Foliar Chemical Protection Against *Pantoea ananatis* in Onion Is Negated by Thrips Feeding

Spencer Stumpf,¹ Leana Leach,¹ Rajagopalbabu Srinivasan,² Timothy Coolong,³ Ron Gitaitis,¹ and Bhabesh Dutta^{1,†}

¹ Department of Plant Pathology, Coastal Plain Experiment Station, University of Georgia, Tifton, GA 31793

² Department of Entomology, University of Georgia, Griffin, GA 30223

³ Department of Horticulture, University of Georgia, Athens, GA 30602

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ABSTRACT

Center rot of onion, caused by *Pantoea ananatis*, is an economically important disease in onion production in Georgia and elsewhere in the United States. Growers rely on frequent foliar applications of bactericides and, in some cases, plant defense inducers to manage this disease. However, regular prophylactic application of these chemicals is not cost-effective and may not be environmentally friendly. Thrips (*Thrips tabaci* and *Frankliniella fusca*) are vectors of *P. ananatis*, and their feeding may compromise the effectiveness of foliar applications against *P. ananatis*. In this study, foliar treatments with acibenzolar-S-methyl (Actigard 50WG), cupric hydroxide (Kocide 3000), and Actigard plus Kocide were evaluated for their effectiveness in the presence and absence of thrips infestation at two critical onion growth stages: bulb initiation and bulb swelling. Onion growth stage had no impact on the effectiveness of either Kocide or Actigard. In the absence of thrips, Kocide application resulted in reduced center rot incidence compared with Actigard, regardless of the

growth stage. However, when thrips were present, the efficacy of both Kocide and Actigard was reduced, with bulb incidence not significantly different from the nontreated control. In independent greenhouse studies in the presence or absence of thrips, it was observed that use of protective chemicals (Kocide, Actigard, and their combinations) at different rates also affected pathogen progression into internal neck tissue and incidence of bulb rot. These results suggest that thrips infestation can reduce the efficacy of protective chemical treatments against *P. ananatis*. Thrips feeding on onion foliage and resulting feeding scars could facilitate *P. ananatis* entry and subsequently compromise the efficacy of protective chemical treatments. Therefore, an effective center rot management strategy should likely include thrips management in addition to bactericides at susceptible growth stages of onion.

Keywords: bacteriology, disease control, pest management

Center rot of onion is caused by the gram-negative bacterium *Pantoea ananatis*. Other *Pantoea* species such as *P. agglomerans*, *P. allii*, and *P. stewartii* subsp. *indologenes* have also been associated with center rot (Brady et al. 2011; Edens et al. 2006; Stumpf et al. 2018). *P. ananatis* can cause significant economic damage and, in severe cases, up to 100% yield loss (Goszczynska et al. 2006; Walcott et al. 2002). Characteristic symptoms of center rot include water-soaked lesions running along the length of the leaf that may gradually wilt, turn necrotic, and leave a bleached appearance (Dutta et al. 2014; Gitaitis and Gay 1997). Initial infections commonly occur on the younger leaves at the center of the plant (Nischwitz et al. 2007). After infection, the pathogen may colonize the foliage and progress into the onion bulb (Carr et al. 2013; Gitaitis and Gay 1997). Infected bulb scales are prone to secondary infections by microbes that can result in further rot and production of a foul odor. Mechanical harvest of infected onions can result in foliage breaking from the necks and bulbs left in the ground (Gitaitis 2014; Nischwitz et al. 2007).

To reduce economic losses caused by center rot of onion, an integrated pest management strategy that limits sources of initial inoculum and spread is necessary. It is important to produce and use

pathogen-free seed because *P. ananatis* can be seedborne (Walcott et al. 2002). Additionally, researchers isolated epiphytic populations of pathogenic *P. ananatis* strains from more than 20 weed species commonly found near onion production fields in Georgia (Gitaitis et al. 2002). Weeds serving as a resident host for epiphytic populations of *P. ananatis* most likely serve as a local source of inoculum for center rot outbreaks (Gitaitis et al. 2002). Dutta et al. (2017) observed that *P. ananatis* survived as an epiphyte on Florida pusley (*Richardia scabra* L.) under different temperature and moisture conditions that mimicked environmental conditions common for the months of March, April, and May (bulb maturation) in the Vidalia onion region of Georgia. Two known insect vectors, tobacco thrips (*Frankliniella fusca*) and onion thrips (*Thrips tabaci*), have been identified as vectors of *P. ananatis* (Dutta et al. 2014, 2016; Gitaitis et al. 2003). Furthermore, the studies found that the bacterium was transmitted passively into foliar wounds via contaminated thrip feces, which resulted in foliar infection (Dutta et al. 2014, 2016). These vectors are abundant in the Vidalia onion growing counties in Georgia with *F. fusca* as the dominant species, averaging 78% of the onion-feeding adult thrips population (Riley et al. 2014).

Managing center rot is a challenge because three sources of inocula (seeds, weeds, and thrips) may contribute to disease onset and further spread of the pathogen. Cultural practices such as implementing drip irrigation to minimize pathogen dispersal through water splashing and the use of mulch to repel insect vectors have been evaluated (Gitaitis et al. 2004). These practices were of little benefit under Georgia growing conditions (Gitaitis et al. 2004). Although some commercial cultivars displayed reduced incidence to center rot infection under greenhouse and field conditions, complete resistance has not been identified (Boyhan et al. 2014; Stumpf et al. 2017). Copper-based compounds may reduce center rot incidence and severity; however, the disease

[†]Corresponding author: B. Dutta; bhabesh@uga.edu

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is not effectively managed when environmental conditions are optimal for its development. Furthermore, efficacy can be hampered by the development of copper-tolerant bacterial strains (Nischwitz et al. 2007).

Acibenzolar-*S*-methyl (Actigard 50WG; Syngenta Crop Protection, Greensboro, NC), an analog of salicylic acid (SA), induces systemic acquired resistance (SAR) in hosts to inhibit the severity of diseases caused by bacterial and fungal pathogens (Babu et al. 2003; Cole 1999). Combinations of Actigard and copper bactericides have been used to manage bacterial diseases on vegetables under field conditions. Previous research investigated the efficacy of Actigard and copper bactericide spray programs in reducing the severity of bacterial blight of onion (caused by *Xanthomonas axonopodis* pv. *allii*) in Colorado (Gent and Schwartz 2005). Researchers found that applications of Actigard reduced epiphytic populations of *X. axonopodis* pv. *allii* on onion as effectively as a copper bactericide amended with an ethylenebisdithiocarbamate (EBDC) fungicide in growth chamber studies. In a multilocation field study, Actigard was as effective as copper mixed with an EBDC fungicide against *X. axonopodis* pv. *allii* at reducing the relative area under the disease progress curve without affecting onion bulb yield or grade (Gent and Schwartz 2005). However, the performance of Actigard and copper bactericides against *P. ananatis* on onion in Georgia has been variable with limited to no efficacy (Dutta, unpublished data).

We investigated the effect of onion growth stage on center rot incidence in bulbs. We identified three susceptible growth stages (first leaf senescence, bulb initiation, and bulb swelling) of onion where foliar infection often results in center rot incidence in the bulb (Stumpf et al. 2017). Based on these observations, we hypothesized that targeted protection specifically during these susceptible growth stages can influence center rot bulb incidence. The targeted protection was investigated using Actigard and cupric hydroxide (Kocide 3000, hereafter referred to as Kocide; DuPont, Wilmington, DE). We also hypothesized that thrips can negate these protection strategies, as disease is most severe when thrips feed during early bulbing stages (Orloff et al. 2016). This study investigated the application of targeted protective foliar sprays at bulb initiation and bulb swelling stages for improved disease management. Furthermore, we evaluated whether different rates of protective chemicals (Kocide, Actigard, and their combinations) affected pathogen colonization and bulb rot incidence in the presence or absence of thrips under greenhouse conditions.

MATERIALS AND METHODS

Bacterial strain, inoculum preparation, and copper sensitivity. *P. ananatis* strain PNA 97-1 (accession DQ777968), isolated in 1997 from the foliage of center rot-infected onion plants in Toombs County, Georgia, was used in this study. For inoculum preparation, a single colony from a 48-h-old culture was used to inoculate 25 ml of nutrient broth, which was shaken on a rotary shaker (Inova; New Brunswick Scientific, Edison, NJ) at 160 rpm. After 12 h of incubation, the bacterial suspension was centrifuged at $10,000 \times g$ (Allegra 25R; Beckman Coulter, Fullerton, CA) for 1 min. The supernatant was discarded, the pellet was resuspended in 0.1 M of phosphate-buffered saline (PBS), and the inoculum was adjusted photometrically using a spectrophotometer (Spectronic 20; Bausch and Lomb, Rochester, NY) to an optical density of 0.3 at 600 nm (approximately 1×10^8 CFU/ml). The final inoculum concentration was obtained by serial dilution in PBS to approximately 1×10^6 CFU/ml. Prior to this study, the copper tolerance status of PNA 97-1 was checked in $\text{CuSO}_4 \cdot 0.5\text{H}_2\text{O}$ -amended nutrient agar (NA) plates. To determine the copper tolerance, the bacterial strain was grown on nutrient broth overnight and resuspended to an optical density of 0.3 at 600 nm (1×10^8 CFU/ml) as previously described, and suspensions were serially diluted to a concentration of 1×10^6 CFU/ml. Later, aliquots of 50 μl were spread onto NA and NA plates amended with 200 ppm of

$\text{CuSO}_4 \cdot 0.5\text{H}_2\text{O}$. Plates were incubated at 28°C for 48 to 72 h and observed for bacterial growth.

***F. fusca* maintenance.** A colony of *F. fusca* was established in 2009 on noninfected peanut leaflets of the cultivar Georgia Green with thrips collected from peanut blooms from the Belflower Farm at the University of Georgia Tifton Campus. Since then, thrips have been maintained in Munger cages (Munger 1942) on peanut (cultivar Georgia Green) leaflets. Munger cages were maintained in a growth chamber (Thermo Scientific, Dubuque, IA) at 25 to 30°C with a 14-h/10-h light/dark photoperiod. To confirm whether the peanut plants were negative for *P. ananatis*, leaflets ($n = 5$) without surface sterilization were rinsed in 2 ml of 0.1 M PBS for 2 min. Aliquots of 0.1 ml were then spread plated on PA-20 semi-selective medium (Goszczyńska et al. 2006) and incubated. Thrips in the colony were also crushed and spread plated on PA-20 medium to check for *P. ananatis* contamination. The majority of individuals in the colonies (>80%) were female. Adult females of relatively the same age were subsequently used for all experiments.

Effect of targeted growth stage chemical applications to mitigate center rot incidence in bulbs. Onion seedlings (cultivar Century) were established in 10 $\times$ 8-cm (diameter $\times$ height) plastic pots (Hummert International, Earth City, MO) containing a commercial potting mix (Scotts Miracle-Gro Company, Marysville, OH). The seedlings were maintained in greenhouse conditions at 24 to 27°C and 70 to 80% RH with ambient light from December to May. Potted plants were kept on metal benches 1 m off the ground and watered at the base of the stem every other day by hand. Plants reached a height of 25 to 30 cm with five to six leaves at the bulb initiation stage and six to eight leaves at the bulb swelling stage. We also evaluated the temporal expression of pathogenesis-related protein *PR1* and *PR5* genes in onion seedlings upon Actigard foliar application under greenhouse conditions (Supplementary File S1).

Foliar treatments with Actigard, Kocide, or Actigard plus Kocide were applied at either the bulb initiation or bulb swelling growth stage. Kocide was mixed with water at a rate of 1.7 kg/ha and Actigard was mixed with water at a rate of 35 g/ha. The sequence of treatments started with Actigard, which was applied 48 h prior to inoculation (based on the gene expression studies; Supplementary Fig. 1), whereas Kocide was applied 24 h prior to inoculation. The treatment that comprised of Actigard and Kocide also followed a similar sequence. Foliar chemical treatments were applied to foliage until runoff using a sterile backpack sprayer. Plants were inoculated with *P. ananatis* (PNA 97-1; 10^6 CFU/ml) at 48 h after Actigard application or 24 h after Kocide application. Plants were spray inoculated with a bacterial suspension until runoff as described above. Plants not treated with chemicals but sprayed with sterile water served as the nontreated noninoculated control (negative control), whereas plants not treated but inoculated with PNA 97-1 served as the nontreated inoculated control.

Twenty-four hours after inoculum was applied, onion seedlings were placed in a 47.5 $\times$ 47.5 $\times$ 93-cm insect rearing cage (BugDorm, Taiwan) and 30 *P. ananatis*-free *F. fusca* adults were released. Overall, 10 insect cages were used for each treatment and each insect cage contained 10 seedlings per treatment. Onion seedlings ($n = 10$ per insect cage per treatment per experiment) without thrips were also placed inside an insect cage. Insect cages were arranged in a randomized complete block design inside a greenhouse and each treatment was replicated 10 times. Three independent experiments were conducted. Onions were watered as necessary at the base of the plant until maturity.

When foliar necks were soft, indicating maturity, bulbs were harvested, stored temporarily in sterile 4.5-kg mesh onion bags (Harris Seeds, Rochester, NY), and transported to the Coastal Plain Experiment Station (CPES) Natural Products Laboratory (University of Georgia, Tifton, GA). Onion bulbs were graded for any visual symptoms prior to curing. Bulbs displaying extreme and suspicious softening of the neck were cut longitudinally and assessed for center rot symptoms. Bulbs that appeared healthy were cured at room temperature (25°C) for 7 days. After 7 days of curing, bulbs were cut

through the center using a sterile knife and assessed for center rot symptoms (discoloration of interior bulb scales). Onion bulbs displaying center rot symptoms were recorded as diseased, and asymptomatic bulbs were recorded as healthy. Bulb incidence of center rot was calculated for each chemical treatment at two growth stages by adding the number of bulbs discarded prior to curing and the number of bulbs displaying symptoms after curing divided by the total number of bulbs assessed $\times 100$. Mean bulb incidences of center rot with chemical treatments at two onion growth stages with and without thrips exposure were analyzed using Proc Glimmix with Beta distribution for percentage response variables in SAS 9.4 software (SAS Institute, Cary, NC) at the $P = 0.05$ level. The interactive effects of chemical application $\times$ growth stage and chemical application $\times$ thrips exposure on center rot incidence in onion bulbs were also analyzed.

Effect of protective chemical application on colonization of onions and thrips by *P. ananatis* and center rot bulb incidence. There were no significant differences between initiating the application of chemicals at the bulb initiation and bulb swelling stages. Therefore, greenhouse experiments were designed to further evaluate the protective chemicals (at different rates) and their combinations at only the bulb initiation stage. Further detailed pathogen population colonization, thrips infestation status, and expression of *PR1* and *PR5* were also evaluated.

Onion seedlings (cultivar Century) were established and maintained in greenhouse conditions as described above. At the bulb initiation stage, onion seedlings were treated with three rates of Actigard, two rates of Kocide, or combinations of Actigard and Kocide (Table 1). The sequence of treatments started with Actigard, which was applied 48 h prior to inoculation, whereas Kocide was applied 24 h prior to inoculation. The treatment that comprised Actigard and Kocide also followed a similar sequence. Actigard was applied 24 h prior to Kocide applications and inoculations with *P. ananatis* were made 48 h after Actigard application (24 h after Kocide application). Three different rates of Actigard (35, 52.5, or 70 g/ha) were mixed in water (5 liters) and applied using a backpack sprayer. Two rates of Kocide (1.7 or 2.2 kg/ha) were applied in a similar manner 24 h after Actigard treatment. Treatments that included a combination of three rates of Actigard and two rates of Kocide also followed a similar scheme. Mixed combinations were

as follows: 35 g/ha of Actigard plus 1.7 kg/ha of Kocide, 35 g/ha of Actigard plus 2.2 kg/ha of Kocide, 52.5 g/ha of Actigard plus 1.7 kg/ha of Kocide, 52.5 g/ha of Actigard plus 2.2 kg/ha of Kocide, 70 g/ha of Actigard plus 1.7 kg/ha of Kocide, and 70 g/ha of Actigard plus 2.2 kg/ha of Kocide. Plants were inoculated with *P. ananatis* (PNA 97-1; 1×10^6 CFU/ml) at 48 h after Actigard treatment or 24 h after Kocide treatment. Onion plants treated in a similar manner (three rates of Actigard, two rates of Kocide, or their combinations) at the bulb initiation stage were exposed to thrips. Twenty-four hours after *P. ananatis* inoculation, treated onion seedlings ($n = 30$ per treatment per experiment) were placed in a cylindrical Mylar film cage with a copper mesh (approximately 200 μ m; Grafix Plastics, Cleveland, OH). After seedlings were placed in individual Mylar cages, *P. ananatis*-free *F. fusca* adults ($n = 30$) were released per cage. Onion seedlings ($n = 30$ per treatment per experiment) without thrips were also placed inside a Mylar film cage. *F. fusca* adult females of relatively the same age were used for all experiments. Onion seedlings in a pot served as a single experimental unit. Onion seedlings were maintained until harvest maturity. Seedlings in Mylar film cages were placed in a randomized complete block design inside a greenhouse and each treatment was replicated 30 times. Two independent experiments were conducted. Onions were watered as necessary at the base of the plant until maturity. Plants sprayed with sterile water and inoculated with *P. ananatis* served as a nontreated inoculated control. Noninoculated and sterile water-treated plants served as a nontreated noninoculated control (negative control). Control plants were either exposed to thrips under the insect cage or left nonexposed.

Temporal dynamics of *P. ananatis* populations in internal neck tissues of onion. Population dynamics of *P. ananatis* for each treatment with and without thrips exposure were determined from the internal neck tissues of onion bulbs. At each sampling period (0, 7, 14, 21, 28, 35, and 40 days postinoculation [dpi]), three onion bulbs were sampled to determine bacterial populations from the internal neck tissue. To achieve this, tunic layers from sampled onion bulbs were removed and surface disinfested with 70% ethanol; the surface was then wiped with sterile water using a sterile paper towel as described previously (Dutta et al. 2014). Internal neck tissue (1 cm above the shoulder of the bulb) was excised using a sterile scalpel, weighed, and used for *P. ananatis* enumeration. For

TABLE 1. Percentage of bulb incidence and relative expression of pathogenesis-related protein genes *PR1* and *PR5* in onion seedlings in response to chemical treatment with and without thrips exposure

Treatments and rate (listed per acre)	Bulb incidence (%) ^a		Relative expression of <i>PR1</i> ^b		Relative expression of <i>PR5</i>	
	Thrips exposure	Without thrips exposure	Thrips exposure	Without thrips exposure	Thrips exposure	Without thrips exposure
Actigard (g/ha)						
35	80.2 Aa ^z	74.6 Aa	2.7 Aa	3.2 Aa	4.4 Aa	3.6 Aa
52.5	82.4 Aa	71.2 Aa	3.8 Aa	3.7 Aa	3.8 Aa	2.8 Aa
70	72.2 Aa	65.8 Aa	3.6 Aa	4.1 Aa	4.2 Aa	3.6 Aa
Kocide 3000 (kg/ha)						
1.7	70.2 Aa	28.5 Bb	0.9 Bb	0.7 Bb	1.4 Ba	0.7 Ba
2.2	73.8 Aa	32.8 Bb	1.2 Bb	0.9 Bb	0.9 Ba	1.6 Ba
Actigard (g/ha) + Kocide 3000 (kg/ha)						
35 + 1.7	78.4 Aa	38.4 Bb	2.9 Aa	2.5 Aa	3.6 Aa	4.1 Aa
35 + 2.2	83.8 Aa	29.2 Bb	4.2 Aa	3.8 Aa	3.8 Aa	2.7 Aa
52.5 + 1.7	82.6 Aa	36.4 Bb	3.8 Aa	4.2 Aa	4.7 Aa	4.8 Aa
52.5 + 2.2	75.4 Aa	37.6 Bb	3.6 Aa	3.8 Aa	4.2 Aa	3.2 Aa
70 + 1.7	72.8 Aa	39.6 Bb	3.2 Aa	2.9 Aa	4.8 Aa	2.6 Aa
70 + 2.2	78.2 Aa	37.4 Bb	2.8 Aa	3.4 Aa	3.6 Aa	4.1 Aa
Control (PNA 97-1)	90.5 Aa	78.5 Ab	1.5 Bb	1.8 Bb	1.7 Aa	1.4 Aa

^a Percentage of bulb incidence was calculated based on the number of bulbs displaying center rot symptoms (number of bulbs with center rot prior to curing + number of bulbs with center rot after 1 week of curing) divided by the total number of bulbs evaluated $\times 100$.

^b Mean relative expression of *PR1* and *PR5* in onion seedlings treated with acibenzolar-S-methyl (Actigard 50WG), copper hydroxide (Kocide 3000), or both at different rates under greenhouse conditions. Relative expressions of *PR1* and *PR5* were determined in onion foliar tissues collected 72 h postapplication. Leaf tissue samples from three replicates per treatment were collected for gene expression analysis and a total of two independent experiments were conducted.

^z Means within the same treatment class followed by different uppercase (column-wise comparison in each section) and lowercase (row-wise comparison in each section) letters are significantly different at $P < 0.05$ according to Fisher's least significant difference test.

enumeration, tissue samples were placed individually in 1 ml of 0.1 M PBS in 2-ml microcentrifuge tubes, macerated using a sterile glass rod, and vigorously agitated for 30 s (Vortex Genie2; Fisher Scientific International). The tissue macerates were 10-fold serially diluted in 1 ml of PBS. Subsequently, 100- μ l aliquants were spread plated onto semi-selective PA-20 and incubated for 5 to 7 days at 28°C. After incubation, bacterial colonies were enumerated and recorded from each sampling point. Area under the growth progress curve (AUGPC) for each replicate per treatment was calculated as follows:

$$\Sigma_{i=1}^n [(Y_{i+n-1} + Y_i)]/2 [X_{i+1} - X_i]$$

where Y_i is the bacterial population at the i th observation, X_i is the time in hours at the i th observation, and n is the total number of observations. Analysis of variance was conducted on the AUGPC values to determine treatment effects on *P. ananatis* populations, and Fisher's least significant difference (LSD) test ($P < 0.05$) was used for mean separations. The same treatments were compared under thrips exposure and without thrips exposure scenarios with the t test.

Determination of the percentage of thrips carrying internal populations of *P. ananatis*. Percentages of thrips that internally harbored *P. ananatis* were determined for each treatment that was exposed to thrips as described above. Using a fine-tip paintbrush, four individual adult *F. fusca* were randomly sampled from the whorl of each plant inside the Mylar cage at 0, 7, 14, and 21 dpi. Thrips sampling for *P. ananatis* was conducted for the two independent greenhouse trials described above. The sampled thrips were placed individually into separate 1.5-ml microcentrifuge tubes and surface sterilized as described previously (Dutta et al. 2014). After surface sterilization, the collected thrips (adult) from each seedling per treatment were macerated individually using a plastic pestle (Polypropylene Pellet Pestle; Grainger Inc., Jacksonville, FL) and the tissue macerates were plated onto PA-20 medium. Individual thrips were assayed for the presence of internal *P. ananatis*. To ensure that *P. ananatis* was absent after the last rinsing step, the surface sterilization rinsate (from last rinsing step with PBS) was also tested for the presence of *P. ananatis* by plating as described above. Mean percentages of thrips harboring internal *P. ananatis* populations were statistically analyzed with repeated-measures analysis ($P < 0.05$) using Proc Glimmix in SAS 9.4 software.

Expression of SA-induced genes (PR1 and PR5) in response to chemical treatment under thrips versus nonthrips exposure. Relative expressions of *PR1* and *PR5* were determined in onion foliar tissues collected at 48 h after thrips exposure (72 h after *P. ananatis* inoculation). Three seedlings from each treatment were sampled and a total of two independent experiments were conducted. Leaf samples were immediately stored in liquid nitrogen and later transferred to a -80°C freezer at CPES until needed for further analysis. Leaves were ground in liquid nitrogen and total RNA was extracted from 50 mg of ground leaf tissue using the manufacturer's protocol (RNeasy Plant Mini Kit). First-strand cDNA was synthesized from 500 ng of total RNA by reverse transcription using the iScript cDNA synthesis kit (Bio-Rad Laboratories Inc., Hercules, CA). Then quantitative PCR was conducted on a Smart Cycler System (Cepheid, Sunnyvale, CA) using iQ SYBR Green Supermix (Bio-Rad) and specific primer pairs for *PR1* and *PR5* as described previously by ElMorsi et al. (2015) with a minor modification in annealing temperatures of 59 and 56°C, respectively. The *beta-actin* gene was used as a control and was assessed using the primer pair described by ElMorsi et al. (2015). The cycle threshold (Ct) values thus obtained were converted into relative fold differences of marker genes in treated samples compared with the water control samples (negative control) and relative to the endogenous control gene using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen 2001; Schmittgen and Livak 2008). This was done after we verified the stability of the endogenous control genes and that the primer pairs had high, comparable PCR efficiencies (Schmittgen and Livak 2008).

Relative fold changes of target genes were calculated and compared. The effect of chemical application and thrips exposure treatment combinations on the relative expression of *PR1* and *PR5* was determined and compared by Fisher's LSD at $P = 0.05$.

Bulb incidence of center rot. At harvest maturity, bulbs were harvested for each treatment (thrips exposed and nonexposed), stored temporarily in surface-disinfested 4.5-kg mesh onion bags (Harris Seeds), and transported to the CPES Natural Products Laboratory. Harvested bulbs were stored at room temperature (25°C) for 7 days without prior grading. After 7 days of curing, bulbs were cut through the center using a sterile knife and assessed for center rot symptoms (discoloration of interior bulb scales). Onions displaying center rot symptoms were recorded as diseased and asymptomatic bulbs were recorded as healthy. Center rot bulb incidence was calculated for each chemical treatment with and without thrips exposure as the number of symptomatic bulbs divided by the total number of stored bulbs $\times$ 100. Mean incidences of center rot for each chemical treatment (thrips exposed versus nonexposed) were calculated using Proc Glimmix with Beta distribution for percentage response variables in SAS 9.4 (SAS Institute). The effects of chemical application and thrips exposure combination on center rot incidence in onion bulbs were also analyzed as described above. The effect of the interaction of chemical treatment and thrips exposure was analyzed using Proc Glimmix in SAS 9.4.

Bacterial identification. To confirm that symptoms observed were caused by *P. ananatis*, bacteria from symptomatic bulbs ($n = 10$) were isolated from margins of necrotic tissue, streaked onto Tryptic soy broth agar plates, and incubated for 48 h at 28°C. After 48 h of incubation, yellow-pigmented colonies were isolated and tested for *P. ananatis* using species-specific TaqMan-based PCR assays as described by Stumpf et al. (2017). The above tests were also utilized for bulbs whose foliage was treated with 0.1 M of PBS but not inoculated with *P. ananatis*.

RESULTS

Bacterial strain, inoculum preparation, and copper sensitivity. *P. ananatis* (PNA 97-1) did not grow on NA media amended with 200 ppm of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. However, confluent colony growth was observed on nonamended NA plates.

***F. fusca* maintenance.** *P. ananatis* colonies were not recovered from the *F. fusca* individuals on PA-20 medium, indicating that thrips were free of *P. ananatis*. These results indicate that *P. ananatis* was not acquired by *F. fusca* and therefore was not spread within or between onion plants through stercorarian transmission (transmission through feces).

Effect of targeted growth stage chemical applications to mitigate center rot incidence in bulbs. The effects of protective foliar chemical treatments (1.7 kg/ha of Kocide, 35 g/ha of Actigard, or 1.7 kg/ha of Kocide plus 35 g/ha of Actigard) at two growth stages (bulb initiation or bulb swelling) on reducing center rot bulb incidence were evaluated for two scenarios: either exposure to thrips or not. There were no significant interactions among chemical foliar treatments at different growth stages and chemical treatments with thrips infestation ($P = 0.721$). However, main effects of chemical treatment ($P < 0.034$) and thrips infestation ($P < 0.001$) significantly affected center rot incidence in bulbs. In addition, the effects of three experiments were not significant ($P = 0.142$); hence, the data were pooled and analyzed.

The effect of chemical application on *P. ananatis* bulb incidence at two onion growth stages without thrips infestation was not significant ($P = 0.362$); hence, bulb incidence data at bulb initiation and bulb swelling stages were pooled. For the treatments that had no thrips exposure but were treated with chemicals at both growth stages, center rot incidence in bulbs was significantly lower than the nontreated *P. ananatis*-inoculated control (bulb incidence = 54.5%; $P = 0.034$) (Fig. 1A). The mean center rot incidence in bulbs treated with Kocide (bulb incidence = 16.5%) and Kocide plus Actigard

(bulb incidence = 18.2%) was significantly lower than Actigard only (bulb incidence = 39.2%) (Fig. 1A). Center rot symptoms were not observed on bulbs whose foliage was treated with sterile water and not inoculated with *P. ananatis*.

The effect of chemical application on *P. ananatis* bulb incidence at two onion growth stages with thrips exposure was not significant ($P = 0.272$) (data not shown); hence, data for bulb incidence at bulb initiation and bulb swelling stages were pooled. In addition, the overall effect of chemical treatment on *P. ananatis* bulb incidence in the presence of thrips was not significant ($P = 0.615$). A mean incidence of 45.2% was observed for plants treated with Kocide only, 45.6% for Kocide plus Actigard treatment, and 52.2% for Actigard-only treatment (Fig. 1B). In addition, the nontreated control (*P. ananatis* only) displayed a mean bulb incidence of 48.5% (Fig. 1B). Center rot symptoms were not observed for bulbs used as a negative control.

Since effects of chemical treatments upon thrips feeding were not anticipated, quantitative assessments of thrips feeding injury were not made. However, qualitative assessments of thrips foliar feeding damage at harvest were made. Irrespective of the chemical treatment applied, onion seedlings that were exposed to thrips had silvery patches or streaks on the leaves, which were likely attributable to feeding. On some foliage, tiny black “tar” spots, which were remnants of thrip excrement, were an indication of thrips feeding. Thrips feeding-associated injury was not observed in plants that were not exposed to thrips.

Effect of protective chemical application on colonization of onions and thrips by *P. ananatis* and center rot bulb incidence. Temporal dynamics of *P. ananatis* populations in internal neck tissue of onion. For seedlings without thrips exposure, onion seedlings treated with only water but challenge inoculated with *P. ananatis* (in foliar tissue) resulted in a gradual increase of bacterial populations in internal neck tissues from 7 to 40 dpi. At 7 dpi, the mean *P. ananatis* population was 3.8×10^3 CFU/g of neck tissue, increased to 1.2×10^6 CFU/g by 21 dpi, and reached a population of 2.6×10^8 CFU/g by 40 dpi (Fig. 2A). Actigard

applications at 35, 52.5, and 70 g/ha followed a similar trend of *P. ananatis* growth in internal neck tissues from 7 to 40 dpi (Fig. 2A). At 7 dpi, *P. ananatis* populations for the three different Actigard rates ranged from 2.1×10^2 to 3.7×10^2 CFU/g, which gradually increased to a final population range of 2.3×10^8 to 4.8×10^8 CFU/g by 40 dpi (Fig. 2A). *P. ananatis* populations were not detected from internal neck tissues until 21 dpi for onion seedlings that were treated with either Kocide (two rates) or its combination with Actigard (six different combinations). For these treatments, *P. ananatis* populations ranged from 1.8×10^2 to 5.2×10^2 CFU/g at 21 dpi, then gradually increased to a final population range of 4.8×10^3 to 2.1×10^4 CFU/g by 40 dpi, which was four to five log₁₀-orders reduced than Actigard only and the nontreated control (*P. ananatis* only) (Fig. 2A). AUGPC values were significantly reduced for onion seedlings that were treated with either Kocide (two rates) or its combination with Actigard (six different combinations) compared with *P. ananatis* (control) and Actigard-only treatments ($P < 0.001$) (Fig. 3). *P. ananatis* populations were not recovered from internal neck tissue of bulbs of the negative control.

For seedlings with thrips exposure, *P. ananatis* populations in internal neck tissues were detected as early as 7 dpi from both treated and nontreated seedlings that were exposed to thrips. Nontreated onion seedlings that were inoculated with *P. ananatis* and later exposed to thrips had a gradual increase in bacterial populations in internal neck tissues from 7 to 40 dpi. At 7 dpi, the bacterial population was 1.7×10^5 CFU/g, gradually increased to 2.6×10^7 CFU/g by 21 dpi, and reached a final population of 3.4×10^{10} CFU/g by 40 dpi (Fig. 2B). Actigard applications at three different rates (35, 55.2, and 70 g/ha) showed a similar trend from 7 to 40 dpi. At 7 dpi, *P. ananatis* populations for the three different Actigard rates ranged from 5.4×10^3 to 3.8×10^4 CFU/g and gradually increased to a final population range of 7.5×10^8 to 8.1×10^9 CFU/g by 40 dpi (Fig. 2B). For the treatments that included Kocide (two rates) or its combination with Actigard (six different combinations), *P. ananatis* populations ranged from 5.8×10^3 to 1.4×10^4 CFU/g at 7 dpi, which gradually increased to a final

Fig. 1. Percent incidence of center rot in bulbs whose foliage was chemically treated either at bulb initiation or bulb swelling stage under greenhouse conditions. **A.** Without thrips. **B.** With thrips. Onion plants ($n = 10$ per treatment per experiment) were treated with acibenzolar-S-methyl (Actigard; 35 g/ha), copper hydroxide (Kocide 3000; 1.7 kg/ha), or Actigard (35 g/ha) plus Kocide 3000 (1.7 kg/ha) prior to spray inoculation with *Pantoea ananatis* (1×10^6 CFU/ml) at either the bulb initiation or bulb swelling stage. Plants were inoculated at 24 and 48 h after Kocide 3000 and Actigard treatments, respectively. Data points represent the mean bulb incidence of center rot in three independent experiments. Bars indicate standard error of the mean. Means with a similar uppercase letter are not significantly different according to Fisher's least significant difference test at $P = 0.05$.

population range of 8.2×10^8 to 5.8×10^9 CFU/g by 40 dpi (Fig. 2B). The effects of chemical treatments on AUGPC values were not significant ($P = 0.423$) (Fig. 3). When effects of thrips exposure and nonthrips exposure were evaluated for the same chemical treatments, significantly higher AUGPC values ($P < 0.05$) were observed for onion seedlings exposed to thrips (Fig. 3). *P. ananatis* populations were not recovered from onions used as the negative control.

Determination of the percentage of thrips carrying internal populations of *P. ananatis*. Thrips were sampled, surface sterilized, and assayed for the presence of internal populations of *P. ananatis*. All thrips sample rinsates from the last sterilization step were negative for *P. ananatis* on PA-20 medium. The percentage of *P. ananatis*-positive thrips did not differ significantly among treatments at 0 and 7 dpi ($P = 0.437$). At 7 dpi, the mean percentage of *P. ananatis*-positive thrips ranged from 46.6 to 60%. By 14 dpi, the percentage of *P. ananatis*-positive thrips ranged from 73.3 to 100%. By 21 dpi, 100% of the sampled thrips across the treatments harbored the bacterium. The effect of treatments on the percentage of *P. ananatis*-positive thrips was not significant at 14 dpi ($P = 0.527$) and 21 dpi ($P = 0.382$).

The chemical treatments did not affect thrips feeding; hence, quantitative assessments of thrips feeding injury were not made. However, qualitative assessments of onion bulbs and associated foliage at harvest were made for thrips feeding as described above. Irrespective of the chemical treatment applied, onion seedlings exposed to thrips had thrips feeding-associated injuries such as silvery patches or streaks on the leaves. Thrips feeding damage was not observed in plants not exposed to thrips.

Expression of SA-induced genes (*PR1* and *PR5*) in response to chemical treatment under thrips versus nonthrips exposure. For seedlings without thrips exposure, the effects of chemical treatments on *PR1* and *PR5* in plants that were not exposed to

thrips were significant ($P < 0.05$). In the case of *PR1*, expression was significantly higher for treatments with Actigard only (three rates) or its combination with Kocide (six combinations) compared with Kocide-only treatments (two rates) and the nontreated control (*P. ananatis* only). Relative expression ranged from 2.5 to 4.2 for Actigard only (three rates) or its combination with Kocide (six combinations), whereas expression ranged from 0.9 to 1.5 for Kocide only and the nontreated control (*P. ananatis* only) (Table 1). Similar trends were observed in the case of *PR5* expression, with significantly higher relative expression with Actigard only (three rates) or its combination with Kocide (six combinations) compared with Kocide-only treatments (two rates) and the nontreated control (*P. ananatis* only). Relative expression ranged from 2.9 to 4.1 for Actigard only (three rates) or its combination with Kocide (six combinations), whereas it ranged from 0.7 to 1.8 for Kocide only and the nontreated control (*P. ananatis* only) (Table 1).

For seedlings with thrips exposure, the effects of chemical treatments on *PR1* and *PR5* expression in plants exposed to thrips were significant ($P < 0.05$). In the case of *PR1*, expression was significantly higher for onions treated with Actigard only (three rates) or its combination with Kocide (six combinations) compared with Kocide only (two rates) and the nontreated control (*P. ananatis* only). Relative expression ranged from 3.6 to 4.8 for Actigard only (three rates) or its combination with Kocide (six combinations), whereas expression ranged from 0.9 to 1.7 for Kocide only and the nontreated control (*P. ananatis* only) (Table 1).

Relative expression of *PR5* was significantly higher for Actigard only (three rates) or its combination with Kocide (six combinations) compared with Kocide only (two rates) and the nontreated control (*P. ananatis* only). Relative expression for *PR5* ranged from 2.6 to 4.1 with Actigard only (three rates) or its combination with Kocide (six combinations), whereas it ranged from 0.7 to 1.6 for Kocide only and the nontreated control (*P. ananatis* only) (Table 1).

Fig. 2. Temporal dynamics of *Pantoea ananatis* populations ($\log_{10}$ CFU/g of fresh tissue weight) in internal neck tissues of onions in response to chemical treatments (different rates) with and without thrips exposure. Population dynamics of *P. ananatis* for each treatment (acibenzolar-S-methyl [Actigard; three rates], copper hydroxide [Kocide 3000; two rates], and their combination [six combinations]) in the **A**, absence and **B**, presence of thrips were determined from the internal neck tissues of onion bulbs. At each sampling period (0, 7, 14, 21, 28, 35, and 40 days postinoculation), three onion bulbs were sampled to determine bacterial populations from the internal neck tissue and enumerated on PA-20 semi-selective medium. Data points represent the mean bacterial populations at each sampling period in two independent experiments. Bars indicate the SEM.

There were no significant differences when row-wise comparisons were made (*PR1* and *PR5*) for Kocide only (two rates), Actigard only (three rates), and their combinations (six different combinations) for plants either exposed to thrips or not (Table 1).

Bulb incidence of center rot. For seedlings without thrips exposure, the effect of foliar treatments on center rot bulb incidence was significant ($P = 0.001$), with 78.5% disease incidence in the nontreated control (*P. ananatis* only). The bulb incidence values of center rot for Actigard (three rates) ranged from 65.8 to 74.6% and were not significantly different (Table 1). The bulb incidence of center rot was significantly higher for the nontreated control (*P. ananatis* only) and the Actigard treatments (three rates) compared with Kocide (two rates) or its combination with Actigard (six different combinations). Bulb incidences were not significantly different for Kocide (two rates) or its combination with Actigard (six different combinations) and ranged from 29.2 to 39.6% (Table 1). Center rot symptoms were not observed on bulbs of the negative control.

For seedlings with thrips exposure, the effect of foliar treatments on center rot bulb incidence was not significant ($P = 0.384$); the nontreated control (*P. ananatis* only) had a disease incidence of 90.5% (Table 1). The bulb incidence values for treatments with Actigard (three rates), Kocide (two rates), and their combination (six combinations) were not significantly different from each other or from the nontreated control (*P. ananatis* only). The bulb incidence for the foliar treatments, including Actigard (three rates), Kocide (two rates), and their combinations (six combinations), ranged from 70.2 to 83.8% (Table 1).

When row-wise comparisons were made for treatments with Kocide (two rates) or in combination with Actigard (six different

combinations) for the plants that were either exposed to thrips or not, bulb incidences were significantly higher for the former scenario than the latter ($P < 0.05$) (Table 1). Bulb incidences of center rot for Actigard treatments under thrips exposure were significantly higher than the nonexposed scenarios. A significantly higher bulb incidence was even observed for the nontreated control (*P. ananatis* only) under thrips exposure than the nonexposure scenario ($P < 0.05$) (Table 1). Center rot symptoms were not observed for bulbs from the negative control. Overall, there was a significant interaction between thrips exposure and chemical treatment ($P = 0.036$).

Presence of *P. ananatis* in infected bulbs. Bacterial colonies isolated from symptomatic onion bulbs ($n = 10$) were tested to confirm their identity. Colonies were yellow pigmented, gram negative, positive for indole, and had their DNA amplified with real-time PCR species-specific primers. One hundred percent of the bulbs expressing symptoms were positive for *P. ananatis*. Asymptomatic bulbs were negative for colonies indicative of *P. ananatis*. Also, *P. ananatis* colonies were not recovered from the bulbs used as the negative control.

DISCUSSION

Previous studies identified three susceptible growth stages (first leaf senescence, bulb initiation, and bulb swelling) that led to the development of bulb symptoms; based on these observations, it was hypothesized that targeted protection during these susceptible growth stages may influence center rot bulb symptoms (Stumpf et al. 2017). Furthermore, it was also hypothesized that thrips feeding may impact the efficacy of foliar treatments. The results of

Fig. 3. Area under the growth progress curve (AUGPC) for *Pantoea ananatis* in internal neck tissues of onions in response to chemical treatments (acibenzolar-S-methyl [Actigard; three rates], copper hydroxide [Kocide 3000; two rates], and their combination [six combinations]) with and without thrips exposure. The AUGPC for each replicate per treatment was calculated as follows: $\sum_{i=1}^n [(Y_{i+1} + Y_i)]/2 [X_{i+1} - X_i]$, where Y_i is the bacterial population at the i th observation, X_i is the time in hours at the i th observation, and n is the total number of observations. Analysis of variance was conducted on AUGPC values to determine the significance of treatment effects on *P. ananatis* populations, and Fisher's least significant difference test ($P < 0.05$) was used for mean separations. In addition, the same treatments were compared under thrips exposure and without thrips exposure scenarios and analyzed with a t test at $P = 0.05$.

the current study demonstrated that center rot bulb incidence at either growth stage (bulb initiation and bulb swelling) was reduced under conditions in which onion seedlings were not exposed to thrips but were protected with foliar chemical treatments such as Kocide (1.7 kg/ha), Actigard (35 g/ha), or Kocide (1.7 kg/ha) plus Actigard (35 g/ha). Among the treatments, Kocide performed significantly better than the Actigard-only treatment when applied at either of the two growth stages. Application of Kocide plus Actigard significantly reduced bulb incidence compared with Actigard alone, indicating that most of the control was attributable to Kocide rather than Actigard. It is also important to note that the strain used (PNA 97-1) was reconfirmed to be copper sensitive prior to the greenhouse studies.

When thrips were present on onion plants, the percentage of onion bulbs with center rot was not significantly different regardless of whether plants were treated with Actigard, Kocide, or both (Fig. 1B). Thrips are important in spreading bacteria between onion plants; therefore, center rot incidence in the nontreated control should have been higher when thrips were exposed versus when they were not. However, this was not evident in this study. These observations can partly be explained by the overwhelming effect of thrips despite chemical (Kocide or Actigard) treatments, as they can carry *P. ananatis* and transmit the pathogen to onion plants. Another aspect to consider is that the chemicals used in this study do not affect thrips; hence, bacterial transmission via thrips and associated bulb rot were not significantly different across treatments and the nontreated control. Also, it is possible that the plant defense mechanism might have been overwhelmed when the onion plants were exposed to thrips and, irrespective of the chemical treatments, bulb rot incidence was not reduced for chemically treated versus nontreated plants.

The targeted timing of chemical application at bulb initiation or bulb swelling was not significantly different. Hence, we concentrated on bulb initiation stage-based protection and evaluated different rates of Kocide (two rates: 1.7 and 2.2 kg/ha) or Actigard (three rates: 35, 52.5, and 70 g/ha) or their combinations (six combinations) on various aspects of *P. ananatis* colonization in onion tissue as well as the bacterial presence or absence internally within the thrips. The aspects that were evaluated included population of *P. ananatis* colonization in neck tissue, percentage of thrips that carried internal *P. ananatis* populations in the neck region, expression of *PR1* and *PR5* in onion tissues (72 h after *P. ananatis* inoculation), and ultimately the percentage of center rot-infected bulbs. Under the scenario where the onions were not exposed to thrips, foliar protection with Actigard at three different rates did not significantly influence the growth of *P. ananatis* in internal neck tissue, as the bacterial population was similar to that of the nontreated control (*P. ananatis* only). In both Actigard-only treatments (three rates) and the nontreated control (*P. ananatis* only), *P. ananatis* populations were detected in the onion neck tissue within 7 dpi. In contrast, bacterial populations were not detected in the onion neck tissues until 21 dpi for seedlings that were protected with either Kocide (two rates) or their combinations with Actigard (six combinations). Interestingly, the final populations of *P. ananatis* at 40 dpi with Kocide only (two rates) or their combinations with Actigard (six combinations) were at least four to five orders of magnitude less than what was observed for the Actigard-only treatments (three rates) and the nontreated control (*P. ananatis* only). This was also evident with significantly higher AUGPC values for the Actigard-only treatments (three rates) and the nontreated control (*P. ananatis* only) compared with Kocide-only treatments (two rates) or their combinations with Actigard (six combinations). These observations indicate that Kocide was effective in reducing foliar *P. ananatis* populations compared with Actigard and thereby reducing bacterial populations in onion neck tissues. Since onion neck tissues are a major conduit to bulb infection for foliar *P. ananatis*, it is imperative that a chemical foliar treatment reduces the bacterial populations in foliage and thereby

limits bacterial ingress or colonization in the neck tissue. Reduced bacterial populations in neck tissues may influence bulb infection and further deterioration in storage. We also surmise that the effectiveness of Kocide and Actigard combination treatments was mainly driven by Kocide, as Actigard-only treatments were not significantly different from the *P. ananatis*-only control.

Under the scenario where onions were exposed to thrips, *P. ananatis* populations in internal neck tissue were detected as early as 7 dpi from both chemically treated onion seedlings and the nontreated control (*P. ananatis* only). Bacterial populations in chemically protected plants and the nontreated control (*P. ananatis* only) reached a level of approximately 10^9 to 10^{10} CFU/g by 40 dpi in internal neck tissues. In addition, AUGPC values for the treated plants and the nontreated control (*P. ananatis* only) were not significantly different from each other. When AUGPC values were compared between each chemical treatment and the nontreated control (*P. ananatis* only) under thrips or nonthrips exposure scenarios, significantly higher values were observed in the former scenario than in the latter. When final bacterial populations in internal neck tissues for the chemically treated plants and the nontreated control (*P. ananatis* only) were compared between thrips and nonthrips exposure scenarios, *P. ananatis* populations were at least two to three orders of magnitude higher in the scenario with thrips. Interestingly, the chemical treatments (Kocide only [two rates] or their combinations [six combinations]) that significantly reduced bacterial populations in neck tissues (1×10^4 CFU/g), compared with the nontreated control (*P. ananatis* only; 1×10^8 CFU/g) or Actigard-only treatments (1×10^8 CFU/g), were ineffective in reducing *P. ananatis* populations compared with the nontreated control in the presence of thrips. These observations indicate that thrips exposure and feeding can overcome the efficacy of chemical treatment with Kocide, Actigard, or their combinations. The feeding wounds can open avenues for *P. ananatis* to gain ingress and colonize internal onion foliar tissues then gradually move downward toward neck tissues. The colonization of internal neck tissues with higher bacterial populations increases the risk of bacterial ingress, colonization, and ultimately rotting of onion bulbs.

Chemical treatments (Kocide, Actigard, or their combinations) did not affect the percentage of internal *P. ananatis*-positive thrips, which were sampled from the neck tissue. It is possible that after chemical treatment followed by a *P. ananatis* challenge inoculation, thigmotactic thrips might carry superficial bacterial populations to the internal neck tissues. Internal neck tissues are often favored by thrips for colonization where feeding-mediated damage and subsequent bacterial infection might occur. Effective chemical treatment (Kocide) may reduce superficial foliar bacterial populations; however, thrips infestations may reduce treatment efficacy by inducing feeding-related injuries in foliar tissues and also by migrating to the internal tissue regions where chemical protection is difficult to achieve. It was also observed that despite chemical treatments, thrips were able to carry *P. ananatis* to the internal neck region within 7 dpi and 100% of the thrips were positive for internal bacterial populations by 21 dpi. Aggressive thrips colonization and bacterial transmission in neck tissues increases the risk of bulb rot incidence.

Actigard is a well-known plant defense inducer that induces host defense through induction of key genes (*PR1* and *PR5*) in the SAR pathway against biotrophic pathogens (Glazebrook 2005). Chemical treatment with Actigard only (three rates) or its combination with Kocide (six combinations) significantly induced expression of *PR1* and *PR5* in foliar tissues (72 h after *P. ananatis* inoculation) compared with Kocide only (two rates) or the nontreated control (*P. ananatis* only). Irrespective of thrips exposure, significant differences in expression levels of *PR1* and *PR5* were not observed for the same group of chemical treatment. These observations indicate that thrips exposure did not influence these key SAR pathway genes, which is not surprising because *P. ananatis* most

likely behaves as a hemibiotroph. Based on controlled greenhouse experiments, it was observed that *P. ananatis* could increase to high populations in both live and necrotic onion leaf tissues. In addition, insect herbivory, including thrips feeding, can induce jasmonic acid (JA) pathway events in plants and a JA inducer would potentially be a better alternative than a SAR inducer in the center rot pathosystem (Rahman et al. 2012). Future greenhouse and field studies will evaluate these questions.

Thrips exposure influenced bulb rot incidence. Irrespective of the rates of Kocide, Actigard, or their combinations, center rot incidence was not significantly different for any of the chemical treatments that were exposed to thrips. In fact, bulb incidences were significantly higher for the same treatments that were exposed to thrips compared with those that were not. These observations indicate that the *P. ananatis* population in neck tissue can reach high levels and colonization of thrips in these tissues most likely plays an important role.

Thrips may carry these bacteria externally or internally from foliar tissues to the tight whorl of onion foliage (at the neck) where they prefer to colonize (Palomo et al. 2015). Feeding and defecating in neck regions can result in bacterial transmission and ingress and in further movement and colonization of internal bulb tissues. We acknowledge that mere expression of the SAR pathway genes by Actigard application is not effective in reducing *P. ananatis* population in neck tissues or center rot incidence in the bulb. Grode et al. (2017) made similar observations with increased symptom expression in onion foliage caused by thrips feeding. The authors observed that onion seedlings infested with thrips and inoculated with *P. ananatis* had more necrotic tissue compared with non-exposed onion seedlings. In addition, Grode et al. (2017) also demonstrated that symptom severity increased with an increase in thrips density, and thrips feeding facilitated center rot development by providing entry sites for the bacterium into leaf tissue. A field study demonstrated that when a bactericide spray program was used in conjunction with a thrips management program, a significant reduction in center rot incidence in bulbs was observed compared with when the same bactericide spray was used without a thrips management program (Dutta et al. 2020). These observations reiterate that thrips management is critical in managing center rot in onion.

Overall, it was observed that chemical protection with Kocide during the bulb initiation and bulb swelling stages was effective in reducing center rot incidence in onion bulbs in the absence of thrips. In contrast, similar levels of growth stage-based protection at either the bulb initiation or bulb swelling stages were not observed in the presence of thrips. Actigard displayed limited efficacy, whereas Kocide showed a higher level of efficacy in reducing center rot symptoms in bulbs when applied at the bulb initiation or bulb swelling stage. Furthermore, bulb initiation stage-based protection with different rates of Kocide, Actigard, or their combinations affected various aspects of the *P. ananatis* bulb infection process in the absence of thrips. In contrast, such differences in the bulb infection process or treatment efficacy were not observed in the presence of thrips, as feeding-induced damage can reduce the efficacy of protective chemical treatments against *P. ananatis*. Hence, it is critical to implement an effective management strategy including protective chemical treatments against *P. ananatis*, thrips management using cultural and chemical tactics, and weed management at both growth stages. Grode et al. (2019) demonstrated the benefit of using an effective thrips management program in reducing bacterial stalk rot and leaf necrosis (causal agent: *P. agglomerans*) in onion under field conditions in Michigan. The authors demonstrated that chemical treatments targeting *P. agglomerans* (NuCop 50, Kasugamycin, or Kocide 3000) used along with a thrips management program significantly reduced disease severity compared with when chemical treatments were used alone. Although protective treatments were ineffective when thrips were present, they could provide some level of protection against

bacterial dissemination through rain splash dispersal and other passive modes of pathogen spread onto foliage. However, it is important to understand the biology of thrips–onion–*P. ananatis* infections, including the modes by which thrips bypass foliar chemical protectants. It should be noted that only a single application of these chemical compounds was made in this study, which is not an usual use pattern in the field. Further studies with multiple applications of these chemical compounds should be evaluated under various thrips exposure regimes that may reflect to some extent the conditions a grower may encounter in the field. Developing an improved integrated management program that emphasizes growth stage-targeted protective chemical treatments for *P. ananatis* and rigorous vector management can reduce the frequency of chemical application (copper-based bactericides) and thus minimize input cost. Further studies are designed to explore different bactericide- and insecticide-based programs for the effective integrated disease management against *P. ananatis*.

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