Arthropods in Relation to Plant Disease

Onion Thrips (Thysanoptera: Thripidae) Feeding Promotes Infection By *Pantoea ananatis* in OnionAri Grode,¹ Shicheng Chen,² Edward D. Walker,^{1,2} and Zsafia Szendrei^{1,3}¹Department of Entomology, Michigan State University, East Lansing, MI 48824, ²Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI 48824, and ³Corresponding author, e-mail: szendrei@msu.edu

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Abstract

Onion thrips, *Thrips tabaci* Lindeman, is a primary insect pest of onions (*Allium cepa*) worldwide. Onion thrips cause feeding damage by destroying epidermal tissue. They are also vectors of *Pantoea ananatis* (Serrano) Mergaert, the bacteria that causes center rot. Onions with center rot develop white streaks with water-soaked margins along the onion leaves, which turn necrotic and lead to bulb rot during storage. The role of thrips feeding on the establishment and progression of bacterial infection in onions has not been investigated. Onions infested with thrips and inoculated with *P. ananatis* had more necrotic tissue and symptoms were more severe with increasing thrips density. We conducted a fluorescence microscopy study that examined how *P. ananatis* (expressing a fluorescence protein gene) colonized a control group of onions without thrips in comparison to a test group of onions with thrips. We found that *P. ananatis* colonized some onions in the control group because of naturally existing wounds in the epidermal tissue but more colonization was found in the thrips infested group because of the increased presence of entry points caused by thrips feeding. Overall, our results demonstrate that wounds caused by thrips feeding facilitate center rot development by providing entry sites for the bacteria into leaf tissue.

Key words: *Thrips tabaci*, bacterial center rot, insect–bacteria interaction, fluorescence microscopy

Onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), is a primary insect pest of onions (*Allium cepa* L.). Onion thrips directly affect the United States' onion industry, which is annually valued at \$1 billion (USDA/NASS, 2015). Feeding by onion thrips results in the destruction of epidermal and parenchyma tissue (Chisholm and Lewis 1984, Hunter and Ullman 1989), which leads to chlorophyll loss causing reduced photosynthetic conductivity (Diaz-Montano et al. 2011, Gill et al. 2015). In severe cases, high thrips infestation rates can result in bulb weight reductions of up to 60% (Kendall and Capinera 1987, Fournier et al. 1995, Rueda et al. 2007, Waiganjo et al. 2008, Diaz-Montano et al. 2011). In addition to directly feeding on plant tissue, onion thrips are also known vectors of several onion diseases (Gill et al. 2015). They are able to acquire and transmit *Pantoea ananatis* (Serrano) Mergaert (Gitaitis and Gay 1997, Lewis 1997, Dutta et al. 2014, Dutta et al. 2016). *Pantoea ananatis* and close members of the onion center-rot complex, *Pantoea agglomerans* (syn. *Erwinia herbicola*) and *Pantoea allii* (Brady et al. 2011), produce leaf-symptoms with white streaks and water-soaked margins along the length of the leaf that turn necrotic as the disease progresses; they also cause center rot during storage (Gitaitis and Gay 1997, Vahling-Armstrong et al. 2016). Yield losses associated with center rot may be as high as 100% under favorable conditions (Gitaitis et al. 2002).

Multiple species of thrips are able to acquire *P. ananatis* through feeding and transmit the bacteria through their feces by stercorarial means (Gitaitis et al. 2003; Dutta et al. 2014, 2016). Under laboratory conditions, 20% of onion thrips acquired *P. ananatis* after 1 h of feeding on infected onions and 48 h of feeding resulted in 100% acquisition (Dutta et al. 2014). Center rot symptoms were also observed on 70% of onion seedlings after 15 d of feeding by *P. ananatis*-infected thrips (Dutta et al. 2014). Similarly, 65% of onion seedlings inoculated with rinsates containing *P. ananatis*-infected thrips feces developed center rot symptoms. This indicates that *P. ananatis* survives in the thrips gut (Dutta et al. 2014). However, this relationship has not been tested under field conditions where more biotic and abiotic factors are at play.

Bacterial infections, such as those caused by *P. ananatis*, are spread in agricultural fields during wind and rain events (Wiriyajitsomboon et al. 2014). Recent research in Michigan determined that thrips in commercial onion fields play a role in the transmission of *Pantoea* sp., where higher abundances of onion thrips were correlated with a higher proportion of necrotic leaves in commercial onion fields (Grode 2017). The bacteria frequently enter plant tissue through mechanical wounds (Gitaitis et al. 1978, Gitaitis et al. 2002). A recent field trial demonstrated that a season-long insecticide program led to lower thrips populations as well as reduced bacterial

symptoms, regardless of whether onions were also treated with bactericides (Grode 2017). This suggests that thrips feeding leads to an increase in *Pantoea* sp. infection in the field. A similar relationship has been documented with onion thrips and purple blotch, a fungal disease, where thrips feeding wounds enhanced the development of purple blotch by providing alternative entry points for it (McKenzie et al. 1993). Similarly, feeding wounds caused by onion thrips may provide entry points for *P. ananatis*, leading to increased necrotic leaf tissue and center rot development.

The objectives of this study were to 1) determine the role of onion thrips feeding injury in facilitating the development of *P. ananatis*, 2) investigate the relationship between thrips abundance on plants and center rot development and, 3) quantify morphological changes in onion leaf tissue from onion thrips feeding and *P. ananatis* infection.

Methods and Materials

Onion Thrips Colony

Onion thrips used in these experiments were from a laboratory colony kept in cages (50 cm by 50 cm by 50 cm; MegaView, Taichung, Taiwan) at Michigan State University (East Lansing, MI). Insects originated from populations of onion thrips collected from a commercial onion farm (Stockbridge, MI) in 2015. The colony was maintained on potted onion plants (cv. 'Sedona'; High Mowing Organic Seeds, Wolcott, VT) and allowed to feed ad libitum; new plants were added every one to two wk allowing thrips to transfer from old to new plants. The colony was kept at room temperature (22–25 °C), with ambient humidity.

Experiment 1

'Sedona' onions were seeded (High Mowing Organic Seeds) in 4 cm square pots (FarmTek, Dyersville, IA) and maintained in an environmental growth chamber (28°C, 40% RH, 16:8 [L:D] h). Plants received water and fertilizer (20-20-20 N-P-K; Jack's Professional, J.R. Peters, Inc., Allentown, PA) every other day. After 5 wk, onion plants of uniform size (3–5 leaf stage) were transplanted into 12 cm² pots (The HC Companies: ITML, Middlefield, OH). An experimental unit consisted of a plant surrounded by a ventilated

cylindrical cage (30 cm tall, 2.5 cm wide) made of polyester plastic (ACCO Brands, Inc., Apollo, Lincolnshire, IL), embedded in the soil (2–3 cm deep) with a top made of nylon netting attached with hot glue (160 µm mesh size, MegaView). Cages prevented the movement of thrips from plant to plant but allowed light and air movement into the cages. Plants in the environmental chamber were arranged in a randomized complete block design with six treatments and 15 replications. The six treatments were: 1) untreated control, 2) thrips only, 3) *P. ananatis* only, 4) thrips that were added to plants 7 d prior to *P. ananatis* inoculation, 5) mechanical injury at the same time as *P. ananatis* inoculation, and 6) thrips added at the same time as *P. ananatis* inoculation. Plants receiving thrips were infested with one adult thrips/leaf (three to five thrips per plant). All treatments receiving bacteria (treatments 3, 4, 5, and 6, see Table 1) were inoculated with a virulent strain of *P. ananatis* (~1 × 10⁸ colony-forming unit [CFU]/ml, Dutta et al. 2014), isolated from symptomatic onion plants grown in a commercial onion field (Stockbridge, MI). Inoculations were done using a 250-ml spray bottle (Meijer Inc., Grand Rapids, MI) until leaves were fully wetted. Mechanical damage was inflicted by poking 5 holes per leaf randomly using a pin to represent mechanical damage that may happen in the field. Plants were grown for a total of 14 d in the environmental growth chamber then returned to the laboratory for rating, where they were removed from the soil and placed singly in zip-top bags to retain thrips for later counting.

The number of leaves per plant and the length of each leaf were recorded prior to adding treatments and after the 14-d experimental period. Total leaf area was calculated using leaf length as an index of leaf area (Gamiely et al. 1991). Growth of each plant was calculated by subtracting the total area of each plant prior to thrips infestation from the total leaf area of each plant measured at the end of the experiment. The percentage of leaf area affected by *P. ananatis* and thrips feeding was visually estimated for each plant (Horsfall and Barratt 1945). Thrips damage was assessed by estimating the percentage of leaf area fed on by thrips and the total number of adults and juveniles were counted on each plant.

Five symptomatic plants were randomly selected from each treatment for bacterial isolation and identification. Leaf tissue from water-soaked leaf margins was excised (1.0 cm²) using a sterile

Table 1. The effects of onion thrips (*T. tabaci*), bacterial leaf blight (*P. ananatis*), and mechanical damage (five pricks using a pin) on onions

Damage Parameters	Treatments ^{a,b} (mean ± SE)					
	–/– (1)	th/– (2)	–/pa (3)	th/pa (4)	md/pa (5)	th/pa [st] (6)
Number of leaves	6.00 ± 0.17a	6.06 ± 0.18a	6.13 ± 0.17a	5.93 ± 0.12a	6.13 ± 0.17a	6.33 ± 0.21a
Total leaf area, cm ² ^c	584.67 ± 26.78a	520.62 ± 33.98a	586.56 ± 31.16a	512.45 ± 44.23a	547.62 ± 23.73a	589.38 ± 38.04a
Growth, cm ² ^d	456.56 ± 18.95a	414.79 ± 19.40a	463.15 ± 19.40a	411.34 ± 35.77a	426.10 ± 14.20a	463.46 ± 20.50a
Estimated % leaf blight ^e	0.00 ± 0.00b	0.00 ± 0.00b	1.93 ± 0.50a	2.13 ± 0.27a	1.46 ± 0.39a	1.80 ± 0.28a
Estimated % thrips injury ^f	0.00 ± 0.00b	9.30 ± 2.41a	0.00 ± 0.00b	10.60 ± 2.75a	0.00 ± 0.00b	2.60 ± 0.69b
Thrips/plant ^g	0.00 ± 0.00c	3.86 ± 0.60b	0.00 ± 0.00c	4.26 ± 0.88b	0.00 ± 0.00c	12.60 ± 1.47a

When thrips and bacteria were present together, bacteria were applied to plants either 7 d after thrips infestation or simultaneously. Thrips (three to five adults per plant) were taken from a laboratory colony. Bacterial solution (1 × 10⁸ CFU) was applied to plants using a hand spray bottle. Experiments (*n* = 15) were conducted for 14 d in a growth chamber at Michigan State University, East Lansing, MI. Numbers (1–6) in the table correspond to treatment numbers for Experiment 1.

^aDifferent letters within a row indicate differences among treatments, Tukey's HSD, α < 0.05.

^bThrips infestation (th), *P. ananatis* inoculation (pa), mechanical damage (md), thrips and bacteria added at the same time [st].

^cGamiely et al. (1991).

^dGrowth = total leaf area prior to rating – total leaf area 7 d after inoculation. ^eEstimated percentage of leaf damage from bacterial leaf blight cause by *P. ananatis* (Horsfall and Barratt 1945).

^fEstimated percentage of leaf damage from thrips feeding.

^gNumber of thrips counted on each plant.

scalpel blade (Bard-Parker, Becton Dickinson Acute Care, Franklin Lakes, NJ), dipped briefly in 95% ethanol, rinsed with sterile distilled water twice, dried for 3 min, and placed on a microscope slide. The disinfected leaf piece was macerated using a sterile scalpel with a no. 10 blade (Bard-Parker). A 50 μ l drop of sterile distilled water was pipetted onto the cut leaf, where bacterial cells were released from the tissue into the water. The suspension was streaked onto nutrient broth yeast extract (NBY) agar using a sterile bacterial loop, and incubated at 28°C for 2 d. Each bacterial colony that had different morphological characteristics (form and color) was selected and transferred once to fresh NBY agar resulting in pure cultures for identification.

Representative colonies were identified to species by sequencing the small subunit of the bacterial ribosomal DNA gene. First, DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). DNA was quantified using a Qubit 2.0 Fluorometer and Qubit dsDNA HS Assay Kit (ThermoFisher Scientific Inc., Waltham, MA). The 16S rDNA gene was amplified with a 16S primer pair (forward primer, AGTTTGATCCTGGCTCAG, reverse primer TACCTTGTACGACTTCGTCCTCA; De Baere et al. 2004). A total volume of 50 μ l polymerase chain reaction (PCR) mixture contained, 38.5 μ l PCR-grade H₂O, 10 $\times$ PCR buffer, 0.2 μ M deoxynucleotide (*dNTP*), 0.2 μ M of each primer, and 0.625 U Taq polymerase (Promega, Madison, WI). PCRs were conducted in a Mastercycler Pro thermocycler (Eppendorf, Hauppauge, NY) with an initial denaturation at 94°C for 5 min, followed by 3 cycles of denaturation at 94°C for 45 s, annealing at 52°C for 2 min, and extension at 72°C for 1 min, and another 30 cycles of denaturation at 94°C for 20 s, annealing at 52°C for 1 min, and extension at 72°C for 1 min. The final extension step was performed at 72°C for 7 min. The PCR products were visualized on 1.5% agarose gel stained with 7.5 μ l GelRed nucleic acid stain (Phenix Research Products, Candler, NC). Reactions with sufficient PCR product were purified using the QIAQuick PCR Purification Kit (Qiagen) and sequenced with a single primer by submitting to the Michigan State University Genomics Core Facility (East Lansing, MI). The nucleotide sequences were compared to the nucleotide collection in NCBI using a BLAST nucleotide search analysis.

Mean plant growth, leaf area, number of leaves, bacterial leaf blight severity, thrips damage, and thrips per plant were compared among treatments using an analysis of variance (ANOVA). All of the variables met the assumptions of ANOVA. Tukey's honest significant difference (HSD) tests were used to determine differences among treatment means. All statistical analyses were conducted using R software (R Core Development Team, 2015).

Experiment 2

Plants in this experiment were grown and maintained as described in Experiment 1 except they were grown for 7 wk (4–6 leaf stage). To measure the effects of varying degrees of thrips feeding damage on bacterial leaf blight severity, plants were arranged in a randomized complete block design with 4 treatments and 11 replications. An experimental unit consisted of a plant surrounded by a ventilated cylindrical cage as described in Experiment 1. The four treatments included: 1) *P. ananatis* only, 2) 4 thrips per plant with *P. ananatis*, 3) 12 thrips per plant with *P. ananatis*, and 4) 20 thrips per plant with *P. ananatis*.

Eight days after thrips infestation, all treatments were inoculated with a virulent strain of *P. ananatis* ($\sim 1 \times 10^8$ CFU/ml) as described in Experiment 1. Onions were rated 7 d following bacterial inoculation as described in Experiment 1.

Mean plant growth, leaf area, number of leaves, bacterial leaf blight severity, thrips damage, and thrips per plant were compared among treatments using an ANOVA. All of the variables met the assumptions of ANOVA. Tukey's HSD tests were used to determine differences among treatment means. Pearson product-moment correlation coefficients were computed to assess the relationships between the number of thrips present on plants or thrips feeding damage (%) and leaf blight severity.

Experiment 3

To visually assess how *P. ananatis* infects onions, plasmid pSCH476, derived from the wide-range vector PBBR1MCS-3 in Gram-negative bacteria, contained a green fluorescent protein (*gfp*) gene expression cassette and a tetracycline resistance gene. It was successfully used by Chen and Hickey 2011 and was, therefore, chosen to label *P. ananatis*.

To make the competent cells for transformation of pSCH476, a pathogenic *P. ananatis* strain was grown for 24 h at 28°C in 10 ml of Luria-Bertani (LB) liquid medium, harvested by centrifugation at 4°C, and resuspended in 25 ml of ultrapure H₂O. After washing three times with water, cells were resuspended in 2 ml of 10% ice cold glycerol (J.T. Baker—Avantor, Center Valley, PA). Aliquots (200 μ l) of cells were electroporated (2.5 kV, 25 mA, 25 μ F, and 400 Ω) with 0.165 ng plasmid. After transformation, 1 ml LB broth was added, incubated for 1 h at 28°C, plated on LB medium supplemented with tetracycline (50 μ g/ml) and grown at 28°C for 48 h. Colonies emitting GFP fluorescence under ultra violet (UV) light were purified and used for later study.

'Sedona' onions were grown and kept in an environmental chamber as described in Experiment 1. Plants were arranged in a randomized complete block design with four treatments and three replications. The four treatments included: 1) an untreated control, 2) thrips injury alone, 3) *P. ananatis* alone, and 4) thrips injury in combination with *P. ananatis* inoculation. Plants receiving thrips were infested with 12 adult thrips per plant.

Eight days after thrips infestation, all treatments were inoculated with the GFP strain of *P. ananatis* ($\sim 1 \times 10^8$ CFU/ml) using a 250-ml spray bottle until leaves were fully wetted and started to drip. Plants were maintained as described in Experiment 1.

Leaf tissue was compared by excising three leaf pieces from each plant ($n = 36$). Areas where thrips feeding injury was present were chosen in those treatments which received thrips (treatments 2 and 4, see Table 1), symptomatic areas were chosen in treatments inoculated with bacteria (treatments 3 and 4), and random areas were chosen in the treatment where plants were untreated (treatment 1). Leaf tissue was excised (1.0 cm²) and surface sterilized as described in Experiment 1. The resulting suspension was diluted 10-fold, twice, and 50 μ l of each concentration of the dilution series was streaked onto LB medium supplemented with tetracycline (25 μ g/ml) and incubated at 28 °C for 48 h. The number of colonies with the GFP fluorescence was identified using UV light, counted on each plate containing the 1:100 dilution and multiplied by 100 in order to calculate total bacterial density. Mean bacterial density (CFU) per cm² of leaf tissue was compared between treatments using a *t*-test. The number of CFUs was log transformed to meet normality requirements of a *t*-test.

For microscopy, onion plants were removed from pots 8 d after inoculation and washed under running tap water. Leaf tissue (1.0 cm²) was excised and mounted on a microscope slide. Areas where thrips feeding injury was present were chosen in those treatments which received thrips (treatments 2 and 4), symptomatic areas

Table 2. Effects of varying numbers of onion thrips (*T. tabaci*) per plant and bacterial leaf blight (*P. ananatis*) on onions

Damage Parameters	Treatments ^{a,b} (mean ± SE)			
	0 th/pa (1)	4 th/- (2)	12 th/pa (3)	20 th/pa (4)
Number of leaves	7.73 ± 0.20a	7.18 ± 0.26a	7.36 ± 0.20a	7.36 ± 0.28a
Total leaf area, cm ² ^c	1006.92 ± 42.78a	821.51 ± 52.79a	877.18 ± 41.09a	956.82 ± 64.90a
Growth, cm ² ^d	558.78 ± 33.90a	374.23 ± 52.57a	446.59 ± 42.00a	486.42 ± 56.65a
Estimated % leaf blight ^e	11.27 ± 0.54b	14.09 ± 0.80b	13.27 ± 0.96b	19.72 ± 1.59a
Estimated % thrips injury ^f	0.00 ± 0.00b	3.45 ± 0.53b	15.72 ± 1.65a	18.09 ± 2.61a
Thrips per plant ^g	0.00 ± 0.00b	1.63 ± 0.53b	6.18 ± 0.67ab	12.27 ± 3.16a

Thrips (0, 4, 12, or 20 per plant) were added to plants from a laboratory colony. Plants were inoculated with a liquid culture of *P. ananatis* (1×10^8 CFU), sprayed onto plants. Experiments ($n = 11$) were conducted for 15 d in a growth chamber at Michigan State University, East Lansing, MI. Numbers (1–4) in the table correspond to treatment numbers for *Experiment 2*.

^aDifferent letters within a row indicate differences among treatments, Tukey's HSD, $\alpha < 0.05$.

^bThrips infestation (th), *P. ananatis* inoculation (pa), mechanical damage (md), thrips, and bacteria added at the same time (st).

^cGamiely et al. (1991).

^dGrowth = total leaf area prior to rating – total leaf area 7 d after inoculation.

^eEstimated percentage of leaf damage from bacterial leaf blight cause by *P. ananatis* (Horsfall and Barratt 1945).

^fEstimated percentage of leaf damage from thrips feeding.

^gNumber of thrips counted on each plant.

were chosen in treatments inoculated with bacteria (treatments 3 and 4), and random areas were chosen on untreated plants (treatment 1). Fluorescence microscopy was carried out on a Nikon Eclipse E800 epifluorescence microscope (Nikon Corporation, Tokyo, Japan) with a Nikon B-2A fluorescence filter (Nikon Corporation). GFP-tagged bacterial cells were excited using a 490 nm filter and the images were captured with a Samsung Galaxy S6 Active camera (Samsung Group, Seoul, South Korea).

Results

Sequencing of the 16S rDNA indicated that *P. ananatis* was present in 100% of our treatments that were inoculated with the bacteria. None of the untreated or thrips only treatments were positive for *P. ananatis*. The most similar GenBank sequence results for our bacterial isolates were *P. ananatis* (96–98% match, accession KT957000.1).

Experiment 1

The number of leaves per plant and total leaf area before manipulation were statistically similar between treatments (leaf number: $F = 0.95$, $df = 5, 70$, $P = 0.45$; leaf area: $F = 0.54$, $df = 5, 70$, $P = 0.75$). We did not find statistical differences among the treatments at the end of the experiment in the number of leaves per plant, total leaf area, and plant growth (leaf number: $F = 0.68$, $df = 5, 70$, $P = 0.64$; leaf area: $F = 1.14$, $df = 5, 70$, $P = 0.35$; growth: $F = 1.35$, $df = 5, 70$, $P = 0.25$; Table 1). All treatments inoculated with *P. ananatis* had significantly more bacterial leaf blight symptoms than treatments which did not receive bacteria ($t > 3.39$, $df = 5$, $P < 0.01$; Table 1). The percentage of tissue damaged by thrips was significantly higher in treatments with thrips alone (treatment 2) and thrips with *P. ananatis* (treatment 4) were compared with all other treatments ($t > 4.60$, $df = 5$, $P < 0.01$; Table 1). Significantly more thrips were counted on all treatments, which received thrips relative to those without thrips ($t > 3.87$, $df = 5$, $P < 0.01$; Table 1). There were approximately three times more thrips on the treatment that received *P. ananatis* with thrips

Fig. 1. Correlation between the number of onion thrips (*T. tabaci*) per plant and the percent leaf area with leaf blight symptoms ($y = 0.4134x + 12.929$) (A). Correlation between the percent leaf area with thrips feeding damage and the percent leaf area with leaf blight symptoms ($y = 0.3314x + 11.580$) (B). Data were combined for all treatments from *Experiment 2*, which received thrips (treatments 2, 3, and 4; 4–20 thrips per plant). Plants were inoculated with a liquid culture of *P. ananatis* (1×10^8 CFU), sprayed onto plants. Experiments ($n = 11$) were conducted in a growth chamber at Michigan State University, East Lansing, MI.

simultaneously (treatment 6) compared with the other two treatments with thrips (treatments 2 and 4) ($t > 8.06$ df = 5, $P < 0.01$; Table 1).

Experiment 2

The number of leaves per plant and total leaf area before manipulation did not differ between treatments (number of leaves: $F = 0.95$, df = 3, 30 $P = 0.45$; leaf area: $F = 0.54$, df = 3, 30 $P = 0.75$). The number of leaves per plant, total leaf area, and plant growth were similar among treatments at the end of the experiment (leaf number: $F = 1.00$, df = 3, 30, $P = 0.41$; leaf area: $F = 2.23$, df = 3, 30, $P = 0.11$; growth: $F = 2.28$, df = 3, 30, $P = 0.10$; Table 2). The treatment that received 20 thrips exhibited the most severe blight symptoms at the end of the experiment, with about 35% more leaf tissue necrosis than any of the other treatments ($t = 5.18$, df = 3, $P < 0.01$; Table 2). The percentage of leaf tissue damaged by thrips was 12 and 15% higher when initial infestation was 12 and 20 thrips per plant, respectively (treatments 3 and 4) ($t = 5.16$, df = 3, $P < 0.01$; Table 2), compared with the treatments with lower numbers of thrips. The number of thrips per plant at the end of the experiment was seven times higher when initial thrips infestation was 20 thrips per plant (treatment 4) ($t = 2.31$, df = 3, $P < 0.01$; Table 2) compared to when initial infestation was 0 or 4 thrips per plant (treatments 1 and 2).

There was a significant positive correlation, across all treatments, between the number of thrips on each plant and the percent leaf area damaged by *P. ananatis* ($R^2 = 0.68$, $P < 0.01$; Fig. 1A). Thrips feeding damage symptoms on leaves (%) and severity of leaf blight symptoms (%) were positively correlated ($R^2 = 0.70$, $P < 0.01$; Fig. 1B).

Experiment 3

P. ananatis was not recovered from any of the treatments which did not receive bacteria (treatments 1 and 2; numbers correspond to treatment numbers in Table 1). *P. ananatis* colonies containing the GFP gene were cultured from symptomatic leaf tissue on plants which received *P. ananatis* without thrips (treatment 3) and *P. ananatis* in combination with thrips (treatment 4) with mean bacterial densities of 5.83×10^5 and 3.01×10^5 CFU, respectively. Fluorescent colonies were also isolated from random nonsymptomatic areas on plants that received *P. ananatis* alone (treatment 3) and from feeding sites on plants that received *P. ananatis* in combination with thrips (treatment 4) with mean bacterial densities of 1.44×10^3 and 1.58×10^5 CFU, respectively. The average bacterial density per plant did not differ between treatments ($t = 1.75$, df = 34, $P = 0.09$). Bacterial density on leaf tissue with disease symptoms from plants which received *P. ananatis* without thrips (treatment 3) compared with symptomatic leaf tissue from plants which received *P. ananatis*

Fig. 2. Representative samples of undamaged onion leaf tissue (A), onion tissue damaged by thrips (*T. tabaci*) feeding (B), necrotic leaf tissue damaged by fluorescent *P. ananatis* (C), and onion leaf tissue damaged by both thrips feeding injury and fluorescent *P. ananatis* (D). White arrows (a) indicate where thrips mandible was inserted into the leaf tissue. Yellow pigmentation is the result of chlorophyll removal by thrips feeding (B, D). Fluorescent *P. ananatis* colonizing intercellular spaces of leaf cells (C) and regions where cells were damaged by thrips feeding (D), indicated by the white circles (b and c, respectively). Plants were inoculated with a liquid culture of *P. ananatis* (1×10^8 CFU), sprayed onto plants. Experiments were conducted in a growth chamber at Michigan State University, East Lansing, MI. Photos were taken at 10x magnification and each square of the reticle is 50 μm^2 .

in combination with thrips (treatment 4) was statistically similar ($t = 0.95$, $df = 16$, $P = 0.36$). On plants that received both *P. ananatis* and thrips, bacterial density was significantly higher in leaf tissue containing thrips feeding damage (treatment 4) compared with areas without thrips feeding damage (treatment 3) ($t = 3.32$, $df = 16$, $P < 0.01$).

P. ananatis was not detected in leaf tissue without bacterial application (Fig. 2A and B). Fluorescence microscopy confirmed the colonization of onion leaf tissue by the GFP strain of *P. ananatis*, mainly in intercellular regions of damaged cells (Fig. 2C). Fluorescent *P. ananatis* colonized thrips feeding sites, resulting in necrotic lesions (Fig. 2D).

Discussion

Our results suggest that onion thrips feeding injury promotes the colonization of onion leaves by *P. ananatis* and bacterial symptoms are more abundant with increasing thrips numbers. Previous research demonstrated that thrips were able to acquire this pathogen after a few hours of feeding on an infected plant and that bacteria was able to survive transstadially without affecting the fitness of the insect host (Dutta et al. 2014, 2016). Thrips are able to spread this pathogen to new plants through their feces (Dutta et al. 2014, 2016), and their feeding injury provides entry points for bacterial infection. A similar relationship was recorded, where the feeding injury of thrips promoted the development of purple blotch by providing alternative penetration sites for *A. porri* (McKenzie et al. 1993). By utilizing fluorescent microscopy, we visualized as the GFP tagged strain of *P. ananatis* colonized areas of the onion leaf tissue damaged by thrips, providing evidence that feeding sites act as disease entry points.

Our results demonstrate that as the number of thrips per plant increases, so do the symptoms of bacterial center rot caused by *P. ananatis* (Fig. 1). These results are similar to previous findings from an insecticide trial where plots of onions receiving insecticides through the growing season had significantly less necrotic leaf tissue compared with plots with unmanaged thrips populations (Grode 2017). Onion thrips reproduce asexually through parthenogenesis and the rate of reproduction is dependent on environmental conditions. Under hot and dry conditions, onion thrips oviposition rates increase and generation time decreases, leading to rapid population growth (Rueda et al. 2007, Gill et al. 2015). Therefore, it is important for onion growers to manage onion thrips populations, particularly during hot weather, in order to minimize the development of bacterial rot in onions. Future research should focus on understanding the application threshold for insecticides for reducing thrips populations along with bacterial symptoms.

The interaction between onion thrips and *P. ananatis* during the early growing season is poorly understood, and while the inoculum source of *P. ananatis* is currently unknown, it has been previously identified from onion seeds (Walcott et al. 2002) and a variety of other crops (Bruton et al. 1986, Wells et al. 1987, Bruton et al. 1991, Azad et al. 2000, Coutinho et al. 2002). This bacterium has also been identified as a common epiphyte of various weed species (Gitaitis et al. 2002). Onion thrips are known to overwinter on many weed species surrounding onion fields (Smith et al. 2011) before colonizing onions in the spring (Larentzaki et al. 2007). Monitoring and managing weed species early in the growing season for both onion thrips and *P. ananatis* may prevent or slow the disease from spreading to the field. A better understanding of this interaction may provide insights for improving early-season management of this pest complex.

Our laboratory results will need to be verified under field conditions in the future since less is understood about *P. ananatis* and thrips relationships in the field. Bacterial pathogens, such as *P. ananatis*, can be disseminated throughout the field via wind, splashing water (Wiriyajitsomboon et al. 2014), and contaminated machinery (Gitaitis et al. 1978), therefore the relative contribution of thrips and different abiotic factors to pathogen spread in onion fields needs to be further explored. In addition, while field research demonstrated that onion thrips are contributing to the spread of a closely related pathogen, *P. agglomerans* (Grode 2017), the acquisition rates for *P. ananatis* and *P. agglomerans* are not the same (Dutta et al. 2014), thus results with one pathogen should not be readily transferred to the other.

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