estimated by hydrolysis of fluorescein diacetate.

[y] The experiment was conducted in a factorial design; repeated measurements analysis was performed. There was significant interaction between soil heating and cabbage amendments. Thus, for each column, uppercase letters denote significant differences between cabbage amendments within each heating treatment, and lowercase letters denote significant differences between heating treatments within each cabbage-amendment treatment, according to Fisher's protected least significant difference test ($P \leq 0.05$).

[z] Microbial activity was determined at the end of the experiment (4 wk) in soil in syringes affected only by volatile compounds released from heated soil (see Fig. 1).

Qualitative differences in volatile compounds were recorded between the vapor phases of nonheated and heated cabbage-amended soil samples. The atmosphere of heated soil contained isothiocyanates and other sulfur-containing compounds, alcohols, and aldehydes, whereas vapors from nonheated soil contained methanethiol, ethanol, and occasionally acetic acid and methanol. Similar volatile compounds were detected during field solarization, which supports the use of our test system to provide reliable estimations of solarization effects, as reported earlier (31). The principal volatile compound found during this study in nonamended heated soil was CO_2, as was also previously reported (25).

The concentration of volatile compounds evolved was also directly related to increased heating of soil in the laboratory experiments and in field soil solarized in microplots. Various sulfur-containing compounds are degradation products of cabbage and other cruciferous plants (5,10,11). A wider range of sulfur-containing volatile compounds was detected when cabbage was cooked or heated (7,18), however. Numerous bacteria and fungi can also decompose cabbage and generate sulfur-containing volatile compounds, mainly sulfides (13,16). In a previous study, sulfur-containing volatile compounds such as CH_3SH, $(CH_3)_2S$, and $(CH_3)_2S_2$ were detected in soil amended with cabbage residues (14). No isothiocyanates were found, however. In the present study, we detected allyl isothiocyanate and phenyl isothiocyanate in the vapor phases of heated or solarized cabbage-amended soils but not in the vapor phase of the corresponding nonheated soil. Apparently, cabbage decomposition and volatile concentration in soil involve physical as well as biological mechanisms. This conclusion is also supported by our results that showed an increase in volatile compounds in solarized soil related directly to soil temperature. Undoubtedly, high temperatures increase the vapor pressure of compounds present in the liquid or solid soil fractions, resulting in greater release into the soil atmosphere. Other mechanisms, such as heat-induced breakdown of more complex compounds and release of polar molecules from clay particles, may also be involved. Several volatile compounds reported from decomposing cabbage (5,14,18) were not detected in our study, however.

Significant relationships between specific groups of volatile compounds generated from heated cabbage-amended soil and reduced viability of *P. ultimum* and *S. rolfsii* were obtained from the controlled-environment experiments. Significant dosage-response curves were obtained when fungal viability was expressed as a function of relative concentrations of isothiocyanates or aldehydes generated. Previous studies have shown that aldehydes, isothiocyanates, and sulfide volatile compounds are toxic to soilborne plant pathogens (15,16,20,30). Although this study included no attempt to determine fungicidal activity of individual compounds or combinations thereof, the correlation between volatile generation and pathogen control was well established.

Microbial activity in cabbage-amended heated soil was rapidly reduced during the first week of incubation, as compared to heated nonamended soil. A considerable portion of the total soil microflora probably was adversely affected by the heat and the toxicity of volatile compounds. In contrast, microbial activity was increased in cabbage-amended nonheated soil during the first week of incubation as the available organic material was metabolized. Microbial activity also increased in soil that was exposed to vapors from heated or nonheated cabbage-amended soil, compared with microbial activity in nonamended soil. Volatile compounds such as alcohols, aldehydes, and others can stimulate germination of fungal propagules and increase microbial activity in soil (13,16,20). Unlike plant pathogens such as *P. ultimum* and *S. rolfsii,* which were vulnerable to the toxic effects of the volatile compounds, other saprophytic soil biota apparently were not as sensitive. Microbial activity in highly organic soils and level of suppressiveness against soilborne pathogens are often correlated (3).

Control of *P. ultimum* and *S. rolfsii* in soil exposed to vapors may have been due to a combination of the direct toxic effect of specific volatile compounds and the antagonistic activity of soil microflora that were activated by such compounds.

Soil solarization with plastic films or sprayable mulches and cruciferous residues or other appropriate soil amendments can be effective tools for controlling soilborne pathogens and other pests. Solarization, a temperature-dependent process, is marginally useful in cool areas or for control of heat-tolerant organisms. The usefulness of soil amendments for pathogen control may be limited by low concentrations of toxic volatile compounds or by insensitivity of resting structures of target organisms. The utility and predictability of both methods may be maximized by combination.

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