Induction of Plant Defense Gene Expression by Plant Activators and *Pseudomonas syringae* pv. *tomato* in Greenhouse-Grown Tomatoes

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ABSTRACT

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Plant activators provide an appealing management option for bacterial diseases of greenhouse-grown tomatoes. Two types of plant activators, one that induces systemic acquired resistance (SAR) and a second that activates induced systemic resistance (ISR), were evaluated for control of *Pseudomonas syringae* pv. *tomato* and effect on plant defense gene activation. Benzothiadiazole (BTH, SAR-inducing compound) effectively reduced bacterial speck incidence and severity, both alone and in combi-

nation with the ISR-inducing product. Application of BTH also led to elevated activation of salicylic acid and ethylene-mediated responses, based on real-time polymerase chain reaction analysis of marker gene expression levels. In contrast, the ISR-inducing product (made up of plant growth-promoting rhizobacteria) inconsistently modified defense gene expression and did not provide disease control to the same level as did BTH. No antagonism was observed by combining the two activators as control of bacterial speck was similar to or better than BTH alone.

Additional keywords: *Bacillus* spp., induced defense responses, *Solanum lycopersicum*.

Plant activators induce plant defense responses known as systemic acquired resistance (SAR) or induced systemic resistance (ISR) (37,41). Commercially available compounds that induce SAR (such as benzothiadiazole) and ISR-inducing plant growth-promoting rhizobacteria (PGPR) have been shown to be effective for disease control in tomato (2,10,18,19,23,27,32). However, few studies examine utilization of these products together as an integrated control strategy or have investigated their combined impact on plant defense responses (2,12,16,27,32).

Marker genes can be used to better understand how these products activate plant defense responses. Induction of salicylic acid (SA)-mediated responses or SAR can be followed using the expression level of the acidic *PR-1* gene as a marker (5,40,42). This gene is activated by compounds such as benzothiadiazole (BTH) and in response to abiotic and biotic stress (11,14). Ethylene (ET) is thought to enhance and stimulate defense responses; activation of these responses can be followed using the basic *PR-1* marker gene (5,40,42,45). ET-mediated responses may be involved in PGPR-plant interactions due to the role of ET-regulated pathways in expression of ISR (44). The expression level of a proteinase inhibitor gene, *Pin2*, can be utilized to measure induction of wound-inducible jasmonic acid (JA) signaling (7,13,30). JA-mediated responses are activated when a plant is wounded, such as by insect herbivory or pathogen lesion formation, and are also thought to be involved in ISR (6,19,31,39).

Plant defense responses are interconnected and both antagonism and synergy between these phytohormone signaling networks is known (6,22). Activation of ISR and SAR simultaneously in a laboratory setting has been shown to provide additive disease control in the *P. syringae* pv. *tomato*-*Arabidopsis* pathosystem

(46). Utilizing this same pathosystem, Ramos Solano et al. (32) also found disease control with additive effects of the SA and ET pathways, depending on which type of PGPR was used. One PGPR strain, *Chryseobacterium balustinum*, induced both the SAR and ISR pathways by initially activating the SA-dependent pathway (SAR), followed by stimulation of the SA-independent pathway (ISR) (32).

In New York State, tomato production is predominantly for fresh market. Tomato seedlings are grown in the greenhouse until 5 to 7 weeks of age and then transplanted into the field (33). Large numbers of plants in a relatively small, confined space with overhead irrigation facilitates rapid spread of bacterial diseases through a greenhouse. *Pseudomonas syringae* pv. *tomato* is the causal agent of bacterial speck disease of tomato (17,24,29). This persistent disease can be economically important under conducive conditions such as cool temperatures (18 to 24°C) and high relative humidity (15,17). Symptomatic plants typically exhibit small, necrotic lesions surrounded by chlorotic halos on foliage and fruit. While this disease does not often kill the plant, symptoms on fruit can decrease marketability. The identification of control strategies that could effectively control bacterial disease in the greenhouse could aid transplant production.

The goal of this study was to determine whether the SAR plant activator (BTH) and the ISR plant activator (*Bacillus* spp.), utilized alone or in combination, would effectively control *P. syringae* pv. *tomato* in greenhouse-grown tomato transplants. Additionally, SA-, JA-, and ET-mediated defenses were followed in response to activators and pathogen inoculation and assessed using quantitative real-time polymerase chain reaction (PCR).

MATERIALS AND METHODS

Plant materials and treatments. Tomato (*Solanum lycopersicon*) cv. Sunchief VF was used for all experiments. The greenhouse used for these experiments was maintained at 23 to 26°C

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(day) and 20 to 22°C (night) with 15 h of natural light and approximately 40% relative humidity. Tomato seeds were sown in 128 cell polystyrene flats in the greenhouse in Cornell mix (a soilless peat mixture), perlite, and vermiculite (4:1:1). Tomatoes were divided into four treatments: untreated control, plants grown in the presence of PGPR, BTH applied to foliage, and the combination of plants grown in the presence of PGPR and foliage treated with BTH. The PGPR treatment, BioYield Concentrate (Bayer CropScience, Research Triangle Park, NC), was mixed evenly into the potting mix at planting (1.2 kg/m³). This PGPR product contains 4.1×10^4 endospores of two bacterial strains, *Bacillus subtilis* GB03 and *B. amyloliquefaciens* IN937a, per cm³. After 4 weeks, all tomatoes were transferred to 4-inch pots. Six- to seven-week-old plants were used for this study. BTH (Actigard 50 WG, Syngenta Crop Protection, Greensboro, NC) was applied at the highest recommended rate (52 g/ha) 4 days prior to pathogen inoculation.

Pathogen inoculation and disease rating. The experiment was arranged in a randomized complete block design, with three plots of each of the four treatments at each of the four tissue collection times (tissue collection described below) per experiment. Each plot consisted of three potted tomato plants; thus, with three replicates per treatment, there were a total of nine plants per treatment and collection time per experiment. From each plot of three plants, one was used for disease rating, while tissue was collected from the other two to test for defense response gene expression (described below). Each experiment consisted of 144 plants (3 plants per plot $\times$ 3 replicates $\times$ 4 collection time points $\times$ 4 treatments) and the entire experiment was conducted three times.

Isolate A9 of *P. syringae* pv. *tomato* was cultured in 100 ml of nutrient broth (35) with shaking at 180 rpm overnight at 28°C. This isolate was collected from a diseased tomato plant in the field in 2003 and stored at -80°C. Five milliliters of this inoculum was transferred to each of 15 flasks containing 500 ml of nutrient broth and cultured overnight as above. Prior to inoculation, bacteria were spun down at 20,000 $\times$ g for 15 min and rinsed with 10 mM MgCl₂. Bacteria were resuspended in 10 mM MgCl₂ containing 200 μ l/liter of the surfactant Silwet L77 (Helena Chemical, Collierville, TN) to a concentration of 10⁸ CFU/ml and applied to runoff with a pump sprayer. All plants (untreated control, PGPR, BTH, and BTH+PGPR) were inoculated.

One plant from each treatment plot was rated for disease incidence and disease severity 7 days after inoculation. The number of diseased leaflets and the total number of leaflets per plant were recorded for disease incidence. To determine severity, all leaflets from each plant were placed into one of six rating categories: 0, 1 to 10, 11 to 25, 26 to 50, 51 to 100, and >100 lesions per leaflet.

Analyses of disease ratings. Bacterial speck incidence, measured by the proportion of leaflets per plant containing bacterial lesions, was analyzed using quasi-likelihood models (25). Means were compared using a chi-square test at $P = 0.05$. The control efficacy of the plant activators, represented by percent disease control of bacterial speck relative to the untreated control was calculated as described by Baider and Cohen (3). Specifically, percent control was calculated using the following formula: $1 - x/y \times 100$, where x = mean bacterial speck incidence in treated plants and y = mean bacterial speck incidence in control plants. Synergism between the two plant activators was assessed using the Abbott method and the Gowing formula (20). The Gowing formula is as follows: expected percent control = $(a + b - a \times b) \times 100$, where a and b = mean bacterial speck incidence of acibenzolar-*S*-methyl- and PGPR-treated plants, respectively. An ordinal proportional odds logistic regression was used to model the relationship between treatment and bacterial speck disease severity (36). The odds of a treatment resulting in less disease severity than the untreated control (having fewer numbers of leaflets in higher disease severity categories) was

determined with a 95% confidence interval for each of the three experiments.

Tissue collection and RNA extraction and purification. All tomato leaflets above the three oldest leaves were collected from two plants per plot at four time points (-12, 12, 36, and 60 h relative to pathogen inoculation). Leaf tissue from both plants was combined in a single bag, flash frozen in liquid nitrogen immediately after collection, and stored at -80°C. RNA was extracted from 1 g of tissue with the SV Total RNA Isolation System (Promega Corporation, Madison, WI) and further DNase treated with Turbo DNA-free (Ambion Inc., Austin, TX). Samples were visualized by electrophoresis on a 1.2% agarose formaldehyde gel.

Analysis of gene expression using quantitative real-time PCR. Two-step real-time PCR was performed using the iCycler iQ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA) and utilized fluorogenic probe technology. Two micrograms of total RNA was used to generate cDNA with the iScript cDNA synthesis kit (Bio-Rad Laboratories) according to the manufacturer's instructions. Controls lacking reverse transcriptase were included to check for DNA contamination. Gene expression was quantified from each cDNA sample using four tomato genes, actin (a housekeeping gene), acidic *PR-1*, basic *PR-1*, and *Pin2*. Real-time PCR primers and probes for tomato acidic and basic *PR-1* were identical to those described by Block et al. (5). Tomato actin and *Pin2* primers and probes were designed using Primer Express 2.0 software (Applied Biosystems, Foster City, CA) and were as follows: actin probe 5'-/6-FAM/C-GTTTGGATCTTGCTGGTCGTGATTAACT/TAMRA/-3'; actin forward primer 5'-TTGCCGCATGCCATTCT-3'; actin reverse primer 5'-TCGGTGAGGATATTCATCAGGTT-3'; *Pin2* probe 5'-/6-FAM/TGTGGAATCTTGGGTTCTGGGATATGCC/TAMRA/-3'; *Pin2* forward primer 5'-TGATGCCAAGGCTTGACTAGAGA-3'; *Pin2* reverse primer 5'-AGCGGACTTCCTTCTGACGT-3' (Integrated DNA Technologies, Coralville, IA). Both probes were labeled with 6-carboxyfluorescein (FAM) at the 5' end and TAMARA quencher dye at the 3' end.

Real-time quantitative PCR reactions were carried out in triplicate; each reaction utilized the iQ Supermix Kit (Bio-Rad Laboratories) and consisted of 1 $\times$ Mastermix, forward and reverse primers (300 nM final concentration) and 200 nM fluorogenic probe. Reaction parameters consisted of 95°C for 4 min, followed by 45 cycles of 95°C for 10 s, 50°C for 1 min, and 72°C for 30 s. In addition to cDNA reaction samples lacking reverse transcriptase, reactions with no cDNA template were also included as negative controls.

Initial transcript levels were determined using the standard curve method (User Bulletin 2, ABI PRISM 7700 Sequence Detection System, Relative quantification of gene expression, 2001, Applied Biosystems). Serial dilutions of tomato total genomic DNA were used to generate standard curves (47). Standard curve construction was accomplished by plotting the threshold cycle (Ct) against the logarithm of the known tomato DNA dilutions. The absolute quantity of the product in each sample was calculated from these curves (9,26). Subsequently, tomato actin (used as an internal control) was utilized to normalize tomato defense gene expression and generate relative expression values (REVs) (4). Relative expression change was calculated by calibrating samples to the mean REV of the three replicates (within each experiment) prior to pathogen inoculation (-12 h time point) for each of the four treatments. The log of the REVs for each of the three marker genes tested was used to determine statistical significance. Analysis of variance using the Proc Mixed procedure and SAS software version 9.1 (SAS Institute, Cary, NC) was used to analyze these values (26). Differences between treatments were evaluated using orthogonal contrasts at each time point and P values of <0.05 were considered statistically significant (26). Data are presented as fold change relative to a calibrator sample, which

was untreated control plants at the -12 h time point (prior to pathogen inoculation).

RESULTS

Bacterial speck incidence. Incidence of *P. syringae* pv. *tomato*, as measured by the mean proportion of infected leaflets per plant, followed a similar pattern in all three experiments ($P = 0.30$, Table 1). Incidence was not significantly different between the untreated control and PGPR-treated plants ($P = 0.119$, 0.9177 , and 0.12 , respectively). Both BTH ($P < 0.0001$, $P = 0.0244$, and $P < 0.0001$, respectively) and BTH in combination with the PGPR product ($P < 0.0001$, $P = 0.0003$, and $P < 0.0001$) significantly reduced bacterial speck incidence in all three experimental replicates. Synergy between BTH and the PGPR product was observed in one of the three experiments (Table 1).

Bacterial speck severity. In the three experiments, disease severity in all treatments differed significantly from the untreated control ($P < 0.0001$, Table 2). The average number of leaflets per plant in each disease severity category is depicted by treatment and experiment in Table 2. Plants grown in the presence of the PGPR product were less severely infected than the control in two of the three experiments ($P < 0.05$). Plants that received BTH treatment consistently exhibited less severe symptoms than untreated control and PGPR-treated plants ($P < 0.05$). In one experiment, BTH+PGPR-treated plants were less severely infected than those treated with BTH alone.

Effect of treatment on marker gene activation. Level of defense gene expression was effectively quantified for each of the three defense response marker genes (relative to actin) via quantitative real-time PCR for each of the four treatments. Complete results for each of the three experiments are divided by marker gene (Figs. 1 to 3) and are presented by treatment (untreated control, PGPR-treated, BTH-treated, and BTH+PGPR-treated) below.

Acidic *PR-1* transcript levels. In all three experiments, the untreated control demonstrated low levels of acidic *PR-1* expression prior to pathogen inoculation (Fig. 1, white bars). Over the course of the experiment, activation of SA-mediated responses increased gradually to highest levels at 60 h after inoculation (16.9-, 10.2-, and 4.9-fold increase from -12 h, $P = 0.0005$, $P < 0.0001$, and $P < 0.0001$).

PGPR-treated plants had similar levels of acidic *PR-1* expression to the untreated control prior to pathogen inoculation in all three experiments (Fig. 1, striped bars). Induction of SA-mediated responses increased to highest expression levels at 60 (Fig. 1A and C) and 36 (Fig. 1B) hours postinfection (6.3-, 4.8-, and 8.8-fold increase from -12 h, $P = 0.0005$, $P < 0.0001$, and $P = 0.0002$). Peak expression relative to the untreated control varied by experiment; no significant differences were observed in experiment 1. PGPR-treated plants had repressed acidic *PR-1* activation compared with the untreated control in experiment two and significantly greater expression in experiment three.

Treatment with BTH 3 days prior to pathogen inoculation activated acidic *PR-1* at the -12 h time point to levels 3- to 13-fold higher than the untreated control (Fig. 1, gray bars). Levels were elevated throughout the course of the experiment, increasing after inoculation to highest levels at the 36 (Fig. 1B and C) or 60 h time points (Fig. 1A). By 60 h, acidic *PR-1* transcript abundance in BTH-treated plants was not significantly different from untreated control plants.

As seen with BTH treatment alone, the combination of plant activators induced acidic *PR-1* activation 3- to 7-fold higher than the untreated control prior to inoculation (Fig. 1, black bars). Transcript abundance increased over the course of the experiment to peak levels at the 36 (Fig. 1B and C) or 60 h time points (Fig. 1A). As observed with BTH treatment, acidic *PR-1* expression did not differ significantly from untreated control at the 60 h time point.

Basic *PR-1* transcript levels. Basic *PR-1* expression followed a similar pattern in untreated control plants as acidic *PR-1*, with

TABLE 1. Disease incidence of *Pseudomonas syringae* pv. *tomato* in greenhouse-grown tomatoes over three experiments

Experiment	Untreated control ^v	Plant growth-promoting rhizobacteria (PGPR)		Benzothiadiazole (BTH)		BTH + PGPR		Expected percent control ^y
	Proportion ^w	Proportion	Percent control ^x	Proportion	Percent control	Proportion	Percent control	
1	0.53 ± 0.04 a ^z	0.44 ± 0.04 a	17	0.18 ± 0.03 b	66	0.15 ± 0.03 b	72	72
2	0.42 ± 0.05 a	0.42 ± 0.05 a	0	0.26 ± 0.05 b	38	0.16 ± 0.04 b	62	38
3	0.46 ± 0.04 a	0.37 ± 0.04 a	20	0.15 ± 0.03 b	67	0.15 ± 0.03 b	67	74
Average			12		57		67	

^v Numbers of infected and total leaflets per plant were recorded from 12 plants per treatment, 7 days after pathogen inoculation. The proportion of infected leaflets per plant was analyzed using quasi-likelihood models and means were compared using a chi-square test at $P = 0.05$.

^w Mean proportion of infected leaflets per plant averaged for three replicates ± standard error.

^x Percent control efficacy of each treatment relative to the untreated control. Percent control was calculated using the following formula: $1 - x/y \times 100$, where x = mean bacterial speck incidence in treated plants and y = mean bacterial speck incidence in control plants.

^y Numbers in this column are used to determine whether there is a synergistic interaction when BTH and PGPR products are used together. Expected percent control was calculated as follows: $(a + b - a \times b) \times 100$, where a , b = mean bacterial speck incidence of acibenzolar-S-methyl- and PGPR-treated plants, respectively. If the percent control is greater than the expected control, a synergistic interaction between the two products has occurred.

^z Means within a row sharing the same letter are not statistically different.

TABLE 2. Disease severity of *Pseudomonas syringae* pv. *tomato* over three greenhouse experiments

Severity ^y	Untreated control			Plant growth-promoting rhizobacteria (PGPR)			Benzothiadiazole (BTH)			BTH + PGPR		
	1 ^z	2	3	1	2	3	1	2	3	1	2	3
0	15	32	29	18	30	32	26	41	48	28	49	48
1 to 10	11	20	19	12	19	16	5	12	8	5	9	8
11 to 25	3	2	4	2	1	3	1	2	0	0	0	1
26 to 50	2	1	1	1	1	0	0	0	0	0	0	0
51 to 100	1	0	0	0	0	0	0	0	0	0	0	0
>100	0	0	0	0	0	0	0	0	0	0	0	0

^y Disease severity classes signifying the number of lesions per leaflet. Leaflets from 12 plants per treatment were placed into one of six severity classes 7 days following inoculation: 0, 1 to 10, 11 to 25, 26 to 50, 51 to 100, and >100 lesions per leaflet.

^z The mean number of leaflets per disease rating category for each experiment (1, 2, and 3) is presented.

low expression levels prior to pathogen inoculation, increasing to highest levels 60 h after inoculation (Fig. 2, white bars). By 60 h, basic *PR-1* expression was significantly higher than levels observed prior to inoculations in each of the three experiments (8.9-, 5.8-, and 5.4-fold increase from -12 h, $P = 0.0005$, $P = 0.0001$, and $P < 0.0001$).

In PGPR-treated plants, activation of ET-mediated responses was similar to acidic *PR-1*, with low expression levels prior to inoculation and increasing to highest levels 36 (Fig. 2B, striped bars) or 60 h (Fig. 2A and C) after inoculation. At peak levels, basic *PR-1* expression was significantly higher than levels observed prior to inoculations in each of the three experiments (4.4-, 4.2-, and 4.9-fold increase from -12 h, $P = 0.0155$, $P < 0.0001$, and $P < 0.0009$). In two experiments, PGPR-treated plants exhibited significantly lower activation of ET-mediated responses (Fig. 2A and B) compared with the untreated control, while no significant differences were found in the third experiment (Fig. 2C).

BTH treatment also induced ET-mediated responses prior to inoculation to levels 3- to 7-fold higher than the untreated control (Fig. 2, gray bars). Peak activation followed a similar pattern to acidic *PR-1* expression in all three experiments, with levels increasing after inoculation to highest levels at 36 (Fig. 2B and C) or 60 h (Fig. 2A) postinoculation. By 60 h, basic *PR-1* expression was not significantly different from the untreated control in two experiments (Fig. 2A and B) and repressed in one (Fig. 2C).

ET-mediated responses were also induced in plants treated with BTH and PGPR prior to inoculation, as expression of the basic *PR-1* gene was 4- to 7.5-fold higher than the untreated control at -12 h (Fig. 2, black bars). A similar pattern to acidic *PR-1* expression was observed following inoculation, with levels increasing to highest levels at 36 (Fig. 2C) or 60 h (Fig. 2A and B). One difference from acidic *PR-1* expression was observed in experiment 2, where peak activation of ET-mediated responses occurred 1 day later, at the 60 h time point (Figs. 1B and 2B). Expression levels were not significantly different from the untreated control

Fig. 1. Expression pattern of tomato gene acidic *PR-1* following inoculation with *Pseudomonas syringae* pv. *tomato*. All treatments were inoculated at time 0. A, Experiment 1; B, experiment 2; and C, experiment 3. Solid white bars represent relative fold change of three replicated plots of untreated control plants, striped bars represent plant growth-promoting rhizobacteria (PGPR)-treated plants, gray bars indicate benzothiadiazole (BTH)-treated plants, and black bars correspond to plants treated with both BTH and PGPR. Bars represent average induction ($\pm$ SE) of gene transcripts normalized to the house-keeping gene actin for three replicate plots. Fold change was calculated by calibrating data to the untreated control prior to bacterial inoculation (-12 h).

Fig. 2. Expression pattern of tomato gene basic *PR-1* following inoculation with *Pseudomonas syringae* pv. *tomato*. All treatments were inoculated at time 0. A, Experiment 1; B, experiment 2; and C, experiment 3. Solid white bars represent relative fold change of three replicated plots of untreated control plants, striped bars represent plant growth-promoting rhizobacteria (PGPR)-treated plants, gray bars indicate benzothiadiazole (BTH)-treated plants, and black bars correspond to plants treated with both BTH and PGPR. Bars represent average induction ($\pm$ SE) of gene transcripts normalized to the house-keeping gene actin for three replicate plots. Fold change was calculated by calibrating data to the untreated control prior to bacterial inoculation (-12 h).

at 60 h in two experiments (Fig. 2A and C) and induced in one (Fig. 2B).

***Pin2* transcript levels.** Wound-induced signaling in untreated control plants dramatically increased from low levels 12 h prior to inoculation to highest levels at 60 h (Fig. 3, white bars). Large changes in transcript abundance were observed in JA-mediated responses at 60 h postinoculation in each experiment (205.7-, 7.2-, and 3.2-fold change from uninoculated plants at -12 h, $P < 0.0001$, $P = 0.0001$, and $P = 0.0001$).

Pin2 transcript levels dramatically increased from 12 h prior to inoculation to highest levels at 60 h in PGPR-treated plants (Fig. 3, striped bars). At peak induction of JA-mediated responses, large changes in transcript levels were observed in each experiment (39.0-, 4.3-, and 10.7-fold increase from -12 h, $P = 0.0001$, $P < 0.0093$, and $P < 0.0049$). Variability between experiments was observed with the PGPR-treated plants not differing by 60 h from the untreated control (Fig. 3A), repressed in experiment 2 (Fig. 3B) and induced in experiment 3 (Fig. 3C).

Fig. 3. Expression pattern of tomato gene *Pin2* following inoculation with *Pseudomonas syringae* pv. *tomato*. All treatments were inoculated at time 0. A, Experiment 1; B, experiment 2; and C, experiment 3. Solid white bars represent relative fold change of three replicated plots of untreated control plants, striped bars represent plant growth-promoting rhizobacteria (PGPR)-treated plants, gray bars indicate benzothiadiazole (BTH)-treated plants, and black bars correspond to plants treated with both BTH and PGPR. Bars represent average induction ($\pm$ SE) of gene transcripts normalized to the housekeeping gene actin for three replicate plots. Fold change was calculated by calibrating data to the untreated control prior to bacterial inoculation (-12 h).

Over the time course, *Pin2* levels significantly increased in BTH-treated plants in two of the three experiments, though expression levels observed were down-regulated by 50 to 80% relative to untreated controls (Fig. 3, gray bars). Induction of JA-mediated responses increased to highest levels by the 60 h time point in each experiment (74.1-, 12.1-, and 1.4-fold change from uninoculated plants at -12 h, $P < 0.0001$, $P < 0.0001$, and $P = 0.2239$).

Expression of *Pin2* increased following inoculation in BTH+PGPR-treated plants, though levels were repressed (41 to 84% lower) relative to the untreated controls (Fig. 3, black bars). Greatest induction of JA-mediated responses occurred at the 60 (Fig. 3A and B) or 36 h (Fig. 3C) time point (44.4-, 4.7-, and 2.2-fold change from uninoculated plants at -12 h, $P < 0.0001$, $P < 0.0001$, and $P = 0.6371$).

DISCUSSION

Data presented in this study provide support for the incorporation of plant activators in greenhouse management of bacterial diseases. Treatment with the PGPR product reduced bacterial speck symptoms relative to the untreated control in two of the three experiments, though not to a commercially acceptable level. In contrast, BTH effectively reduced bacterial speck incidence and severity both alone and in combination with the PGPR product, in agreement with previous studies (21,23,34). Usage of both activators together resulted in lower disease incidence than BTH alone in one experiment and lower infection severity in two experiments.

Symptom development correlated to wound-induced gene expression (*Pin2*). Plants exhibiting higher levels of disease (untreated control, PGPR-treated) demonstrated a larger increase in *Pin2* transcript levels. Previous studies have found production of coronatine, a JA mimic and phytotoxin, by *P. syringae* pv. *tomato* to be correlated with induction of wound-responsive genes in susceptible tomato (38,48). In contrast, BTH-treated plants displayed significantly lower levels of *Pin2* gene expression, alone or in combination with the PGPR product, than the untreated control at 60 h. This reflects the reduced disease severity in plants treated with BTH. Expression levels of PGPR-treated plants did not reflect the level of disease relative to the untreated control, as response ranged from the same, lower, and higher *Pin2* expression in PGPR plots. Part of this variation may be due to high variability between PGPR-treated plants in experiment 3 at the 60 h time point. One replicate demonstrated much higher *Pin2* transcript levels than the other two, consequently inflating the mean expression and standard error. While gene expression differences between the three experiments may also be due to environmental differences in the greenhouse that influenced the growth of the pathogen, the outcome was still the same. Treatment with BTH effectively controlled disease, whereas the PGPR product did not provide commercially acceptable control. Utilization of both compounds in concert did not negatively impact SAR as acidic *PR-1* expression was similar between BTH+PGPR- and BTH-treated plants with disease control as good as or better than using the BTH compound alone.

Previous studies have found that SA- and ET-mediated responses increase during compatible interactions between bacterial pathogens and tomato (5,45,48). Plants that exhibited higher levels of bacterial speck lesions (untreated control and PGPR-treated) also demonstrated increasing acidic and basic *PR-1* levels over the course of the experiment following inoculation. Untreated controls reached peak expression of both acidic and basic *PR-1* at 60 h after inoculation, whereas the response of PGPR-treated plants was variable.

Treatment with BTH induced SA- and ET-mediated responses prior to inoculation, alone or in combination with the PGPR product, in all three experiments. A 3- to 13-fold increase in both

acidic and basic *PR-1* transcript levels was observed at the –12 h collection time for plants treated with BTH compared with untreated control plants. Plants remained activated throughout the duration of the experiment and each marker gene was induced to a greater degree following pathogen inoculation. There was no evidence for antagonistic effects of activating the SAR and ISR responses in concert as no consistent differences were found in expression of the three marker genes between BTH- and BTH+ PGPR-treated plants. Synergy between the two products is possible as disease incidence and severity was reduced in one experiment.

In previous studies, PGPR products have not been associated with major changes in defense gene expression (43) though some strains of *Bacillus* and *Chryseobacterium* have been found to activate defense-related marker genes (28,32). It is possible that the PGPR product primed plants to respond more quickly and to a greater degree following inoculation (Figs. 1C, 2C, and 3A and C) though the effect was inconsistent. Alfano et al. (1) found several tomato genes upregulated via *Trichoderma* induction of systemic resistance, including extensin and osmotin. Possibly these markers could provide more information regarding the PGPR–tomato interaction as *Trichoderma* spp. have been found to induce defenses in a manner similar to PGPR (8).

Although the PGPR product used in this study failed to provide sufficient bacterial speck control, growth-promoting benefits of this product were not analyzed. Previous work has shown that PGPR can be effective in transplant and field production (18,19). While copper is currently the most widely utilized control measure, incorporation of BTH into greenhouse transplant production could be useful for bacterial speck control. Combining the two products may provide the disease control benefits of BTH while boosting plant yield; however, further investigation of these plant activators in a production system is necessary.

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