Compost and Compost Water Extract-Induced Systemic Acquired Resistance in Cucumber and Arabidopsis

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ABSTRACT

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A biocontrol agent-fortified compost mix, suppressive to several diseases caused by soilborne plant pathogens, induced systemic acquired resistance (SAR) in cucumber against anthracnose caused by *Colletotrichum orbiculare* and in Arabidopsis against bacterial speck caused by *Pseudomonas syringae* pv. *maculicola* KD4326. A peat mix conducive to soilborne diseases did not induce SAR. The population size of *P. syringae* pv. *maculicola* KD4326 was significantly lower in leaves of Arabidopsis plants grown in the compost mix compared to those grown in the peat mix. Autoclaving destroyed the SAR-inducing effect of the compost mix, and inoculation of the autoclaved mix with nonautoclaved compost mix or *Pantoea agglomerans* 278A restored the effect, suggesting the SAR-inducing activity of the compost mix was biological in nature. Topical sprays with water extract prepared from the compost mix reduced symptoms of bacterial speck and the population size of pathogenic KD4326 in Arabidopsis grown in the peat mix but not in the compost

mix. The peat mix water extract applied as a spray did not control bacterial speck on plants grown in either mix. Topical sprays with salicylic acid (SA) reduced the severity of bacterial speck on plants in the peat mix but did not further reduce the severity of symptoms on plants in the compost mix. The activity of the compost water extract was heat-stable and passed through a 0.2- μ m membrane filter. β -1,3-Glucanase activity was low in cucumber plants grown in either mix, but when infected with *C. orbiculare*, this activity was induced to significantly higher levels in plants grown in the compost mix than in plants grown in the peat mix. Similar results were obtained for β -D-glucuronidase (GUS) activity driven by a *PR2* (β -1,3-glucanase) gene promoter in transgenic Arabidopsis plants grown in the compost or peat mix. GUS activity was induced with topical sprays of the compost water extract or SA in plants not inoculated with the pathogen, suggesting that compost-induced disease suppression more than likely involved the potentiation of resistance responses rather than their activation and that compost-induced SAR differed from SAR induced by pathogens, SA, or compost water extract.

Additional keywords: compost-amended potting mix.

Several reports suggest that composts may alter resistance of plants to disease. Tränkner (39) observed that powdery mildew of wheat and barley was less severe in compost-amended than in unamended soils. Roe et al. (36) reported lower incidence of early blight and bacterial spot of tomato on field-grown plants in compost-amended soil than in the control, even though the compost was applied under polyethylene mulch. Furthermore, Zhang and Paulitz (52) found that composted spruce and pine bark-amended mixes induced systemic acquired resistance (SAR) in cucumber against *Pythium* root rot and anthracnose caused by *Colletotrichum orbiculare* (Berk. & Mont.) Arx.

SAR can be induced by chemicals, pathogens, and beneficial soil microorganisms (9,22,24,29,32). It is unknown how composts induce SAR; however, plants in compost-amended substrates are colonized by a variety of bacterial taxa (2,8,13,46,48,51) from which strains capable of inducing systemic resistance in plants have been described (25,29,42,45). Such specific strains must be present above a certain threshold population size in the rhizosphere to induce this effect (34). Highly decomposed peat mixes conducive to root rots caused by *Pythium* and *Phytophthora* spp. do not support activity of bacterial taxa that act as biological controls (2,17,28), and they also do not induce SAR (52).

Compost water extracts have been used for years as topical sprays to control foliar diseases of plants (38,46,50). They reduce

the severity of foliar diseases, such as powdery mildew of grape caused by *Uncinula necator*, downy mildew of grape caused by *Plasmopara viticola*, grey mold of strawberries, and late blight of potato (11,30,50). These treatments often are variable in efficacy, however. The effect of the substrate (soil) in which the plant is grown may have an impact on the efficacy of compost water extracts, but this potential interaction has not been investigated. Compost extracts contain biocontrol agents, as well as unidentified chemical factors that appear to play a role in efficacy (7, 38,46,50). Although the mechanisms by which these extracts provide control remain largely a mystery, Cronin et al. (7) demonstrated that a low molecular weight compound was critical for in vitro lysis of conidia of *Venturia inaequalis*. It has been postulated that the protective effects of compost extracts is due, at least in part, to the induction of systemic resistance in plants (46). Chemicals, such as salicylic acid (SA), can induce SAR in plants (9, 20,37). Comparison of the disease control achieved by compost water extracts with a putative chemical inducer for SAR, such as SA, may aid in understanding the mechanisms of disease suppression induced by these extracts.

Some rhizobacteria capable of inducing systemic resistance induce the accumulation of pathogenesis-related (PR) proteins in plants (29), whereas other strains do not (32). Zhang et al. (52) reported that peroxidase activity, a putative SAR marker in cucumber (12,35), was significantly higher in plants grown in a compost mix than in plants grown in a peat mix. They concluded that the interaction of the compost and pathogen infection appeared critical for rapid activation of SAR-associated gene expression in cucumber plants grown in compost mix (52).

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The objectives of this work were to determine (i) whether composts can induce SAR in *Arabidopsis thaliana* (L.) Heynh. against bacterial speck caused by *Pseudomonas syringae* pv. *maculicola* KD4326; (ii) whether compost-induced SAR is biological in nature; (iii) the efficacy of biocontrol agents from suppressive composts in inducing SAR against anthracnose of cucumber caused by *C. orbiculare*; (iv) the efficacy of water extracts from compost and peat mixes relative to that of SA for control of bacterial speck on plants grown in compost-amended versus peat mixes; and (v) whether β -1,3-glucanase activity in cucumber and *Arabidopsis* is affected by any of these compost treatments.

MATERIALS AND METHODS

Potting mixes and water extract preparation. A composted pine bark mix, fortified with the biocontrol agents *Trichoderma hamatum* 382 (Bonord.) Bainier and *Flavobacterium balustinum* 299 ($\approx 10^5$ and 10^6 CFU g⁻¹ dry weight, respectively) to induce suppression of diseases caused by *Pythium*, *Rhizoctonia*, and *Fusarium* (16,23,40), was obtained from Earthgro, Inc. (Glastonbury, CT). The mix was prepared as described previously (17). The mix, referred to hereafter as compost mix, was autoclaved for some tests (1 h on three consecutive days in 2-liter autoclavable bags) to eliminate suppressiveness (23,40). To restore suppressiveness, 10% (vol/vol) of the compost mix was added to the autoclaved mix.

A sphagnum peat mix, used as a control, was prepared with peat that rated H₄ on the von Post decomposition scale (33). This mix was consistently conducive to many soilborne pathogens (17) and did not induce SAR against *Pythium* root rot and anthracnose in cucumber (52). All mixes were amended with slow-release fertilizer (Osmocote, 14-14-14 [N-P-K], Grace-Sierra Chemical Co., Milpitas, CA), as described previously (17), to maintain sufficient nutrients for plant growth.

Water extracts from the compost or peat mix were prepared by adding tap water to the mixes (1:1, vol/vol). These mixtures (4 liters) were stirred thoroughly and allowed to ferment for 1 week, according to a procedure developed by Weltzien (46), filtered through cheesecloth, and passed through Whatman (Maidstone, England) No. 4 filter paper (particle retention, >20 μ m). Sterilization of extracts was achieved by either autoclaving (35 min at 120°C) or filtration through a Nalgene presterilized bottle-top filter unit (0.2 μ m, surfactant-free cellulose acetate membrane; Nalge Co., Rochester, NY). Sterility of the extracts was confirmed by plating 0.1 ml on 0.1-strength Difco (Detroit) trypticase soy agar. All extracts were sprayed at full-strength onto plants until leaves were wet at 7 days and again at 2 days before inoculation.

Cucumber anthracnose bioassay. Cucumber seeds (*Cucumis sativus* L. 'Straight Eight') were germinated in the compost and peat mixes, as described previously (6). Plant growth conditions and fertilizer treatments were as described earlier (52). An isolate of *C. orbiculare* obtained from J. Kuć (Department of Plant Pathology, University of Kentucky, Lexington) was used to induce classic SAR in cucumber. Fourteen days after planting cucumber seeds a conidial suspension of *C. orbiculare* (10- μ l drops, 10^5 CFU ml⁻¹) was infiltrated at 30 locations into the abaxial side of the first leaf. This treatment is referred to as "classic-induced," according to Kuć and Richmond (22). Pots were arranged in a completely randomized design, with five replications (two plants per pot) per treatment. SAR was detected via challenge-inoculation by placing 30 10- μ l drops of a conidial suspension of *C. orbiculare* (10^4 to 10^5 CFU ml⁻¹) 7 days after inoculation on the second leaf of the same plants, followed by incubation in a moisture chamber for 24 h in the dark (22,52). The severity of anthracnose was rated 7 days after challenge-inoculation, as described previously (52). Control plants were treated with water on both the first and second leaves. In some experiments, the first true leaf was treated 2 days before challenge-inoculation with 5 mM SA as a SAR-positive control (14,41).

Bacterial biocontrol agents with efficacy against several soilborne pathogens (23,40) were tested for their ability to induce SAR in cucumber against anthracnose. Bacteria were cultured for 48 h at 25°C on a rotary shaker in 0.1-strength Difco trypticase soy broth (0.1 TSB). Cells were centrifuged (10 min) at $10,000 \times g$ and resuspended in autoclaved tap water to establish a concentration of 10^9 CFU ml⁻¹. These suspensions were injected into cucumber stems just below the cotyledons 7 days after seeding in the peat mix, according to the procedure described by Wrather and Elrod (49). In a follow-up bioassay, the most efficacious strains were cultured for 24 h at 25°C in 0.1 TSB, washed, resuspended in sterilized tap water, as described above, to a concentration of 10^9 CFU ml⁻¹, and added to the autoclaved compost mix to establish an initial population density of 10^6 CFU g⁻¹ dry weight of the mix. Colonization of the compost mix by these strains was as described earlier (23). Each treatment consisted of 2 liters of potting mix distributed in five 400-ml pots, with one plant per pot. Pots were arranged in a completely randomized design, with five replications per treatment. The efficacy of each strain in the compost mix in inducing SAR in cucumber was tested by rating anthracnose severity on the second leaf 7 days after challenge-inoculation by determining the diameter (millimeters) and number of lesions, as described earlier (52). Disease severity was expressed as mean lesion diameter (millimeters). Data were subjected to analysis of variance (ANOVA). When a significant ($P \leq 0.05$) *F* test was obtained for treatments, separation of means was accomplished by Fisher's least significant different (LSD_{0.05}) test. This experiment was performed three times.

***Pseudomonas* bacterial speck bioassay.** Induction of SAR in *Arabidopsis* was tested with *P. syringae* pv. *maculicola* KD4326 (43). Seeds of transgenic *Arabidopsis* (*A. thaliana* ecotype Columbia) containing a chimeric reporter gene made up of the *Arabidopsis* PR gene (*BGL2*) promoter and the coding region of β -D-glucuronidase (*GUS*) (3,5,10) were germinated in 240-ml 8-cm-tall polystyrene pots (≈ 50 to 60 seeds per pot, six pots per treatment) containing the compost or peat mix. Plants were grown at 25 to 27°C and 60 to 70% relative humidity in a growth chamber with a 14-h photoperiod (light intensity of 100 to 200 μ E m⁻² s⁻¹). Inoculum of *P. syringae* pv. *maculicola* KD4326 resistant to rifampicin was produced on a rotary shaker (72 h and 25°C) in 0.1-strength King's medium B broth (Difco) containing rifampicin at 50 mg liter⁻¹ (10). Cells were washed by centrifugation at $10,000 \times g$ and resuspended in dilution buffer (7 g of K₂HPO₄, 3 g of KH₂PO₄, and 1 liter of distilled water, pH 6.8) to establish 10^6 , 10^7 , and 10^8 CFU of *P. syringae* pv. *maculicola* KD4326 per ml. The concentration of 10^8 CFU of KD4326 per ml was used for inoculation of *Arabidopsis* plants in all experiments unless otherwise specified. The foliage of 28-day-old seedlings (six pots for each treatment) was sprayed to runoff with this inoculum and incubated in a moisture chamber at 25°C for 2 days to facilitate symptom development. Control plants were sprayed with distilled water. Additional treatments included plants sprayed 7 and 2 days before inoculation with KD4326 with (i) 1 mM SA (a SAR-positive control); (ii) compost water extract; (iii) peat water extract; (iv) filter-sterilized compost water extract; or (v) filter-sterilized peat extract. Treatments were arranged in a randomized complete block design and replicated six times each.

Severity of bacterial speck was determined 4 days after inoculation by determining the percentage of leaves in each pot that developed lesions. Population sizes of *P. syringae* pv. *maculicola* KD4326 (colony forming units per gram fresh leaf weight) were determined immediately after rating leaves for disease severity. Ten leaves were selected randomly from each pot, weighed, and homogenized (Polytron CH6010, Krins-LU, Kinematica GmbH, Switzerland; 60 Hz, 710 W) in phosphate buffer (pH 6.8). Aliquots of the homogenate were plated in duplicate on 0.1-strength King's medium B amended with rifampicin at 50 mg liter⁻¹. Colonies were counted after 72 h of incubation at 25°C, and the

number of colony forming units of *P. syringae* pv. *maculicola* KD4326 per gram fresh leaf weight was determined. Data were subjected to ANOVA, and if a significant ($P \leq 0.05$) *F* test was obtained for spray treatments, potting mix, or their interactions, separation of means was accomplished by Fisher's $\text{LSD}_{0.05}$ test. Moreover, a regression analysis was done to determine the increase in severity with increasing inoculum. This experiment was performed twice.

β -1,3-Glucanase activity in cucumber. The second leaf of cucumber plants grown in both mixes, as described above, was sampled from a separate set of experiments described previously (52) and immediately frozen in liquid nitrogen. β -1,3-Glucanase activity in the leaf was determined according to the procedure of Abeles and Forrence (1). Laminarin was used as substrate, and dinitrosalicylate reagent solution was used to stop the enzyme reaction. Absorbance was read at a wavelength of 500 nm with a spectrophotometer, and β -1,3-glucanase activity was expressed as micromoles glucose equivalent released per milligram of protein per minute. Protein concentration was measured, using the Bio-Rad (Richmond, CA) protein assay kit with bovine gamma globulin (Sigma Chemical Co., St. Louis) as a standard. Data were subjected to ANOVA. When a significant ($P \leq 0.05$) *F* test was obtained for treatments, separation of means was accomplished

by Fisher's $\text{LSD}_{0.05}$ test. This experiment was performed four times.

GUS activity assays. Expression of the chimeric GUS reporter gene driven by the β -1,3-glucanase gene promoter (*BGL2*) in transgenic *Arabidopsis* was determined histochemically by staining whole leaves of plants grown in both mixes, described above. Topical spray treatments, also as described above, were applied at 21 and 26 days after seeding. Five randomly selected leaves per pot (six pots for each treatment) of 28-day-old *Arabidopsis* plants were excised, surface-sterilized in 1% sodium hypochlorite solution for 15 min, washed four times with distilled water, and stained overnight at 37°C. The staining solution contained 0.5 mg of 5-bromo-4-chloro-3-indolyl β -D-glucuronide per milliliter in 0.1 M Na_2HPO_4 (pH 7.0), 10 mM Na_2EDTA , 0.5 mM potassium ferricyanide and ferrocyanide, and 0.06% Triton X-100 (3,18). Leaves were dehydrated in 75% ethanol overnight and photographed.

GUS activity in the leaf extracts was measured quantitatively in a separate set of randomly selected leaves from the experiment described above, using a fluorometric assay (5). Leaves were excised (10 leaves per pot), weighed, and immediately frozen in liquid nitrogen. The frozen samples were ground with a high-speed homogenizer (Rae Corporation, McHenry, IL) in 0.5 ml of pre-chilled extraction buffer in glass tubes containing 50 mM NaH_2PO_4 , pH 7.0, 10 mM EDTA, 0.1% Triton X-100, 0.1% sodium lauryl sarcosine, and 10 mM β -mercaptoethanol, according to the procedure of Jefferson et al. (18). Protein concentrations were determined with Bio-Rad dye reagent (4), and specific GUS activity was calculated as picomoles 4-methyl umbelliferone produced per minute per milligram of protein. Data were subjected to ANOVA to determine the significance of potting mix, spray treatment, and their interaction. When a significant ($P \leq 0.05$) *F* test was obtained for one of the factors or the interaction, separation of means was accomplished by Fisher's $\text{LSD}_{0.05}$ test. This experiment was performed twice.

RESULTS

Bacterial speck of Arabidopsis. The severity of bacterial speck was significantly lower ($P \leq 0.05$) on *Arabidopsis* plants grown in the compost mix than on plants grown in the peat mix (Table 1). A regression analysis of disease severity on the log of inoculum concentration showed there was a significantly larger slope for

TABLE 1. Severity of bacterial speck and population densities of *Pseudomonas syringae* pv. *maculicola* KD4326 in *Arabidopsis* (*Arabidopsis thaliana* ecotype Columbia) grown in a compost or peat mix

Inoculum concentration (CFU ml ⁻¹)	Disease severity (% necrotic leaves/pot) ^w		<i>P. syringae</i> pv. <i>maculicola</i> KD4326 (10 ⁷ CFU g ⁻¹ fresh leaf) ^x	
	Peat	Compost	Peat	Compost
0	0	0	ND ^y	ND
10 ⁶	8.0*	0.7	... ^z	...
10 ⁷	20.8*	1.2	1.36*	0.51
10 ⁸	65.8*	9.7	4.01*	2.28

^w Mean percent necrotic leaves per pot ($n = 6$) were determined 4 days after inoculation. Peat means followed by an asterisk are significantly ($P \leq 0.05$) different from compost means at the same inoculum concentration according to Fisher's least significant difference test ($\text{LSD}_{0.05} = 6.4\%$).

^x Populations of KD4326 determined by dilution plating. Colony counts (colony forming units per gram of fresh leaf) were made 72 h later. Ten leaves were randomly collected from each pot (six pots per treatment) after disease severity was rated and immediately ground in a high-speed homogenizer (Polytron CH6010, Krins-LU, Kinematica GmbH, Switzerland; 60 Hz, 710 W). Peat means followed by an asterisk are significantly ($P \leq 0.05$) different from compost means at the same inoculum concentration according to Fisher's LSD test ($\text{LSD}_{0.05} = 1.7 \times 10^6$).

^y Not detected.

^z Not measured.

TABLE 2. Efficacy of compost water extract and salicylic acid for control of bacterial speck of *Arabidopsis* plants (*Arabidopsis thaliana* ecotype Columbia), caused by *Pseudomonas syringae* pv. *maculicola* KD4326, grown in a compost or peat mix

Spray treatment	Disease severity (% necrotic leaves/pot) ^y	
	Peat	Compost
Uninoculated control	0	0
Distilled water	74.8 a	14.8 ab
Compost water extract	28.1 c	19.9 a
Sterilized compost water extract ^z	35.9 b	19.4 a
Salicylic acid	19.6 d	10.9 b

^y Disease severity of bacterial speck (*P. syringae* pv. *maculicola* KD4326) was expressed as percent necrotic leaves per pot ($n = 6$). Statistical analysis indicated a significant ($P \leq 0.05$) interaction between spray and mix treatments. Means within the same column followed by different letters are significantly ($P \leq 0.05$) different according to Fisher's least significant difference test ($\text{LSD}_{0.05} = 5.5$).

^z Compost water extract was filter-sterilized (0.2 μm). Sterility was confirmed on 0.1-strength trypticase soy agar.

TABLE 3. Effects of the compost and selected biocontrol agents on induction of systemic acquired resistance (SAR) against anthracnose of cucumber (*Cucumis sativus* cv. Straight Eight) in the autoclaved compost mix

Mix treatment	Mean lesion diameter (mm) ^y	
	Test 1	Test 2
Nonautoclaved mix	2.9 b ^w	... ^x
Autoclaved mix	3.4 c	5.0 c
Autoclaved + 10% nonautoclaved mix	2.0 a	...
Autoclaved + <i>Pantoea agglomerans</i> 278A ^y	...	3.7 a
Autoclaved + <i>Pseudomonas putida</i> 371 ^y	...	4.5 bc
"Classic-induced" SAR in autoclaved mix ^z	1.6 a	3.9 ab
$\text{LSD}_{0.05}$	0.4	0.8

^y Lesion diameter determined 7 days after challenge-inoculation (five replications per treatment, two plants per replication) with 30 10- μl drops of *Colletotrichum orbiculare* conidial suspensions (10^4 to 10^5 CFU ml⁻¹) placed on the second leaf in a moisture chamber and incubation for 24 h in the dark.

^w Within each column, means followed by different letters are significantly ($P \leq 0.05$) different according to Fisher's least significant difference test ($\text{LSD}_{0.05}$).

^x Not tested

^y Biocontrol agents *Pantoea agglomerans* 278A and *Pseudomonas putida* 371 were inoculated into the autoclaved compost mix to establish an initial population density of 10^6 CFU g⁻¹ dry weight mix.

^z "Classic-induced" plants were inoculated 14 days after seeding by infiltrating a conidial suspension of *C. orbiculare* on the abaxial side of the first leaf. Resistance was detected via challenge-inoculation 7 days later by placing a conidial suspension of *C. orbiculare* on the second leaf.

peat (29.0; standard error [SE] = 9.0) compared to compost (4.0; SE = 2.0) ($P < 0.001$). Disease was most severe on plants grown in the peat mix sprayed with an inoculum concentration of 10^8 CFU of *P. syringae* pv. *maculicola* KD4326 per ml. Most plants in the compost mix inoculated with the lower concentrations (10^6 and 10^7 CFU ml⁻¹) did not develop symptoms. Control plants were disease-free. In addition, population sizes of the pathogen recovered from leaves of inoculated plants grown in the compost mix were significantly ($P \leq 0.05$) lower than those from plants grown in the peat mix. The pathogen was not recovered from non-inoculated control plants. These results were observed consistently in two experiments.

Preliminary experiments revealed that water extracts from the peat mix failed to reduce bacterial speck severity on Arabidopsis in each of two tests. However, the compost water extract was effective, and its activity was analyzed further. A significant ($P \leq 0.05$) interaction between potting mix and topical spray treatments on disease severity was observed (Table 2). The compost water extract significantly reduced bacterial speck severity on Arabidopsis plants grown in the peat mix. However, severity of bacterial speck on plants grown in peat still was significantly greater than on any of the plants grown in the compost mix. Sterilized compost extracts also significantly ($P \leq 0.05$) reduced bacterial speck severity on plants grown in the peat mix. SA was significantly more effective than compost water extract on plants in either mix. Even so, disease severity on plants grown in the peat mix sprayed with SA was not significantly different from that on plants grown in the compost mix sprayed with water (inoculated control). Control plants not inoculated with *P. syringae* pv. *maculicola* KD4326 remained free of disease symptoms. Similar results were obtained in a second experiment.

The population size of *P. syringae* pv. *maculicola* KD4326 recovered from plants grown in the compost mix was significantly ($P \leq 0.05$) lower than from plants grown in the peat mix. The population size of the pathogen in inoculated plants was significantly reduced from 2.3×10^7 CFU g⁻¹ of fresh leaf tissue for the water control to 6.9×10^6 CFU g⁻¹ of fresh leaf tissue for plants sprayed with the compost water extract. The sterilized compost water extract also significantly ($P \leq 0.05$) reduced the population size of the pathogen in plants grown in the peat or compost mix.

Anthracnose severity on cucumber plants. The severity of anthracnose on the second leaf of cucumber plants grown in the autoclaved compost mix was significantly ($P \leq 0.05$) more severe than anthracnose on plants grown in the nonautoclaved compost mix (Table 3, test 1). Anthracnose was consistently less severe on classic-induced plants. However, disease severity on classic-induced plants and plants grown in the autoclaved mix inoculated

with 10% (vol/vol) nonautoclaved mix did not differ significantly (Table 3, test 1).

In three preliminary screening bioassays with selected biocontrol agents (stem injection assay), *Pantoea agglomerans* E278A significantly ($P \leq 0.05$) reduced anthracnose severity on plants grown in the peat mix. The mean diameter of anthracnose lesions was reduced from 5.2 mm in the water controls to 1.4 mm in plants injected with E278A. Classic-induced plants had a mean lesion diameter of 0.8 mm. *Pseudomonas putida* 371 significantly reduced mean lesion diameter in one experiment but not in two others, and strains of 15 other bacterial taxa, including *F. balustinum* 299, an effective biocontrol agent against *Rhizoctonia damping-off* (23), had no effect in any of three experiments.

Inoculation of the autoclaved compost mix with *Pantoea agglomerans* E278A significantly ($P = 0.05$) reduced anthracnose severity (Table 3, test 2) compared to the autoclaved mix. This response was consistent among three experiments. There was no significant difference in disease severity between classic-induced plants and plants grown in the autoclaved compost mix inoculated with *Pantoea agglomerans* E278A. Biocontrol agents *Pseudomonas putida* 371 and *F. balustinum* 299 were not effective.

β -1,3-Glucanase activity in cucumber. β -1,3-Glucanase activity in controls (not inoculated with *C. orbiculare*) was low and not affected significantly ($P \geq 0.05$) by mix treatments (Table 4). Inoculation of the second leaf with *C. orbiculare* not only increased β -1,3-glucanase activity but to a significantly higher level in plants grown in the compost compared to plants grown in the peat mix. In classic-induced plants, activity also was significantly ($P \leq 0.05$) higher in plants grown in the compost mix. These results were consistent among four experiments.

GUS activity in Arabidopsis plants. GUS activity, as indicated by more distinct blue staining of leaves, was not detected by histochemical staining of leaves of control Arabidopsis plants sprayed with water and grown in either the compost or peat mix. Comparison of overall means of quantitative GUS activity for all spray treatments indicated that plants grown in the compost mix exhibited significantly ($P \leq 0.05$) higher GUS activity than plants grown in the peat mix (Table 5). The compost water extract significantly increased GUS activity above the activity found in plants sprayed with water or peat water extract. GUS activity in plants sprayed with SA also was significantly ($P \leq 0.05$) higher than in plants sprayed with water or peat water extract. The activity of plants sprayed with the peat water extract did not differ from the control plants sprayed with water. These results were consistent in two experiments.

TABLE 4. Effect of *Colletotrichum orbiculare* on β -1,3-glucanase activity in cucumber (*Cucumis sativus* cv. Straight Eight) plants grown in a compost or peat mix

Inoculation procedure ^x	β -1,3-Glucanase activity ^y	
	Peat	Compost
Water control	0.60	0.55
Second-leaf inoculation only	4.30* ^z	5.00
"Classic-induced" SAR	1.95*	3.70

^x Second leaf was inoculated with 30 10- μ l drops of a conidial suspension of *C. orbiculare* (10^5 CFU ml⁻¹). "Classic-induced" plants were inoculated with *C. orbiculare* on the first leaf 17 days after seeding to induce systemic acquired resistance (SAR) and challenged 7 days later on the second leaf.

^y β -1,3-Glucanase activity expressed as micromoles glucose equivalents released per minute per milligram of protein.

^z Statistical analysis indicated a significant ($P \leq 0.05$) interaction between inoculation procedure and potting mix. Peat means followed by an asterisk are significantly ($P \leq 0.05$) different from compost means within the same inoculation procedure according to Fisher's least significant difference test (LSD_{0.05} = 0.61).

TABLE 5. β -D-Glucuronidase (GUS) activity induced by potting mix water extracts or salicylic acid in Arabidopsis plants (*Arabidopsis thaliana* ecotype Columbia) grown in a compost or peat mix

Spray treatment	GUS activity ^x		Spray mean ^y
	Peat mix	Compost mix	
Water control	12.2	25.8	19.0 b
Compost water extract	33.3	48.4	40.8 a
Sterilized compost water extract	21.8	35.8	28.8 ab
Peat water extract	22.5	21.1	21.8 b
Sterilized peat water extract	18.8	21.3	20.0 b
Salicylic acid	32.5	53.1	42.8 a
Mix mean	23.5* ^z	34.2	

^x Picomoles 4-methyl umbelliferone per minute per milligram of protein. Both spray and mix treatments significantly ($P \leq 0.05$) affected GUS activity. GUS activity determined by fluorometric assay (TKO 100 Mini Fluorometer, Hoefer Scientific Instruments, San Francisco) according to the procedure described by Jefferson et al. (18).

^y Statistical analysis indicated no significant ($P > 0.05$) interaction between mix and spray treatments. According to Fisher's least significant difference test, spray means followed by different letters are significantly ($P \leq 0.05$) different.

^z Asterisk indicates peat mix mean is significantly ($P \leq 0.05$) different from the compost mix mean.

DISCUSSION

This work demonstrates that the activity in the compost mix that induced SAR in cucumber was biological in nature. The effect was destroyed by autoclaving and consistently restored by inoculation of the mix with either the nonautoclaved compost mix or the biocontrol agent *Pantoea agglomerans* E278A originally isolated from a suppressive composted hardwood bark mix (23). Beneficial rhizobacteria, as well as fungi, can induce systemic resistance in plants (24,25,27,29,31,32,42,53). Bacterial taxa possibly harboring such strains are abundant in compost mixes (2,8,13,23). *F. balustinum* 299, which was developed as a binary compost inoculant with *T. hamatum* 382 to suppress Rhizoctonia damping-off (23) and Fusarium wilt of radish (40), failed to induce SAR in cucumber. The microflora in the compost mix that induced SAR remains unknown. These results suggest, however, that significant opportunities exist for the development of improved inoculants for composts yielding novel disease control strategies.

The activity of the compost water extract against bacterial speck of Arabidopsis grown in the peat mix (Table 2) was almost as effective as SA. The microflora in the extract had a significant effect, because sterilization through filtration reduced activity. Even so, the significant activity of the filtered extract suggests that an unidentified chemical factor in the extract also played a role. Others also have reported filterable activity in compost extracts (7,21,38,46), and most of the published research on compost water extracts also attributes efficacy to the activities of biocontrol agents (10,38,46,50). Direct interaction of inhibitors in the extracts with the pathogen in the sprayed leaves could not be excluded, however.

Zhang et al. (52) concluded that the effect of composts on peroxidase activity in cucumber was most pronounced after the pathogen infects the plant. In this work, we showed that β -1,3-glucanase activity was enhanced to the greatest extent in cucumber plants grown in the compost mix after infection with *C. orbiculare* (Table 4). GUS activity was higher in Arabidopsis plants grown in the compost mix than in plants grown in the peat mix (Table 5). Even so, activity in plants grown in the compost mix generally was lower than in plants induced by pathogen infections (3,5,14). In the peat mix, on the other hand, this response to infection was much less pronounced in both cucumber and Arabidopsis plants. This suggests that the microflora in the compost had an effect on this PR protein in both plant types and that much of the activation required infection with a pathogen.

The pattern of β -1,3-glucanase induction observed in this work suggests that SAR induced by compost differed from that induced by pathogens. For example, in classic pathogen and chemical-induced SAR, β -1,3-glucanase activity rapidly increases to high levels (20,22,47). In compost-induced SAR, however, most of the increase in β -1,3-glucanase activity did not occur until after plants became infected with the pathogen (Table 4).

The mechanism by which the compost extract reduced the severity of anthracnose of cucumber and bacterial speck of Arabidopsis remains unknown. SAR probably played a role, however, because the compost extract, like SA, significantly increased GUS activity in Arabidopsis (Table 5). Even so, inhibitors in the extract with direct activity against the pathogens may have played a role. We did not test for such effects.

Systemic resistance induced by various agents leads to complex host responses to the inducers. These responses determine the outcome of the interaction between the host plant and the pathogen, between the host plant and biotic inducers, and between abiotic inducers and the pathogen (12,19,26,41). The timing and magnitude of these responses to pathogen attack have a great impact on the outcome of such interactions (12,44,49). In this sense, very little is known about how beneficial rhizosphere microorganisms induce systemic resistance in plants. Maurhofer et al. (29) showed

that SAR induced in tobacco by strain CHA0 of *Pseudomonas fluorescens* against tobacco necrosis virus coincided with the accumulation of PR2 (β -1,3-glucanase) and PR3 (chitinase) proteins. On the other hand, Pieterse et al. (32) showed that systemic resistance in Arabidopsis induced by *P. fluorescens* strain WCS417r is independent of the accumulation of PR gene products. Therefore, the possibility of other yet to be discovered PR proteins that are distinct from SA-dependent and pathogen-induced PRs that play a role in host defense (15) cannot be excluded.

In conclusion, molecular mechanisms responsible for compost-induced SAR remain largely unknown. Compost-amended mixes potentially support high populations of microorganisms important in the induction of systemic resistance, whereas non-SAR-inducing peat mixes support lower populations of such agents (2). Our work strongly suggests that disease resistance induced in plants grown in the compost mix results from specific interactions between microorganisms and the plant. We found that compost-induced disease suppression involved low-level induction of resistance responses rather than high-level activation and that it may differ from suppression induced by pathogens and SA or compost water extract. Detailed information on this process, leading to the identification of compost-induced in planta biomarkers for SAR, would greatly facilitate research on soil organic matter quality relevant to biological control of plant diseases.

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