ELSEVIER

Physiological and Molecular Plant Pathology 63 (2003) 65–77

PMPP

www.elsevier.com/locate/pmpp

Plant foliar disease suppression mediated by composted forms of paper mill residuals exhibits molecular features of induced resistance

Gary E. Vallad[a,1], Leslie Cooperband[b], Robert M. Goodman[a,c,*]

[a]*Department of Plant Pathology, University of Wisconsin-Madison, 1630 Linden Drive, Madison, WI 53706, USA*
[b]*Department of Soil Science, University of Wisconsin-Madison, Madison, WI, USA*
[c]*The Gaylord Nelson Institute for Environmental Studies, University of Wisconsin-Madison, Madison, WI, USA*

Accepted 15 October 2003

Abstract

Arabidopsis thaliana grown in soil from field plots amended with composted forms of paper mill residuals (PMR) exhibited reduced symptoms of bacterial speck caused by *Pseudomonas syringae* pv. *tomato* (*Pst*) compared with plants grown in soil from field plots amended with a non-composted PMR or non-amended soils. Similar results were obtained with tomato (*Lycopersicon esculentum* Mill.). No relationship between foliar disease suppression and plant nutrition or stature was observed. In *Arabidopsis*, the reduction of foliar disease symptoms ranged between 34 and 65%, depending on the type of composted PMR amendment, and was associated with reduced *Pst* titers *in planta*. An *Arabidopsis npr1* defense mutant and a *NahG* transgenic line, both of which exhibit disrupted systemic acquired resistance, were also disrupted in their suppression of *Pst* disease symptoms in composted PMR treatments. *Arabidopsis* grown in soil amended with composted PMR also displayed an increased expression of pathogenesis-related defense genes prior to pathogen inoculation. We conclude that plants grown in soils with composted PMR-amendments were more resistant to disease caused by *Pst* due to the induction of plant defenses, similar to systemic acquired resistance. The identity of the PMR elicitor(s) is as yet unknown, but was shown to be heat labile.

Keywords: Paper mill residuals; Organic matter; Soil amendments; Compost; Disease suppression; *Arabidopsis thaliana* (L.) Heynh; *Lycopersicon esculentum* (Mill.); Tomato; *Pseudomonas syringae* pv. *tomato* (Okabe) Young; Dye and Wilkie; Bacterial speck; Benzothiadiazole; Systemic acquired resistance (SAR); Non-expressor of *PR-1*; *npr1*; Salicylate Hydroxylase; *NahG*

1. Introduction

From improved soil structure to increased plant nutrition, soil organic matter influences a wide array of environmental and agronomic characteristics and is, therefore, a crucial component of any sustainable agriculture system [5]. Management of soil organic matter with the addition of organic amendments, whether in a composted or raw form, is not a revolutionary concept and has probably existed in some rudimentary form for as long as agriculture itself. Numerous studies have demonstrated the effectiveness of composted organic amendments on various plant diseases, especially against soil-borne pathogens in container systems [14,25,38,40,43,44,55,56,57,60,74]. Nevertheless, our scientific understanding of the processes by which organic amendments influence the outcome of plant disease is still marginal, owing in part to the inherent complexities of soil and the organic material itself.

Previous work with compost amended potting mixes or light-colored peat mixes demonstrated the suppression of foliar disease symptoms caused by *Colletotrichum orbiculare* (Berk. and Mont.) Arx. and *Pseudomonas syringae* pv. *maculicola* on cucumbers (*Cucumis sativus* L.) and

Abbreviations: *Atvsp*, *Arabidopsis thaliana* vascular storage protein; *BGL2*, β-1,3-glucanase; BTH, benzo (1,2,3) thiadiazole-7-carbothioic acid *S*-methyl ester; *ein-1*, ethylene insensitive mutant 1; *Hel*, hevein-like protein; ISR, induced systemic resistance; *jar1-1*, jasmonate response mutant 1-1; *NahG*, salicylate hydroxylase; *npr1*, non-expressor of *PR-1*; *Pdf1.2*, plant defensin 1.2; PGPR, plant growth-promoting rhizobacteria; PMR, paper-mill residuals; PMRBC, paper mill residuals and bark compost; PMRC, paper mill residuals compost; PR, pathogenesis-related; *Pst*, *Pseudomonas syringae* pv. *tomato*; SAR, systemic acquired resistance.

* Corresponding author. Present address: Department of Plant Pathology, University of Wisconsin-Madison, 1630 Linden Drive, Madison, WI 53706, USA. Tel.: +1-608-262-9162; fax: +1-608-262-8643.
E-mail address: rgoodman@facstaff.wisc.edu (R.M. Goodman).
[1] Present address: Department of Plant Pathology, University of California-Davis, c/o U.S. Agricultural Research Station, 1636 E. Alisal St., Salinas, CA, 93905, USA.

0885-5765/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.pmpp.2003.10.003

A. thaliana (L.) Henyh., respectively, relative to plants grown in dark peat mixes conducive to disease [73,74]. Molecular and biochemical assays associated an increase in two key plant defense enzymes, β-1,3-glucanase [73] and peroxidase [74], with reduced foliar disease symptoms following pathogen inoculation. These results suggest that the compost amended potting mixes may have potentiated plant defenses to create an increased state of resistance similar to systemic acquired resistance [73,74]. However, the authors did not rule out other confounding factors, such as differences in plant nutrition or the effects of specific biological control agents added to many of the pine bark composts [73], and lacked the proper tools to pursue the issue further.

Induced resistance is a 'state of enhanced defensive capacity' triggered by specific contact stimuli whereby the plant's active defenses are potentiated against subsequent pathogen challenge [67]. The resistance responses are usually systemic [52], but localized forms also exist, and are effective against a broad range of pathogens [51,61]. Induced resistance can be triggered by exposure of plants to virulent, avirulent, or non-pathogenic microbes [61,67], or artificially by various chemical agents like salicylic acid, 2,6-dichloro-isonicotinic acid (INA), or benzo (1,2,3) thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) [61].

Of particular interest to us are the various soil-borne, nonpathogenic microorganisms that are referred to as plant growth-promoting rhizobacteria (PGPR), which are capable of stimulating plant defenses [47,48,67]. Several of these PGPR incite plant defenses through the production of siderophores [36,37,39] or salicylic acid [16,37]. A comparative study of culturable microflora from the rhizosphere of tomato plants grown in different organic amendments found a significant increase in the incidence of bacteria antagonistic to several soil pathogens in in vitro assays [15]. These antagonistic effects corresponded to an increase in the percentage of siderophore-producing bacteria present. Similar changes in populations of fluorescent pseudomonads were observed in soils suppressive to bacterial wilt (*Ralstonia solanacearum* (Smith) Yabuuchi et al.) of tomato as compared to soils conducive to disease [53]. Boehm et al. [7] also documented an increase in the number of bacterial isolates capable of suppressing *Pythium ultimum* Trow, in a cucumber seedling assay, in disease suppressive peat mixes compared to peat mixes conducive to disease. Substrate availability was determined to play a crucial role in sustaining the efficacy of these bacterial isolates to suppress *P. ultimum* [6,7]. These reports demonstrate that it is possible to enrich the soil environment for organisms with biological control potential through the addition of organic amendments.

Our research is focused on elucidating the mechanisms of foliar disease suppression associated with soils from field plots amended with two composted forms of paper mill residuals (PMR) [62]. A series of field experiments was initiated in 1998 to study the effects of PMR-derived soil amendments on various aspects of soil and plant health [17,62]. PMR (sludges), the by-products of local paper mills, are an ideal source of organic matter for the production of soil amendments, due to their consistency relative to other sources of organic matter and their local abundance [5].

2. Materials and methods

2.1. Soil collection and PMR amendment characterization

Soil (Plainfield loamy sand) was collected on October 10, 1999, September 4, 2000 and May 12, 2001 from experimental plots at the University of Wisconsin's Hancock Agricultural Research Station (Hancock, WI, USA) that were annually amended each spring (late March to early April) with either a high (H) or low (L) rate of (a) non-composted PMR (PMR H, 44.8 dry Mg/ha; PMR L, 22.4 dry Mg/ha), (b) PMR composted alone (PMRC H, 78.4 dry Mg/ha; PMRC L, 38.1 dry Mg/ha), or (c) PMR composted with bark (PMRBC H, 78.4 dry Mg/ha; PMRBC L, 38.1 dry Mg/ha). Soil from non-amended, control plots was also collected. The amendments' chemical characteristics are described in Table 1 and further detailed, along with soil characteristics, in Foley and Cooperband [17] and Stone and colleagues [62]. Soil samples were collected arbitrarily from the top six inches of the experimental plots using a spade, while carefully removing plant and other large debris by hand, following the end of

Table 1
Chemical characteristics of amendments used in 1999

Amendment[a]	Solids ($g\ kg^{-1}$)	Ash ($g\ kg^{-1}$)	Total nitrogen ($g\ kg^{-1}$)	Total carbon ($g\ kg^{-1}$)	NH_4-N ($mg\ kg^{-1}$)	NO_3-N ($mg\ kg^{-1}$)	Salts[b] ($S\ m^{-1}$)	pH	C/N[c]
PMR	227.0	366.1	15.5	296.9	66.4	4.0	0.29	7.0	19.2
PMRC	414.0	676.4	12.3	144.8	76.5	0.3	0.16	7.9	11.7
PMRBC	370.2	356.8	13.8	322.4	16.6	4.1	0.15	8.0	23.4

[a] Amendments include non-composted PMR, composted paper mill residuals (PMRC) and paper mill residuals composted with bark (PMRBC).
[b] Electrical conductivity.
[c] Ratio of carbon to nitrogen.

the growing season between the months of August and October. Soil samples were then stored at room temperature in 30.5 cm × 91.4 cm plastic gusseted bags with the ends held loosely shut by clothespins. For growth chamber experiments, replicate soil samples were pooled by treatment and slightly moistened with distilled water (10 ml/l of soil) prior to use in bioassays.

2.2. Arabidopsis bioassay

Seeds of the wild type, *npr1* [9], *jar1* [59], and *ein2* [20] mutant lines, and a *NahG* transgenic line [18] of *A. thaliana* ecotype Columbia, were sown directly into 3.8 cm diameter by 21.0 cm Cone-tainers™ (Hummert Int., Earth City, MO, USA) each containing approximately 150 cm^3 of soil from the various treatments. Plants were cultivated at 20 °C under a 9 h photoperiod (≈300 μE/m^2/s provided by 90 W cool white fluorescent lights) and 65% RH in controlled environment chambers at the University of Wisconsin-Madison Biotron. Plants were watered every other day and fertilized every fourth day with half-strength Hoagland's nutrient solution [21]. A 300 μM solution of BTH (Syngenta, formerly Novartis, Research Triangle Park, NC, USA) was applied as an aerosol to the aerial portions of 4 week-old plants grown in non-amended soil. Nutrient analysis was carried out at the University of Wisconsin Plant and Soil Analysis Laboratory (Madison, WI, USA) on 5 week-old foliar tissue collected prior to inoculation.

Five week-old plants were inoculated with a bacterial suspension of *P. syringae* pv. *tomato* strain DC3000 (2×10^7 cfu/ml; prepared from an overnight culture grown at 28 °C in King's B medium [31] amended with rifampicin (50 mg/l)) in 10 mm $MgSO_4$ and 0.01% (vol/vol) Tween20, and sprayed onto leaf surfaces using a RL Flomaster, Model 1998, home and garden sprayer (Root-Lowell Manufacturing Co., Lowell, MI, USA). Additional plants were mock inoculated with 10 mM $MgSO_4$ and 0.01% Tween20. Plants were maintained in a chamber at >95% RH for 24 h before and after inoculation, and kept at 85% RH thereafter. Four to five days after inoculation, disease severity was assessed as the proportion of total leaves per plant with symptoms of bacterial speck. Bacterial titer was determined by arbitrarily excising three fully expanded leaves from three or five individual plants at the time of inoculation and 4 days after inoculation, respectively. Leaves were placed in pre-weighed 1.5 ml microcentrifuge tubes and homogenized with a micropestle (Fisher Scientific, Pittsburgh, PA, USA). Leaf homogenates were serially diluted in 10 mm $MgSO_4$ and plated onto Rifampicin amended (50 mg/l) King's B media [31] with an AutoPlate® Model 3000 (Spiral Biotech Inc., Norwood, MA, USA). Plates were incubated at 28 °C for 24 h prior to counting the number of colony forming units (cfu). Previous experiments monitoring bacterial growth found that maximum bacterial growth *in planta* of *P. syringae* pv. *tomato* DC3000 was reached 4 days following inoculation, corresponding with the onset or symptoms (data not shown).

2.3. Tomato bioassay

Seed of the tomato cultivar 'M82' was sown into soil in Cone-tainers™. Plants were cultivated at 24 °C with a 16 h photoperiod at 65% RH. At 4 weeks, plants were challenged by dipping into a bacterial suspension of *P. syringae* pv. *tomato* strain SM78 (2×10^7 cfu/ml; prepared from an overnight culture grown at 28 °C in King's B medium [31] amended with rifampicin (50 mg/l)) in 10 mm $MgSO_4$ and 0.01% (vol/vol) Tween20. Prior to inoculation, plants were placed at 100% RH for 24 h and kept at 65% RH thereafter. A model LI-3100 leaf meter (LI-COR, Lincoln, NE, USA) was used to measure leaf area in cm^2. Disease severity was assessed 4 to 5 days after inoculation as the number of lesions per leaf area (cm^2) for all leaves.

2.4. Gene expression analysis

Prior to inoculation, leaf tissue was collected from five *Arabidopsis* plants per treatment, frozen in liquid nitrogen and stored at −80 °C for future gene expression analysis. Tissue samples were pooled (in equal quantities) by treatment and homogenized using a mortar and pestle. Total RNA was extracted from homogenized tissue samples using Trizol Reagent (Invitrogen, Carlsbad, CA, USA). RNA was treated twice with RQ1 DNase (Invitrogen) prior to cDNA synthesis using 200 units of SuperScript II reverse transcriptase per 5 μg of total plant RNA (Invitrogen) as suggested by the manufacturer. Subsequent PCR reactions consisted of five units of *Taq* polymerase (Promega, Madison, WI, USA), 500 nm of each deoxynucleoside triphosphate, 500 nm of each oligonucleotide primer, 1 μl of cDNA from 5 μg of RNA and 1 × reaction buffer (Promega) in a total volume of 20 μl. Amplifications were performed in a Stratagene Robo40cycler (Stratagene, La Jolla, CA, USA). Primers were designed from sequences within the GenBank database (http://www.ncbi.nlm.nih.gov) for the following genes: hevein-like protein (Hel), plant defensin (Pdf1.2), *A. thaliana* vegetative storage protein (Atvsp), and PR-1 (see Table 2 for sequences). PCR products were separated by electrophoresis on 1.5% agarose gels in 1 × TBE buffer, extracted using QIAquick gel extraction columns (Qiagen, Valencia, CA, USA), cloned into pGEM-T (Promega), and sequenced (University of Wisconsin Biotechnology Center, Madison, WI, USA) to verify primer specificity.

For northern analysis, 5 μg of total RNA was separated by electrophoresis on a 1.0% agarose/formaldehyde gel (2.2 M formaldehyde and 1 × MOPS buffer (0.2 M MOPS, 0.5 M sodium acetate, and 0.01 M EDTA)) in 1 × MOPS buffer. RNA was transferred overnight in 10 × SSPE (1.5 M sodium chloride, 0.1 M sodium phosphate, and 0.01 M EDTA) to a MagnaGraph nylon transfer membrane

Table 2
Gene-specific primer sequences used in RT-PCR and in constructing probes for Northern hybridizations

Target gene (GenBank #)[a]	Primer sequences	Application
PR1 (M90508)	5′-ttc ttc cct cga aag ctc aa-3′ 5′-cgc tac ccc agg cta agt tt-3′	RT-PCR
BGL2 (M90509)	5′-tgt ctg aat caa gga gct tag-3′ 5′-cat act aca cgc atg aaa gc-3′	RT-PCR, Northern Hybridizations
Pdf1.2 (AY063779)	5′-cat ggc taa gtt tgc ttc ca-3′ 5a′-aca ctt gtg tgc tgg gaa ga-3′	RT-PCR, Northern Hybridizations
Atvsp (D85190)	5′-ctc ctc gaa tcg aac acc at-3′ 5′-gca agt cct ttg gcg tag aa-3′	RT-PCR
Hel (AF370536)	5′-cgt gag tgc tta ttg ctc ca-3′ 5′-tag cca aaa cca tcg gtg tc-3′	RT-PCR, Northern Hybridizations
PR1 (583) (M90508)	5′-agc tct tgt agg tgc tct tg-3′ 5′-gat tct cgt aat ctc agc tc-3′	Northern Hybridizations
18S rRNA[b] (X51576)	5′-taa cga gga tcc att gga gg-3′ 5′-ttc ctc gtt gaa gac caa ca-3′	Northern Hybridizations

[a] http://www.ncbi.hlm.nih.gov.
[b] 18S rRNA sequence was applied from tomato cDNA; the 18S rRNA sequences of *Arabidopsis* and tomato share 96% identity within this amplified region.

(Osmonics, Minnetonka, MN, USA), and covalently bound to the membrane using a Stratalinker UV-crosslinker (120 mJ/cm^2 (Stratagene)). Radiolabelled RNA probes were transcribed in vitro from cloned RT-PCR fragments (Table 2) using the StripAble RNA Probe Synthesis and Removal Kit (Ambion, Austin, TX, USA) with [α-P^{32}]-UTP. Probes were hybridized to membranes overnight using ULTRAhyb hybridization solution (Ambion) at 68 °C. Final membrane washes were performed at 68 °C in a solution of 0.2 × SSPE. Probes were detected by scanning membranes with a Molecular Dynamics PhosphorImager (Sunnyvale, CA, USA). Membranes were then stripped, before reusing, using reagents supplied in the StripAble RNA Probe Synthesis and Removal Kit (Ambion) as recommended by the manufacturer.

2.5. Statistical analysis

Experimental units in each independent experiment were arranged in a completely randomized design. Treatment effects were assessed using analysis of variance in the PROC GLM procedure of SAS (SAS Institute, Cary, NC, USA). Fisher's protected LSD test at $P \leq 0.05$ was used to compare treatment means.

3. Results

Plants grown in soils amended with composted forms of PMR were more resistant to foliar diseases caused by *Pst*. In disease assays, using soils collected in 1999, both *Arabidopsis* and tomato plants exhibited reduced disease symptoms caused by *Pst* when grown in soils amended with composted forms of PMR (PMRC or PMRBC), compared with results in non-amended soils (control) or soil amended with a non-composted PMR (Table 3). In *Arabidopsis*, the reduction of disease symptoms ranged, on average, from 34 to 65% depending on the soil amendment and was consistent over several independent experiments. Similarly, results with tomato demonstrated a reduction of foliar symptoms associated with PMRC and PMRBC amended soils (62 and 47%, respectively) relative to plants grown in non-amended soil or soil amended with PMR (Table 3).

Plants exhibited few differences in stature or nutrient content when grown in soils amended with PMR. Soil amendment type did not influence shoot biomass (Table 3) or the number of rosette leaves per plant in *Arabidopsis* (data not shown) under the experimental conditions used. However, tomatoes (cv. M82) grown in soil amended with PMRC exhibited nearly a 50% reduction in leaf area and shoot biomass compared to those grown in the other soil treatments (Table 3). Experiments employing a second tomato cultivar, Moneymaker, lacked these probable allelopathic effects (data not shown).

To assess potential nutrient effects, *Arabidopsis* foliar tissues were sampled for nutrient analysis prior to inoculation with *Pst*. Of the macro-and micro-nutrients measured, significant differences in the levels of phosphorous, potassium, and magnesium in plant tissues were observed among soil treatments ($P < 0.05$; Table 4). No statistical differences in the levels of calcium, sulfur, zinc, boron, manganese, iron, copper, aluminum, or sodium were observed among treatments (data not shown). Similar results were obtained in a replicated experiment. Because insufficient quantities of tissue were available, nitrogen levels in *Arabidopsis* were not determined. Most differences in plant nutrient content were observed between plants grown in amended versus non-amended soils, while nutrient content was similar among plants grown in the amended soil treatments (Table 4). Overall, these differences in plant nutrient content did not influence plant growth, or correspond to the reduced symptoms of *Pst*. No signs indicative of nutrient stress were ever observed on plants.

An increased level of pathogenesis-related gene expression in *Arabidopsis* was associated with composted forms of PMR. The expression of several *Arabidopsis* defense genes was assessed by RT-PCR just prior to inoculation. The results demonstrated an increase in the expression of *PR-1* in plants grown in soils amended with PMRC or PMRBC as compared to plants grown in PMR or non-amended (control) soils (Fig. 1). The increase in *PR-1* expression between the non-composted and composted PMR treatments was similar to the effect seen between the control and BTH treatments. Little treatment effect was observed among the expression of several genes encoding a jasmonate-inducible vegetative storage protein (*Atvsp*) [3,47], an ethylene-inducible hevein-like protein

Table 3
Severity of disease symptoms of bacterial speck caused by *P. syringae* pv. tomato on *Arabidopsis* and tomato

Treatment[a]	*Arabidopsis*		Tomato			
	Disease severity (%)[b]	Relative reduction (%)[c]	Disease severity [d]	Relative reduction (%)[c]	Leaf area (cm^2)	Biomass (g)
Control	49.1 a[e]	–	1.40 a[e]	–	75.1 a[e]	2.96 a[e]
PMR H	32.4 b	34.0	1.25 a	10.7	80.7 a	3.14 a
PMRBC H	17.3 cd	64.7	0.74 b	47.1	84.6 a	3.41 a
PMRC H	19.1 c	61.0	0.53 b	62.1	43.9 b	1.65 b
PMRC L	34.5 b	29.9	–	–	–	–
BTH	7.5 d	84.7	0.67 b	52.1	72.7 a	2.85 a
$LSD_{0.05}$	9.9		0.43		18.3	0.85

[a] Plants were grown in a non-amended soil (control), or a soil amended with low (L) or high (H) rates of either non-composted PMR, composted paper mill residuals (PMRC) or paper mill residuals composted with bark (PMRBC). A second set of plants grown in non-amended soil was treated with BTH 1 week prior to inoculation. Soils were collected from field plots on October 10, 1999, and were stored for 3 months prior to the beginning of these experiments.
[b] Percentage of leaves with disease symptoms.
[c] Reduction of disease severity relative to control plants grown in a non-amended field soil.
[d] Average number of lesions per cm^2 of leaf area at 5 days post-inoculation.
[e] Values followed by the same letter are not statistically significant by Fisher's protected LSD test at $P \leq 0.05$.

(*Hel*) with antifungal activity [47,49], and a plant defensin (*Pdf1.2*) that encodes a small protein with antifungal activity that is inducible by ethylene and jasmonate [45,47].

Northern analysis demonstrated a similar, but varied, pattern of defense gene expression across independent experiments (Fig. 2). Plants grown in soils amended with PMRC exhibited gene expression patterns similar to plants treated with BTH, with the expression of the pathogenesis-related genes *PR-1* and β-1,3-glucanase (*BGL2*), and the plant defensin *Pdf1.2* consistently greater relative to the other soil treatments. In most cases, foliar induction with BTH elevated defense gene expression to a level higher than that observed for the PMRC soil treatment.

Defense gene expression varied among experiments for plants grown in soils amended with PMR or PMRBC (Fig. 2). In most cases, the expression level for those defense genes analyzed in the PMRBC soil treatment was equal to or greater than observed for the PMR soil treatment across experiments. The expression of *BGL2* was elevated in the PMRBC soil treatment when compared to the non-amended control and PMR soil treatment at 8 and 16 months, while at 18 months it was similar to those same treatments. Overall, the non-amended control, PMR, and

Table 4
Nutrient analysis of *Arabidopsis* leaf tissue prior to inoculation

Treatment[b]	Nutrient[a]		
	% P	% K	% Mg
Control	0.66 a[c]	2.54 a[c]	1.14 b[c]
PMR H	0.58 bc	2.96 ab	0.63 c
PMRBC H	0.52 c	2.91 bc	0.57 cd
PMRC H	0.42 d	2.56 a	0.43 d
PMRC L	0.51 c	2.86 bc	0.68 c
BTH	0.62 ab	2.67 bc	1.12 b
$LSD_{0.05}$	0.08	0.38	0.16

[a] No statistical differences in the percentage of Ca, S, Zn, B, Mn, Fe, Cu, Al, or Na were observed among treatments (data not shown).
[b] Plants were grown in a non-amended soil (control), or soil amended with low (L) or high (H) rates of either non-composted PMR, composted paper mill residuals (PMRC) or paper mill residuals composted with bark (PMRBC). A second set of plants grown in non-amended soil was treated with BTH 1 week prior to the collection of leaf tissue. Soils were collected from field plots on October 10, 1999, and were stored for 3 months prior to the beginning of these experiments.
[c] Values followed by the same letter are not statistically significant by Fisher's protected LSD test at $P \leq 0.05$.

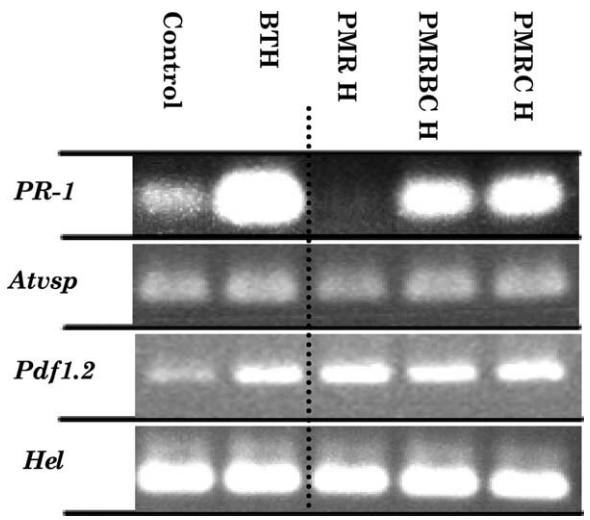

Fig. 1. Effect of 1999 amended and non-amended field soils on the expression of several *Arabidopsis* defense genes, as assessed by RT-PCR analysis of plant samples collected before inoculation with *P. syringae pv. tomato*. Host defense genes assessed include *pathogenesis-related protein 1* (*PR-1*), *Arabidopsis thaliana* vascular storage protein (*Atvsp*), *plant defensin 1.2* (*Pdf1.2*), and *hevein-like protein* (*Hel*). Plants were grown in a non-amended soil (control), or a soil amended with a high (H) rate of either non-composted paper mill residuals (PMR), composted paper mill residuals (PMRC) or paper mill residuals composted with bark (PMRBC). A second set of plants grown in non-amended soil was treated with BTH 1 week prior to inoculation. Soils were collected from field plots 3 months prior to the beginning of this experiment (see corresponding data in Fig. 5). Repeated RT-PCR assessment of *PR-1* and *Pdf1.2* expression gave similar results.

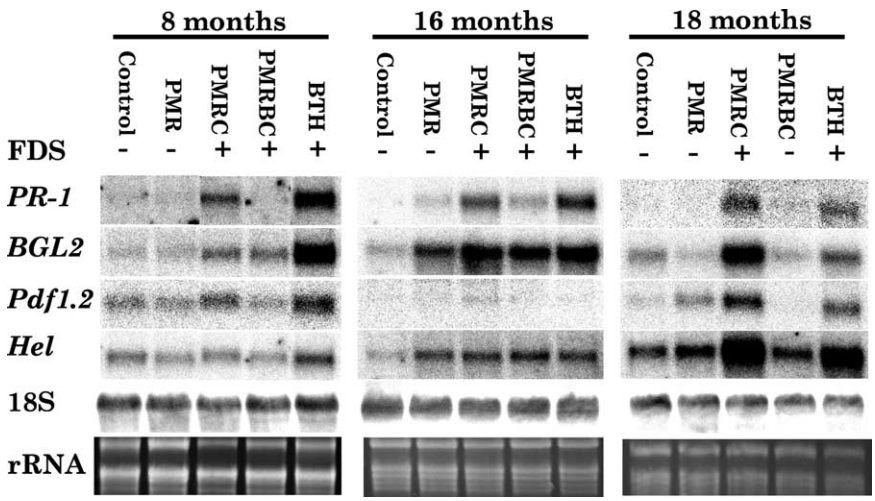

Fig. 2. Effect of 1999 amended and non-amended field soils after 8, 16, and 18 months of storage on the expression of several *Arabidopsis* defense genes, as assessed by northern hybridizations of plant samples collected before inoculation with *P. syringae pv. tomato*. Plants were grown in a non-amended soil (control), or a soil amended with a high (H) rate of either non-composted paper mill residuals (PMR), composted paper mill residuals (PMRC) or paper mill residuals composted with bark (PMRBC). A second set of plants grown in non-amended soil was treated with BTH 1 week prior to inoculation. The presence or absence of foliar disease suppression (FDS) activity as determined in subsequent inoculations is denoted below experimental treatments by (+) or (−), respectively (see corresponding data in Fig. 5). The expression of host defense genes *pathogenesis-related protein 1* (*PR-1*), β-1,3-glucanase (*BGL2*), *plant defensin 1.2* (*Pdf1.2*), and *hevein-like protein* (*Hel*) was assessed with in vitro transcribed probes. The ethidium bromide staining of agarose gels (rRNA) and an in vitro transcribed 18S rRNA probe were used to confirm equal loading and proper transfer of RNA samples to membranes.

BTH treatments exhibited the most consistent patterns of gene expression across experiments.

Disruption of *NPR1*, and expression of the *NahG* transgene in *Arabidopsis* inhibited the disease suppressive effects of composted forms of PMR. To address the importance of several biochemical pathways implicated in induced resistance in foliar disease suppression mediated by the composted forms of PMR, several *Arabidopsis* mutants were grown on non-amended soils and soils amended with either PMRC or PMRBC. Wild type plants exhibited a reduction in the disease severity of *Pst* when grown in soils amended with PMRC and PMRBC relative to the non-amended control, whereas *npr1* plants did not (Fig. 3). Among *jar1* and *ein2* plants, no differences in disease severity were observed across soil treatments (Fig. 3). However, *jar1* and *ein2* plants were severely stunted relative to wild type plants (data not shown). In addition, *jar1* plants exhibited symptoms of damping-off across all treatments, and succumbed to symptoms in the non-amended soil. Damping-off is a symptom typical of plant diseases caused by soil-borne oomycetes.

Disease assays utilizing a *NahG* transgenic *Arabidopsis* line demonstrated that plants unable to accumulate salicylic acid due to the expression of *NahG* were unresponsive to

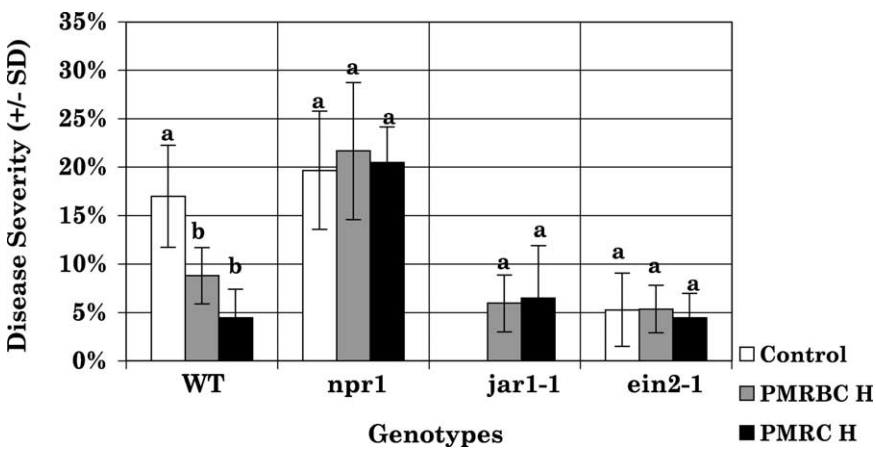

Fig. 3. Effect of wild type and three *Arabidopsis* mutants on the foliar disease suppression of symptoms caused by *P. syringae pv. tomato* associated with plants grown in 1999 amended and non-amended field soils. Plants were grown in a non-amended soil or soil amended with a high (H) rate of either composted paper mill residuals (PMRC) or paper mill residuals composted with bark (PMRBC). Soils were collected from field plots on October 10, 1999, and were stored for 6 months prior to the beginning of these experiments. Values represent the mean percentage of total leaves exhibiting symptoms of bacterial speck per plant 5 days after inoculation. Bars represent the standard deviation of $n = 8$ replicate plants per treatment. Within each soil treatment, means with the same letter are not significantly different by Fisher's protected LSD test at $P \leq 0.05$.

Table 5
Bacterial titer and severity of disease symptoms of bacterial speck caused by *P. syringae* pv. tomato on wild type (WT) and *NahG Arabidopsis* lines

Treatment[a]	Genotype	Disease severity[b]	Bacterial titer Log cfu/g ± SD[c]
PMR H	WT	80.3 a[d]	8.12 ± 0.04 a[d]
PMRBC H	WT	55.4 b	7.86 ± 0.11 b
PMRC H	WT	45.0 c	7.77 ± 0.07 b
	$LSD_{0.05}$	8.4	0.24
PMR H	*NahG*	77.0 a[d]	9.48 ± 0.07 a[d]
PMRBC H	*NahG*	78.3 a	9.26 ± 0.05 a
PMRC H	*NahG*	70.2 a	9.42 ± 0.09 a
	$LSD_{0.05}$	8.1	0.24

[a] Plants were grown in soil amended with a high (H) rate of either non-composted PMR, composted paper mill residuals (PMRC), or paper mill residuals composted with bark (PMRBC). Soils were collected from field plots on October 10, 1999, and were stored for 6 months prior to the beginning of these experiments.
[b] Percentage of leaves with disease symptoms 4 days post-inoculation.
[c] Bacterial titer was determined 4 days after inoculation.
[d] Values followed by the same letter are not significantly different by Fisher's protected LSD test at $P \leq 0.05$.

soils amended with composted forms of PMR (Table 5). Bacterial growth of *Pst in planta* was also examined in the same bioassay. Four days following inoculation, bacterial titers averaged 50% less in wild type plants grown in PMRBC or PMRC compared to wild type plants grown in non-composted PMR. The *NahG* plants did not show any significant differences in disease severity or bacterial titer among treatments.

The disease suppressive effect of PMR amendments was heat labile. In a first step toward determining the nature of the eliciting factor(s) in composted PMR amendments, non-amended field soil was autoclaved and amended (10% w/v) with either a PMRBC amendment (collected in the spring of 2000) or an autoclaved PMRBC amendment for use in the *Arabidopsis-Pst* disease assay (Fig. 4). Wild type *Arabidopsis* exhibited a 60% reduction in disease severity caused by *Pst* when grown in an autoclaved soil amended with PMRBC as compared to plants grown in an autoclaved soil amended with autoclaved PMRBC. No reduction in disease severity caused by *Pst* was observed when the *Arabidopsis npr1* mutant was grown in autoclaved soil amended with PMRBC as compared to the autoclaved soil amended with autoclaved PMRBC. The foliar disease suppression imparted by the PMRBC amendment in autoclaved soil was comparable to the foliar disease suppression associated with soil from the PMRBC-amended field plots (Table 3), demonstrating that the use of autoclaved soil did not diminish the ability of the PMRBC amendment to reduce disease symptoms associated with *Pst*.

The suppressive effect of PMR amendments diminished over time and varied with serial additions. Relative to the non-amended controls, the compost amended field soils

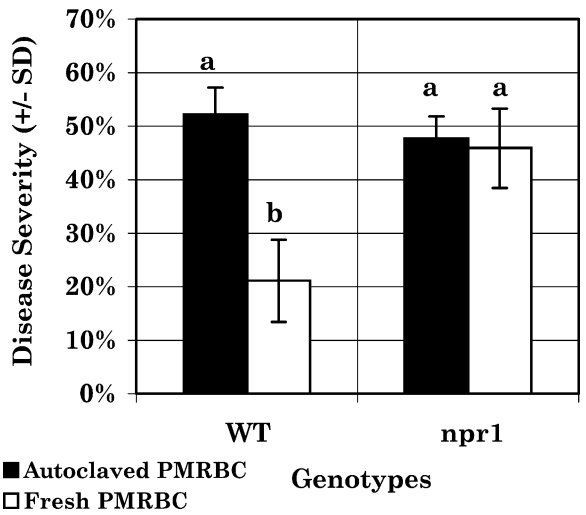

Fig. 4. Effect of autoclaving on foliar disease suppression activity associated with the 2000 PMRBC amendment. The PMRBC amendment was collected May 28, 2000 and stored for 6 months prior to the beginning of this experiment. Wild type *Arabidopsis* and *npr1* mutant were grown in autoclaved field soil amended (10% w/v) with autoclaved (1 h at 121 °C) or non-autoclaved PMRBC amendment. Values represent the mean percentage of total leaves exhibiting symptoms of bacterial speck per plant 5 days after inoculation. Bars represent the standard deviation of $n = 8$ replicate plants per treatment. Means with the same letter are not significantly different by Fisher's protected LSD test at $P \leq 0.05$.

collected in 1999 consistently suppressed foliar disease in the *Arabidopsis-Pst* system for about 18 months when stored at room temperature (Fig. 5). Disease symptoms were reduced 40–80% in composted PMR treatments (PMRC and PMRBC) relative to the non-amended controls. PMR treatments only reduced disease symptoms 20% or less relative to the controls, with one exception. Overall, PMRC reduced disease severity more than PMRBC. After 21 months of storage, all disease suppressive activity was lost in the 1999 amended soils, as determined with the *Arabidopsis-Pst* disease assay.

Following the loss of foliar disease suppression activity in field soils collected in 1999, soils collected in 2000 and 2001 were assessed for their potential to suppress foliar symptoms of bacterial speck (Fig. 6). Only the PMRC treatment soil collected in 2000 was able to suppress significantly foliar disease symptoms caused by *Pst* on *Arabidopsis* to an extent similar to the BTH treatment. This suppressive effect was also reflected by a reduction in bacterial titer *in planta*. To date, no other amendment derived from PMR that we have tested has been capable of suppressing foliar disease symptoms in the *Arabidopsis-Pst* disease assay.

4. Discussion

Our previous field experiments documented the suppression of symptoms of foliar brown spot (causal agent *P. syringae* pv. *syringae*) of snap bean and angular leaf

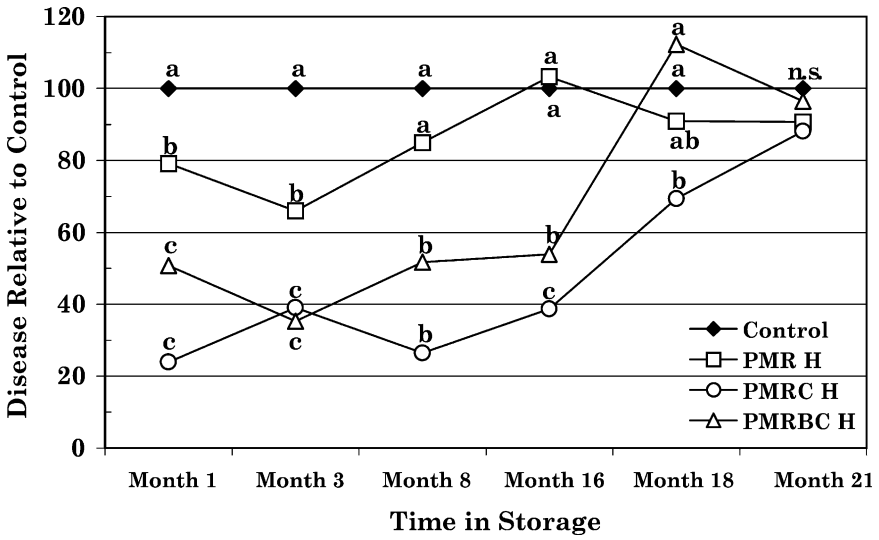

Fig. 5. Stability of foliar disease suppression activity associated with the 1999 amended field soils, as assessed using the *Arabidopsis-Pst* DC3000 disease assay. Plants were grown in a non-amended soil (control), or a soil amended with a high (H) rate of either non-composted paper mill residuals (PMR), composted paper mill residuals (PMRC) or paper mill residuals composted with bark (PMRBC). Values shown are means converted to represent the percentage of disease severity relative to the control for each time point. Disease severity was rated as the percentage of leaves with symptoms of bacterial spot 5 days after inoculation; control means are 20.8, 49.1, 17.0, 63.4, 56.2, and 67.6% for month 1, 3, 8, 16, 18, and 21, respectively. Treatment means ($n = 8$ plants) within a single time point with the same letter are not significantly different by Fisher's protected LSD test at $P \leq 0.05$; n.s. = no significance.

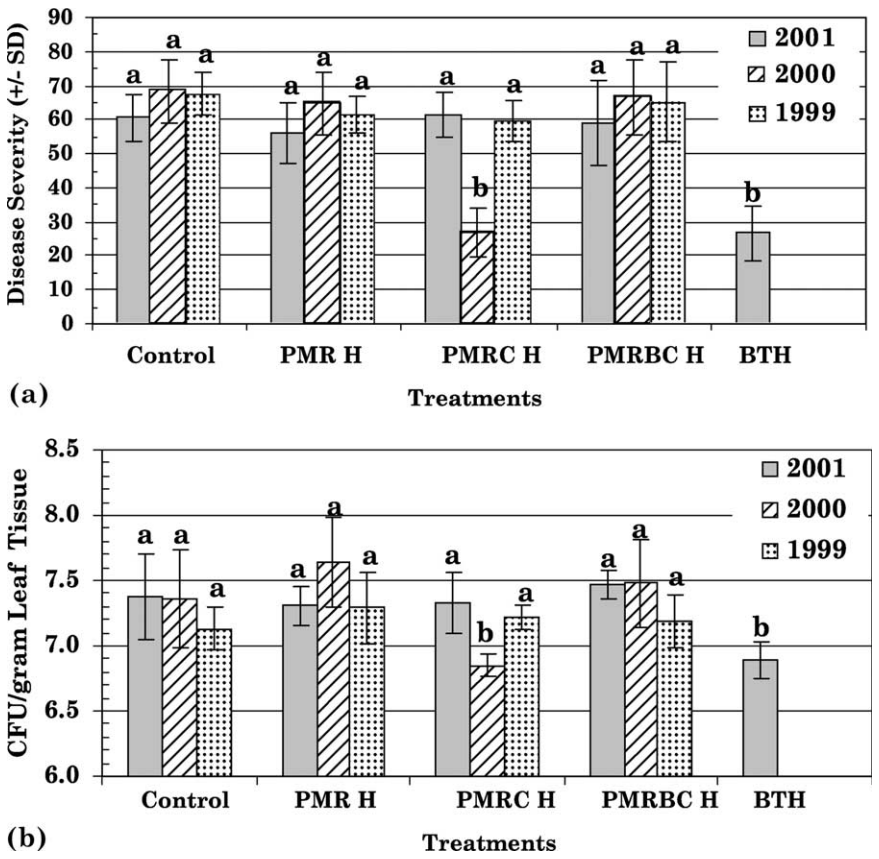

Fig. 6. Foliar disease suppression activity associated with the 1999, 2000, and 2001 amended and non-amended field soils, following 23, 12 and 4 months of storage, respectively, as assessed using the *Arabidopsis-Pst* DC3000 disease assay. Plants were grown in a non-amended soil (control), or a soil amended with a high (H) rate of either non-composted paper mill residuals (PMR), composted paper mill residuals (PMRC) or paper mill residuals composted with bark (PMRBC). BTH treatment was applied to plants grown in 2001 non-amended field soil. (a) Values shown are mean disease severity of $n = 8$ plants rated as the percentage of leaves with symptoms of bacterial speck 4 days after inoculation. (b) Mean *in planta* titer of *Pst* DC3000 at 4 days post-inoculation in $n = 5$ plants per treatment. Treatment means across years with the same letter are not significantly different by Fisher's protected LSD test at $P \leq 0.05$, bars represent the standard deviation.

spot (causal agent *P. syringae* pv. *lachrymans*) and anthracnose (causal agent *Colletotrichum lindemuthianum*) of cucumber grown in soil amended with composted PMR [62]. We have further documented this phenomenon here in replicated experiments with the same field soils showing foliar disease suppression in a controlled environment on two unrelated plants, tomato and *Arabidopsis*, using *P. syringae* pv. *tomato* as the pathogen. Using the *Arabidopsis-Pst* disease model system, we demonstrated that the foliar disease suppression observed with the composted forms of PMR was an induced form of resistance, exhibiting several characteristic features of SAR.

In plants, induced forms of systemic resistance can be broadly categorized into SAR [51,65] and ISR [47,48], based on several characteristics (reviewed in Refs. [61,67]). ISR is potentiated by PGPR, of which the best characterized are several species of *Pseudomonas* that cause no visible damage to the plant's root system [67]. Classical SAR, as induced by a biotic factor, requires some sort of necrotic symptom on the plant's aerial surfaces. SAR is associated with a salicylic acid-dependent pathway [18,35,69] and the induction of pathogenesis related (PR)-proteins [65,71]. ISR, based mostly on the interaction between *A. thaliana* and *Pseudomonas fluorescens* WCS417r, is salicylic acid-independent, does not involve the induction of PR-genes or other defense related genes [23,47,48], and appears to rely on jasmonate-and ethylene-dependent pathways [47]. However, few ISR systems have been characterized to this extent, and some plant-PGPR interactions exhibit features of SAR [16,39,67], implying that the origin of the eliciting agent is not as critical in the classification of induced resistance phenomena as the biochemical responses incited within the plant [61,67]. In addition, SAR is effective across a wide array of plant species, whereas there is some demonstrated specificity in the ability of strains of PGPR to induce systemic resistance at the plant species and genotype level [68]. To date, this type of specificity has not been documented in SAR systems.

We present evidence for the induced expression of two SA-dependent, pathogenesis-related genes, *PR-1* and β-1,3-glucanase (*BGL2*) prior to challenge inoculation in plants grown in soils amended with composted PMRs (PMRC and PMRBC; Figs. 1 and 2). Even though the gene expression data were not replicated at each timepoint (since replicate plant RNA samples were pooled by treatment in each experiment), qualitative differences among treatments were consistent in replicated experiments over time, with a few exceptions. Plants grown in the PMRC treatment showed consistent elevated levels of expression of *PR-1* and *BGL2* relative to the non-amended control and the non-composted PMR treatment. Plants grown in the PMRBC treatment did not differ from the non-amended control or non-composted PMR treatments in the expression of *PR-1*. However, an elevated level of *BGL2* was associated with PMRBC treatment relative to the control or the PMR treatment until after 16 months, coinciding with the loss of foliar disease suppression in the PMRBC treatment (Figs. 2 and 5). This discrepancy in the expression of *PR-1* and *BGL2* in plants grown in PMRC and PMRBC treatments may represent a possible dose response, since the PMRBC treatment generally was not as effective as the PMRC treatment at suppressing foliar symptoms caused by *Pst* on *Arabidopsis* (Fig. 5).

The disruption of foliar disease suppression in *Arabidopsis* plants with an *npr1* mutation or the *NahG* transgene when grown in PMRC-and PMRBC-amended soils implies that this phenomenon is an inducible host defense response similar to SAR [9,47]. In comparison, a functional *NPR1* was also important for ISR [47], while the disruption of salicylic acid signaling by the *NahG* transgene was not [47,48]. Whether PMR-mediated foliar disease suppression is independent of ethylene or jasmonate regulated plant defenses needs to be explored further, since there is evidence that the action of ethylene and jasmonate together can stimulate salicylic acid action [35]. A synergistic effect on the expression of *PR-1* and osmotin (*PR-5*) was documented in tobacco plants exposed to combinations of either salicylic acid and methyl jasmonate, or ethylene and methyl jasmonate [72]. An increased level of expression of *Pdf1.2* was associated with the PMRC and BTH treatments as compared to the control and PMR treatments, similar to *PR-1*, in replicate experiments at 8 months and 18 months (Fig. 2), suggesting a possible role for ethylene and jasmonate-dependent host responses in foliar disease suppression mediated by composted PMR amendments. We have no explanation for the weak expression of *Pdf1.2* at 16 months (which was also observed in a replicated hybridization using the same RNA sample) or the increased expression of *Hel* in PMRC and BTH treatments at 18 months, except that these inconsistencies may reflect other gross changes occurring within the soil over time that are influencing the plant.

We were unable to use *Arabidopsis* mutants to test the importance of ethylene-and jasmonate-dependent defenses in PMR-mediated foliar disease suppression, since *jar1* and *ein2* plants either succumbed to symptoms of damping-off or were severely stunted. The difficulties incurred while growing the *jar1* and *ein2* mutants in field soil are not surprising. Disruptions in the jasmonate and ethylene signaling pathways often lead to susceptibility to various opportunistic oomycetes [19,32,58,70].

There are other examples of improved plant defense responses in relation to foliar disease suppression mediated by composted materials, but these are based on studies using only potting mix systems. Zhang et al. [73,74] showed that several formulations of a disease suppressive, pine bark compost were able to increase the levels of the plant defense-related enzymatic activities of peroxidase and β-1,3-glucanase compared to a peat-based potting mix conducive to disease. These increases in enzyme activities were only observed following inoculation of the plant with the pathogen. In addition, the disease suppression observed

in several experiments appeared to be more associated with a biological control agent that was added to the compost during its preparation, than the compost itself [73]. It is also possible that the increases in enzymatic activity and disease suppression could be attributed to plant nutritional status, which would also be systemic. Another possibility is that the amendments utilized by Zhang et al. [73,74] 'primed' the plant's defenses to respond more rapidly and to higher levels when elicited during pathogenesis. The priming of host defenses has been documented in plants through low exposures to chemical elicitors, such as salicylic acid [13,27,28,41,42,54], methyl jasmonate [29] and β-aminobutyric acid [75]. Priming of *Arabidopsis* defenses with BTH [33] and β-aminobutyric acid [75] was still dependent on *NPR1*.

Another source of composted pulp and PMR was also investigated for its ability to suppress crown and root rot of tomato, caused by *Fusarium oxysporum* f.sp. *radicis-lycopersicon* [46]. Several induced cytological responses indicative of a potentiated host response to *F. oxysporum* occurred when plants were grown in a peat moss potting mix amended with composted pulp and PMR. No apparent cytological changes were observed prior to pathogen introduction in non-compost or compost amended potting mixes [46]. This also suggests a possible priming effect on host defenses.

One possible confounding effect that might explain the apparent disease suppression observed in our experiments is differences in plant nutrient status. However, nutrient analysis of *Arabidopsis* tissue revealed little variation among PMR treatments (Table 4). Detected differences in plant nutrient status were generally between the non-amended and amended treatments, which would be expected because of the improved nutrient content, water holding capacity, and soil structure imparted to the soil by the amendments.

Even though we were unable to assess nitrogen content, due to the small stature of *Arabidopsis*, no differences in biomass or leaf number among soil treatments was ever observed in experiments using *Arabidopsis*. With the exception of the allelopathic interaction observed when tomato cv. M82 was grown on PMRC, there also were no treatment effects on biomass in tomato (Table 3). Elevated nitrogen content was found to increase the susceptibility of tomato plants to *Pst* [22]. Through chemical characterization of the amendments (Table 1) and amended soils [17], we also know that there is an increase in plant-available nitrogen in soils amended with PMR and PMRC amendments. Assuming nitrogen content would be highest in plants grown in PMR and PMRC treatments [17]; the decreased disease severity associated with PMRC and PMRBC treatments, and similarity in disease severity between the PMR and non-amended control seem to rule out nitrogen as a factor.

Experiments using autoclaved amendments demonstrated that the potentiating effect of PMRBC is heat labile and independent of the field soil (Fig. 4). Additionally, in the *Arabidopsis npr1* mutant, foliar disease suppression associated with growth in autoclaved soil amended with PMRBC was disrupted. However, it is inconclusive whether this potentiating effect is biotic or abiotic, since many abiotic substances are also heat labile. A better approach to this question would be to use irradiation, which should 'sterilize' the amendment while preserving its physical and chemical composition. Zhang et al. [73] found that the disease suppressive activity of a fortified pine bark potting medium was also heat labile, but oddly, a fermented water extract (compost tea) from the same medium that also suppressed foliar disease was still active even following autoclaving and passage through a 0.2 μm membrane filter.

Successive disease assays over a 21-month period demonstrated the longevity of foliar disease suppression in amended soils originally collected in 1999 when stored at room temperature (Fig. 5). However, the stability of foliar disease suppression in fields amended with composted forms of PMR is unknown. Field soils collected annually following reapplication of amendments over 3 consecutive years were assessed for foliar disease suppression. From the soils collected in 2000, only the 12-month old PMRC-amended soil exhibited foliar disease suppression (Fig. 6), which corresponded to the suppression of angular leaf spot of cucumber caused by *P. syringae* pv. *lachrymans* in field experiments [62]. Interestingly, the soil amended with PMRBC in 2000 failed to show any foliar disease suppression activity in *Arabidopsis* bioassays (Fig. 6) or in field experiments [63], even though the PMRBC amendment itself demonstrated foliar disease suppression activity when amended to autoclaved soils (Fig. 4). It is possible that the foliar disease suppression associated with the 2000 PMR amendments was not as durable as in the 1999 amendments or may have been residual of the 1999 PMR amendments. No foliar disease suppression was associated with amended soils in 2001 (Fig. 6) or 2002 (data not shown) in our experiments or in field experiments (L.R. Cooperband and D. Rotenberg, personal communication). However, all amended soils described in these experiments and others [62], regardless of amendment type, suppressed root diseases caused by soilborne oomycetes (L.R. Cooperband and D. Rotenberg, personal communication). These observations agree with those of Krause et al. [34] who found that less than 10% of composts tested in a potting mix system were able to suppress foliar diseases, suggesting that foliar disease suppression mediated through composts is a less frequent phenomenon than the suppression of diseases caused by soilborne oomycetes [62].

Why is foliar disease suppression so difficult to generate and maintain with organic amendments? PMR are heterogeneous mixtures of wood pulp, paper fillers and microbial biomass recovered during wastewater purification and are chemically composed of cellulose, hemicellulose, lignin, and other complex carbohydrates, in

addition to a vast assortment of other complex organic compounds, nutrient elements, trace elements, and complex fatty acids [1,12]. Any number of these chemical components could directly elicit a SAR-like response in plants, or indirectly following some sort of biologically driven chemical modification. In addition, numerous examples exist of microorganisms capable of inducing systemic forms of resistance in plants [67], so it is possible that the differences in foliar disease suppression observed among these serially amended soils were due to the presence or absence of specific microorganisms, or the availability of substrates that support these microorganisms [6,7,24]. Finally, because the field soils were serially amended, it is quite possible that over time changes in soil physicochemical properties [17] disrupted chemical or biological conditions required for the maintenance or reestablishment of foliar disease suppression.

Hoitink and Boehm [6,24] postulated that biological control in soil microbial communities is a substrate-dependent phenomenon. That is, microorganisms capable of biological control through mechanisms such as antibiosis, competition, parasitism (or predation) or induced resistance, have specific nutritional requirements that must be met in order to sustain populations and physiological states necessary to maintain microbial activities that impart biological control. However, our data, in addition to other anecdotal observations, beckons a different interpretation of Hoitink and Boehm's [6,24] substrate-dependent hypothesis, beyond providing the essential 'carbon' requirements necessary for growth and persistence of specific microorganisms.

We hypothesize that the SAR activity associated with the composted PMR amendments was an indirect result of the biological degradation of polycyclic aromatic hydrocarbons (PAHs) or other complex molecules by various microorganisms in the soil and plant rhizosphere, producing chemical intermediates, such as salicylic acid, capable of eliciting an induced resistance response in plants. Chemical analysis of the PMR produced during wastewater treatment is performed quarterly by Stora Enso North America, the paper company that produced the non-composted PMR amendments used in this research. The polycyclic aromatic hydrocarbon naphthalene and the aromatic hydrocarbons *p*-isopropyl toluene and xylene were often present in PMR at varying levels (data not shown). Other researchers have also documented the presence of aromatic hydrocarbons (including PAHs) in PMR and other municipal sludges [1,2,4]. However, none of the PMR amendments or parental PMR used to generate the composted PMR amendments for this research were directly tested for the presence of PAHs or other aromatic hydrocarbons.

The differences in foliar disease suppression activity observed between composted and non-composted PMR amendments could be due to the sorption or modification of PAHs during the composting process. For example, when added to soil, researchers have found that a significant proportion of PAHs, such as naphthalene, are sorbed to the various organic fractions of the soil where they are protected from further degradation [26,50]. Therefore, it is possible that PAHs in composted PMR amendments were sorbed to the various organic fractions or modified during the composting process, protecting the PAHs from further degradation. In contrast, the PAHs in the non-composted PMR amendments were not sorbed or modified, making them more sensitive to volatilization or rapid biodegradation by a broad array of soil microorganisms under a variety of environmental conditions [10,11,64]. This hypothesis predicts that the loss of foliar disease suppression in soils amended with composted PMR was not due to the loss of substrate responsible for growth or persistence of any particular microorganism, *per se*, but due to the depletion of PAHs. Once the PAHs were degraded, no secondary intermediates of PAH degradation, such as salicylic acid, would be produced and foliar disease suppression would be lost, but the PAH degrading microbial population could potentially remain unchanged. It is well documented that plant defenses can be induced with the application of salicylic acid or other chemical elicitors of SAR to plant roots directly or as a soil drench [16,30,36,48,63,66]. In addition, some aromatic compounds, including salicylic acid, decompose when heated [8], another potential explanation for why foliar disease suppression was lost when the amendment was autoclaved (Fig. 4).

In summary, we have presented data demonstrating the suppression of two plant foliar diseases caused by *P. syringae* pv. *tomato* in soils amended with composts derived from PMR. These results corroborate similar findings from field experiments [62]. In *Arabidopsis*, foliar disease suppression was associated with the potentiation of plant defenses prior to pathogen inoculation, disrupted by plants expressing an *npr1* mutation or a *NahG* transgene, and destroyed upon autoclaving. Our data suggest that PMR-mediated foliar disease suppression is an induced plant defense response that shares molecular features most similar to SAR. Since the eliciting factor(s) responsible for this phenomenon is yet to be identified, it is important for future research to focus on chemical factors, in addition to microbial factors, that correspond to these disease suppression phenomena.

Acknowledgements

We thank Alex Stone and Dorith Rotenberg for their in-depth discussions about research findings; Andrew Bent, Craig Grau and Jo Handelsman for their guidance during the preparation of this manuscript; Andy Gilbert of Stora Enso, North America for supplying our project with PMR; and Chris Upper and Susan Hirano for *P. syringae* pv. *tomato* strains DC3000 and SM78. This research was funded by the University of Wisconsin CALS Research Division (Hatch Funds), the Wisconsin Potato and Vegetable Growers'

Association and the Wisconsin PURR (Pesticide Use Risk Reduction) Program. Seeds of wild-type and mutant *Arabidopsis* lines were obtained from the *Arabidopsis* Seed Stock Center (Columbus, OH, USA). Seed of the *NahG Arabidopsis* line and BTH were gifts from Syngenta (formerly Novartis, Research Triangle Park, NC, USA). Tomato seeds were obtained from the Tomato Genomics Resource Center (University of California, Davis, CA, USA).

References

[1] Beauchamp CJ, Charest M-H, Gosselin A. Examination of environmental quality of raw and composting de-inking paper sludge. Chemosphere 2002;46:887–95.

[2] Beck AJ, Johnson DL, Jones KC. The form and bioavailability of non-ionic organic chemicals in sewage sludge-amended agricultural soils. The Science of Total Environment 1996;185:125–49.

[3] Bellamy KL, Chong C, Cline RA. Paper sludge utilization in agriculture and container nursery culture. Journal of Environmental Quality 1995;24:1074–82.

[4] Berger S, Bell E, Sadka A, Mullet JE. *Arabidopsis thaliana* Atvsp is homologous to soybean VspA and VspB, genes encoding vegetative storage protein acid phosphatases, and is regulated similarly by methyl jasmonate, wounding, sugars, light and phosphate. Plant Molecular Biology 1995;27:933–42.

[5] Brady NC, Weil RR. The nature and properties of soils. Upper Saddle River, NJ: Prentice Hall; 2000.

[6] Boehm MJ, Hoitink HAJ. Sustenance of microbial activity in potting mixes and its impact on severity of Pythium root rot of pointsettia. Phytopathology 1992;82:259–64.

[7] Boehm MJ, Madden LV, Hoitink HAJ. Effect of organic matter decomposition level on bacterial species diversity and composition in relationship to Pythium damping-off severity. Applied and Environmental Microbiology 1993;59:4171–9.

[8] Budavari S, O'Neil MJ, Smith A, Heckelman PE. The Merck index: an encyclopedia of chemicals, drugs, and biologicals, 11th ed. Rahway, NJ: Merck and Co; 1989. pp. 1324.

[9] Cao H, Bowling SA, Gordon AS, Xinnian D. Characterization of an *Arabidopsis* mutant that is nonresponsive to inducers of systemic acquired resistance. The Plant Cell 1994;6:1583–92.

[10] Cerniglia CE. Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation 1992;3:351–68.

[11] Chang BV, Shiung LC, Yuan SY. Anaerobic biodegradation of polycyclic aromatic hydrocarbon in soil. Chemosphere 2002;48: 717–24.

[12] Chantigny MH, Angers DA, Beauchamp CJ. Decomposition of de-inking paper sludge in agricultural soils as characterized by carbohydrate analysis. Soil Biology & Biochemistry 2000;32: 1561–70.

[13] Conrath U, Thulke OU, Katz VA, Schwindling S, Kohler A. Priming as a mechanism in induced systemic resistance of plants. European Journal of Plant Pathology 2001;107:113–9.

[14] Daft GC, Poole HA, Hoitink HAJ. Composted hardwood bark: a substitute for steam sterilization and fungicide drenches for control of poinsettia crown and root rot. HortScience 1979;14:482–530.

[15] De Brito Alvarez MA, Gagne S, Antoun H. Effect of compost on rhizosphere microflora of the tomato and on the incidence of plant growth-promoting rhizobacteria. Applied and Environmental Microbiology 1995;61:194–9.

[16] De Meyer G, Höfte M. Salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 induces resistance to leaf infection by *Botrytis cinerea* on bean. Phytopathology 1997;87:588–93.

[17] Foley BJ, Cooperband LR. Paper mill residuals and compost effects on soil physical properties in an irrigated vegetable rotation. Journal of Environmental Quality 2002;31:2086–95.

[18] Gaffney T, Friedrich L, Vernooij B, Negrotto D, Nye G, Uknes S, Ward E, Kessmann H, Ryals J. Requirement of salicylic acid for the induction of systemic acquired resistance. Science 1993;261:754–6.

[19] Geraats BPJ, Bakker PAHM, van Loon LC. Ethylene insensitivity impairs resistance to soilborne pathogens in tobacco and *Arabidopsis thaliana*. Molecular Plant–Microbe Interactions 2002;15:1078–85.

[20] Guzmán P, Ecker JR. Exploiting the triple response of *Arabidopsis* to identify ethylene-related mutants. The Plant Cell 1990;2:513–23.

[21] Hoagland DR, Arnon DI. The water culture method for growing plants without soil. California Agricultural Experiment Station Bulletin 1938;347:36–9.

[22] Hoffland E, Jeger MJ, van Beusichem ML. Effect of nitrogen supply rate on disease resistance in tomato depends on the pathogen. Plant and Soil 2000;218:239–47.

[23] Hoffland E, Pieterse CMJ, Bik L, van Pelt JA. Induced systemic resistance in radish is not associated with accumulation of pathogenesis-related proteins. Physiological and Molecular Plant Pathology 1995;46:309–20.

[24] Hoitink HAJ, Boehm MJ. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. Annual Review of Phytopathology 1999;37:427–46.

[25] Hoitink HAJ, Van Doren Jr. DM, Schmitthenner AF. Suppression of *Phytophthora cinnamomi* in a composted hardwood bark potting medium. Phytopathology 1977;67:561–5.

[26] Kästner M, Steibich S, Beyrer M, Richnow HH, Fritsche W. Formation of bound residues during microbial degradation of [^{14}C]anthracene in soil. Applied and Envrionmental Microbiology 1999;65:1834–42.

[27] Kauss H, Jeblick W. Pretreatment of parsley suspension cultures with salicylic acid enhances spontaneous and elicited production of H_2O_2. Plant Physiology 1995;108:1171–8.

[28] Kauss H, Theisinger-Hinkel E, Mindermann R, Conrath U. Dichloroisonicotinic and salicylic acid, inducers of systemic acquired resistance, enhance fungal elicitor responses in parsley cells. The Plant Journal 1992;2:655–60.

[29] Kauss H, Krause K, Jeblick W. Methyl jasmonate conditions parsley suspension cells for increased elicitation of phenylpropanoid defense responses. Biochemical and Biophysical Research Communications 1992;189:304–8.

[30] Kempster NK, Davies KA, Scott ES. Chemical and biological induction of resistance to the clover cyst nematode (*Heterodera trifolii*) in white clover (*Trifolium repens*). Nematology 2001;3:35–43.

[31] King EO, Ward MK, Raney DE. Two simple media for the demonstration of phycocyanin and fluorescin. Journal of Laboratory and Clinical Medicine 1954;44:301–7.

[32] Knoester M, van Loon LC, van den Heuvel J, Hennig J, Bol JF, Linthorst HJM. Ethylene-insensitive tobacco lacks nonhost resistance against soil-borne fungi. Proceedings of the National Academy of Science, USA 1998;95:1933–7.

[33] Kohler A, Schwindling S, Conrath U. Benzothiadiazole-induced priming for potentiated responses to pathogen infection, wounding, and infiltration of water into leaves requires the NPR1/NIM1 gene in *Arabidopsis*. Plant Physiology 2002;128:1046–56.

[34] Krause MS, DeCeuster TJJ, Han DY, Musselman CA, Hoitink HAJ. Systemic acquired resistance induced by composts: a highly specific phenomenon. Phytopathology 1998;88:S49.

[35] Lawton K, Weymann K, Friedrich L, Vernooij B, Uknes S, Ryals J. Systemic acquired resistance in *Arabidopsis* requires salicylic acid but no ethylene. Molecular Plant–Microbe Interactions 1995;6: 863–70.

[36] Leeman M, den Ouden JA, van Pelt JA, Dirkx FPM, Steijl H, Bakker PAHM, Schippers B. Iron availability affects induction of systemic resistance to Fusarium wilt of radish by *Pseudomonas fluorescens*. Phytopathology 1996;86:149–55.

[37] Leeman M, Van Pelt JA, Hendrickx MJ, Scheffer RJ, Bakker PAHM. Biocontrol of Fusarium wilt of radish in commercial greenhouse trials by seed treatment with *Pseudomonas fluorescens* WCS374. Phytopathology 1995;85:1301–5.
[38] Lumsden RD, Lewis JA, Millner PD. Effect of composted sewage sludge on several soilborne pathogens and diseases. Phytopathology 1983;73:1543–8.
[39] Maurhofer M, Hase C, Meuwly P, Metraux JP, Defago G. Induction of systemic resistance of tobacco to tobacco necrosis virus by the root-colonizing *Pseudomonas fluorescens* strain CHA0: Influence of the gacA gene and of pyoverdine production. Phytopathology 1994;84: 139–46.
[40] Millner PD, Lumsden RD, Lewis JA. Controlling plant disease with sludge compost. Biocycle 1982;23:50–2.
[41] Mur LA, Brown IR, Darby RM, Bestwick CS, Bi Y-M, Mansfield JW, Draper J. A loss of resistance to avirulent bacterial pathogens in tobacco is associated with the attenuation of a salicylic acid-potentiated oxidative burst. The Plant Journal 2000;23:609–21.
[42] Mur LA, Naylor G, Warner SAJ, Sugars JM, White RF, Draper J. Salicylic acid potentiates defense gene expression in tissue exhibiting acquired resistance to pathogen attack. The Plant Journal 1996;9: 559–71.
[43] Nelson EB, Hoitink HAJ. Factors affecting suppression of *Rhizoctonia solani* in container media. Phytopathology 1982;72:275–9.
[44] Papavizas GC, Lewis JA. Effect of amendments and fungicides on *Aphanomyces* root rot of peas. Phytopathology 1971;61:215–20.
[45] Penninckx IAMA, Eggermont K, Terras FRG, Thomma BPHJ, De Samblanx GW, Buchala A, Métraux J-P, Manners JM, Broekaert WF. Pathogen-induced systemic activation of a plant defensin gene in *Arabidopsis* follows a salicylic acid-independent pathway. The Plant Cell 1996;8:2309–23.
[46] Pharand B, Carisse O, Benhamou N. Cytological aspects of compost-mediated induced resistance against Fusarium crown and root rot in tomato. Phytopathology 2002;92:424–38.
[47] Pieterse CMJ, van Wees SCM, van Pelt JA, Knoester M, Laan R, Gerrits H, Weisbeek PJ, van Loon LC. A novel signaling pathway controls induced systemic resistance in *Arabidopsis*. The Plant Cell 1998;10:1571–80.
[48] Pieterse CMJ, van Wees SCM, Hoffland E, van Pelt JA, van Loon LC. Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. The Plant Cell 1996;8:1225–37.
[49] Potter S, Uknes S, Lawton K, Winter AM, Chandler D, DiMaio J, Novitzky R, Ward E, Ryals J. Regulation of a hevein-like gene in *Arabidopsis*. Molecular Plant–Microbe Interactions 1993;6:680–5.
[50] Ressler BP, Kneifel H, Winter J. Bioavailability of polycyclic aromatic hydrocarbons and formation of humic acid-like residues during bacterial PAH degradation. Applied Microbiological Biotechnology 1999;53:85–91.
[51] Ross AF. Localized acquired resistance to plant virus infection in hypersensitive hosts. Virology 1961;14:329–39.
[52] Ross AF. Systemic acquired resistance induced by localized virus infections in plants. Virology 1961;14:340–58.
[53] Shiomi Y, Nishiyama M, Onizuka T, Marumoto T. Comparison of bacterial community structures in the rhizoplane of tomato plants grown in soils suppressive and conducive towards bacterial wilt. Applied and Environmental Microbiology 1999;65:3996–4001.
[54] Shirasu K, Nakajima H, Rajasekhar VK, Dixon RA, Lamb C. Salicylic acid potentiates an agonist-dependent gain control that amplifies pathogen signals in the activation of defense mechanisms. The Plant Cell 1997;9:261–70.
[55] Spencer S, Benson DM. Pinebark, hardwood bark compost, and peat amendment effects on development of *Phytophthora* spp. and lupine root rot. Phytopathology 1982;72:346–51.
[56] Spencer S, Benson DM. Root rot of *Aucuba japonica* caused by *Phytophthora cinnamomi* and *P. citricola* and suppressed with bark media. Plant Disease 1981;65:918–21.
[57] Spring DE, Ellis MA, Spotts RA, Hoitink HAJ, Schmitthenner AF. Suppression of the apple collar rot pathogen in composted hardwood bark. Phytopathology 1980;70:1209–12.
[58] Staswick PE, Yuen GY, Lehman CC. Jasmonate signaling mutants of *Arabidopsis* are susceptible to the soil fungus *Pythium irregulare*. The Plant Journal 1998;15:747–54.
[59] Staswick PE, Su W, Howell SH. Methyl jasmonate inhibition of root growth and inductions of a leaf protein are decreased in an *Arabidopsis thaliana* mutant. Proceedings of the National Academy of Science, USA 1992;89:6837–40.
[60] Stephens CT, Herr LJ, Hoitink HAJ, Schmitthenner AF. Suppression of Rhizoctonia damping-off by composted hardwood bark medium. Plant Disease 1981;65:796–7.
[61] Sticher L, Mauch-Mani B, Métraux JP. Systemic acquired resistance. Annual Review of Phytopathology 1997;35:235–70.
[62] Stone AG, Vallad GE, Cooperband LR, Rotenberg D, Darby HR, James RV, Stevenson W, Goodman RM. The effect of organic amendments on soil-borne and foliar diseases in field-grown snap bean and cucumber. Plant Disease 2003;87:1037–42.
[63] Sundar AM, Velazhahan R, Viswanathan R, Padmanaban P, Vidhyasekaran P. Induction of systemic resistance to *Colletotrichum falcatum* in sugarcane by a synthetic signal molecule, acibenzolar-*S*-methyl (CGA-245704). Phytoparasitica 2001;29:231–42.
[64] Sutherland JB. Detoxification of polycyclic aromatic hydrocarbons by fungi. Journal of Industrial Microbiology 1992;9:53–62.
[65] Uknes S, Mauch-Mani B, Moyer M, Potter S, Williams S, Dincher S, Chandler D, Slusarenko A, Ward E, Ryals J. Acquired resistance in *Arabidopsis*. The Plant Cell 1992;4:645–56.
[66] van Loon LC, Antoniw JF. Comparison of the effects of salicylic acid and ethephon with virus-induced hypersensitivity and acquired resistance in tobacco. Netherlands Journal of Plant Pathology 1982; 88:237–56.
[67] van Loon LC, Bakker PAHM, Pieterse CMJ. Systemic acquired resistance induced by rhizosphere bacteria. Annual Review of Phytopathology 1998;36:453–83.
[68] van Wees SCM, Pieterse CMJ, Trijssenaar A, van Westende YAM, Hartog F, van Loon LC. Differential induction of systemic resistance in *Arabidopsis* by biocontrol bacteria. Molecular Plant–Microbe Interactions 1997;10:716–24.
[69] Vernooij B, Friedrich L, Morse A, Reist R, Kolkitz-Jawhar R, Ward E, Uknes S, Kessmann H, Ryals J. Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. The Plant Cell 1994;6:959–65.
[70] Vijayan P, Shockey J, Lévesque CA, Cook RJ, Browse J. A role for jasmonate in pathogen defense of *Arabidopsis*. Proceedings of the National Academy of Science, USA 1998;95:7209–14.
[71] Ward ER, Uknes SJ, Williams SC, Dincher SS, Wiederhold DL, Alexander DC, Ahl-Goy P, Métraux J-P, Ryals JA. Coordinate gene activity in response to agents that induce systemic acquired resistance. The Plant Cell 1991;3:1085–94.
[72] Xu Y, Chang Pi FL, Liu D, Narasimhan ML, Raghothama KG, Hasegawa PM, Bressan RA. Plant defense genes are synergistically induced by ethylene and methyl jasmonate. The Plant Cell 1994;6: 1077–85.
[73] Zhang W, Han DY, Dick WA, Davis KR, Hoitink HAJ. Compost and compost water extract-induced systemic acquired resistance in cucumber and *Arabidopsis*. Phytopathology 1998;88:450–5.
[74] Zhang W, Dick WA, Hoitink HAJ. Compost-induced systemic acquired resistance in cucumber Pythium root rot and anthracnose. Phytopathology 1996;86:1066–70.
[75] Zimmerli L, Jakab G, Métraux J-P, Mauch-Mani B. Potentiation of pathogen-specific defense mechanisms in *Arabidopsis* by β-amino-butyric acid. Proceedings of the National Academy of Science, USA 2000;97:12920–5.