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Root Exudation by Aphid Leaf Infestation Recruits Root-Associated *Paenibacillus* spp. to Lead Plant Insect Susceptibility

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Aphids are a large group of hemipteran pests that affect the physiology, growth, and development of plants by using piercing mouthparts to consume fluids from the host. Based an recent data, aphids modulate the microbiomes of plants and thereby affect the overall outcome of the biological interaction. However, in a few reports, aboveground aphids manipulate the metabolism of the host and facilitate infestations by rhizosphere bacteria (rhizobacteria). In this study, we evaluated whether aphids alter the plant resistance that is mediated by the bacterial community of the root system. The rhizobacteria were affected by aphid infestation of pepper, and a large population of gram-positive bacteria was detected. Notably, *Paenibacillus* spp. were the unique gram-positive bacteria to respond to changes induced by the aphids. *Paenibacillus polymyxa* E681 was used as a rhizobacterium model to assess the recruitment of bacteria to the rhizosphere by the phloem-sucking of aphids and to test the effect of *P. polymyxa* on the susceptibility of plants to aphids. The root exudates secreted from peppers infested with aphids increased the growth rate of *P. polymyxa* E681. The application of *P. polymyxa* E681 to pepper roots promoted the colonization of aphids within 2 days of inoculation. Collectively, our results suggest that aphid infestation modulated the root exudation, which led to the recruitment of rhizobacteria that manipulated the resistance of peppers to aphids. In this study, new information is provided on how the infestation of insects is facilitated through insect-derived modulation of plant resistance with the attraction of gram-positive rhizobacteria.

Keywords: Tritrophic interaction, root exudate, *Paenibacillus* spp., *Myzus persicae*, gram-positive rhizobacteria

Introduction

The study of the interactions between insects and other animals, plants, and microorganisms increases the understanding of ecological systems in nature [7]. The interactions that are mediated by plants between aboveground insects and belowground root-associated bacteria (rhizobacteria) are of particular interest [21]. Specific rhizobacteria elicit defensive responses in plants against insect attack through the modulation of plant hormones, primarily jasmonic acid (JA), and ethylene signaling [9, 19, 23, 35]. For example, root treatment with *Pseudomonas fluorescens* WCS417r reduces the growth and development of the leaf-chewing caterpillar *Spodoptera exigua* on *Arabidopsis* [36], and on tomatoes, the inoculation of roots with *Bacillus subtilis* retarded the development of phloem-sucking whiteflies *Bemisia tabaci* [34]. By contrast, increases in insect performance following root colonization by rhizobacteria were recently reported [5, 22, 28], although the details of the modes of action have not been studied extensively. For example, the pre-inoculation of roots with *P. fluorescens* increased the weight gain and intrinsic growth rate of aphids on *Arabidopsis* and promoted the rate of development and survival of whiteflies on tomato [22, 28].

Besides belowground soil bacteria altering the fitness of aboveground insects, foliar insects, primarily piercing hemipterans such as aphids and whiteflies, also modulate the community of rhizosphere bacteria [6, 11, 15, 28, 37, 40].

The barley aphid *Rhopalosiphum padi* increased the number of rhizobacteria depending on the phase of plant growth [37], and similarly, a whitefly *B. tabaci* infestation led to the accumulation of a specific bacterial community cluster on the roots of tomatoes [28]. In addition to alterations in the number and composition of the rhizobacterial community by phloem-sucking insects, many unanswered questions remain; for example, (i) what are the specific bacterial groups that are affected by phloem-sucking insects within the bacterial community of the rhizosphere; and (ii) how does the phloem-sucking insects modulate the signal transduction from aboveground to belowground and change root exudates to modify the bacteria community?

In a series of recent studies, the feeding of insects mediated the attraction of specific soil bacteria to the proximity of the root [11, 28, 37, 40]. With phloem-sucking whiteflies on pepper leaves, the numbers of gram-positive soil bacteria increased, including *Bacillus* and actinomycete groups, whereas the total population of bacteria and the gram-negative bacteria were not significantly affected [11, 40]. In a further evaluation of aphids phloem-sucking on pepper plant leaves, among the introduced diversity of rhizobacteria, only the population of *B. subtilis* was significantly larger than that on control plants without aphids [11]. However, except for the introduced bacteria, the *Bacillus* species that are recruited by aboveground insect infestations remain unknown. The effects of leaf-feeding insects on root rhizobacteria populations are likely attributed to modifications of root exudates [1, 19], and the effects of aboveground herbivory on changes in the composition of root exudates were reported previously [29, 32]. The cabbage looper on *Centaurea maculosa* stimulates allelopathic exudations from the roots [32], and in a recent study, when tobacco leaves are infested by whiteflies, the concentration of salicylic acid (SA) is augmented in root exudates leading to suppression of the virulence of the soil-pathogenic bacterium *Agrobacterium tumefaciens* [29].

Few studies have examined the role of root exudates in the selection of *Bacillus* groups or the role of exudates in affecting plant fitness by altering insect pathogenicity on plant hosts. Similarly, few studies examined the effects of an aboveground insect herbivory on modulation of the belowground microbiota and plant immunity. In this study, we used aphids and pepper plants as the model insect-plant system to determine which *Bacillus* species in the rhizosphere of pepper seedlings grown in field soil were recruited by the aboveground aphid infestation on leaves. Based on our results, *Paenibacillus* spp. was the dominant bacilli group that was dependent on aphid phloem-sucking. Additionally, the pretreatment with *P. polymyxa*, as a model *Paenibacillus* spp., increased the number of aphids when the insects were subsequently inoculated on newly developed leaves. Thus, new insights were gained in this study, and the aboveground phloem-sucking by aphids affected the secretions of root exudates and attracted *Paenibacillus* spp. to the rhizosphere. Moreover, with large population of *Paenibacillus* spp. in the vicinity of the root system, the susceptibility of the plant to aphids increased.

Materials and Methods

Plant Preparations and Growth Conditions

The pepper (*Capsicum annuum* L. 'PR'; Heung-Nong Seed Co., Korea) seeds were surface-sterilized with 6% sodium hypochlorite for 5 min, washed five times with sterile distilled water (SDW) and then incubated on water agar media at 25°C for 7 days with a photoperiod of 8 h light/16 h dark until germination. The germinated seeds were planted on soil-free medium (Punon Horticulture Nursery Media Low; Punong Co. Ltd., Gyeongju, Korea) and grown at 25°C under fluorescent light (12 h day/12 h night cycle, approx. 7,000 lux light intensity) in a controlled-environment growth room. The 3-week-old pepper seedlings were transplanted to unsterilized field soil in which peppers are routinely grown at 36°26'26''N 126°26'46''E (14, Mukdong-gil, Cheongyang-eup, Cheongyang-gun, Chungcheongnam-do, Korea) and placed in the KRIBB greenhouse facility in Daejeon, South Korea. The chemical and physical properties of the field soil were evaluated by Industry-University Cooperation Foundation at Chungbuk National University in Cheongju, South Korea as described previously [27, 33, 38]. To avoid contamination from fungi or other insects, the pepper seedlings were placed in transparent acrylic cylinders with the tops covered by stockings [40].

Inoculation of Aphid

Ten green peach aphid Myzus persicae (Sulzer) aphids were applied to one leaf per plant, a week after transplanting the peppers to the field soil, as described previously [11]. Soil drenches of 50 ml of 1 mM suspension of benzo (1,2,3) thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) (Syngenta, NC, USA) and 50 ml of SDW were used as the positive and the negative controls, respectively.

Measurement of Root-Associated Bacteria Populations

To assess the effect of aphid infestation on the rhizobacterial community, the plant roots were collected at 0, 1, and 2 weeks after treatment with the aphids, BTH, and SDW. The plant roots were transferred to a 30 ml conical tube (SPL Life Sciences, Pocheon, S. Korea) that contained 10 ml of SDW and shaken for 30 min. To select the gram-positive bacteria, the samples were treated at 70°C for 30 min and then plated on 1/10 strength TSA (Tryptic Soy Broth agar; Difco Laboratories, Detroit, MI, USA) using a dilution plating method [40]. The plated samples were cultured at 30°C for

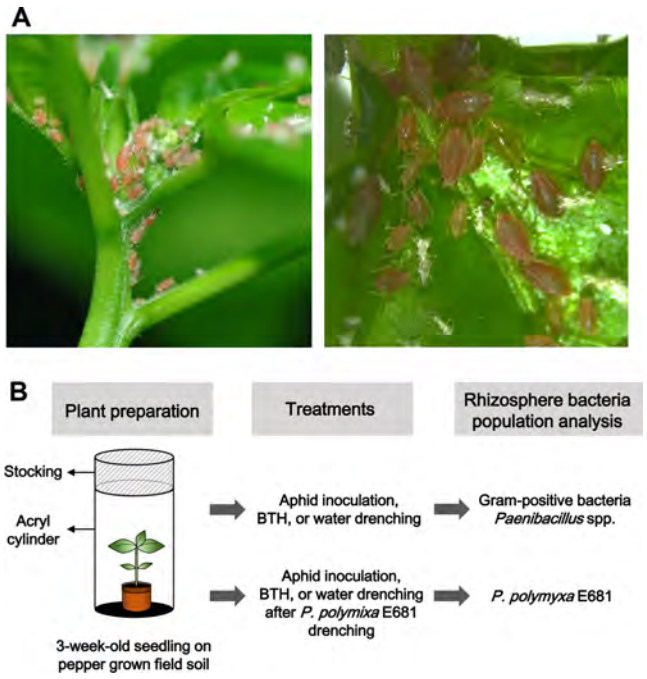

Fig. 1. Aphid infestation and experimental design in the greenhouse.
(**A**) Seven days after aphid inoculation. (**B**) Schematic representation of the experiment in the greenhouse. Pepper seedlings at 3 weeks of age were transplanted to field soil used to grow peppers and placed in acrylic cylinders, which were covered with stockings to prevent attack from other insects. Populations of the gram-positive bacteria, *Paenibacillus* spp. and *P. polymyxa* E681, were determined after aphid, BTH, and water treatments. Plants were pretreated with *P. polymyxa* E681 before aphid, BTH, and water treatments.

2 days. Counts of bacterial colonies were used to calculate the colony-forming units (CFU) [38]. Morphology was used to select the *Paenibacillus* spp. colonies, which were whitish, circular to slightly irregular, convex, and mucoid, with entire to undulate margins [3, 24]. The identifications were verified with 16S rRNA sequencing. Rifampicin-resistant *P. polymyxa* E681 was grown for 24 h at 30°C on TSA medium that contained 100 μg/ml of rifampicin [26]. The E681 cell suspension was adjusted to OD_{600} = 1 (9.9×10^8 CFU/ml) and was applied as a 50 ml drench on the plant roots. A week after drenching the E681 cell suspension, the plants were treated with aphids, BTH, or SDW. The plant roots were collected 2 weeks after the treatments, incubated in SDW for 30 min in a shaking incubator at 30°C, and then spread on TSA medium that contained 100 μg/ml of rifampicin [11]. The experiment had a completely randomized design with five replications and was independently repeated three times.

Harvest of Root Exudates Under Axenic Conditions

Sterile, narrow-neck glass bottles (100 ml; Duran) were filled with a half-strength basal salt mixture of Murashige and Skoog (BSMS; Duchefa Biochemie, the Netherlands) broth medium. The necks of the bottles were covered with UV-sterile parafilm. To transplant a 1-week-old pepper seedling, a hole was made in the center of the parafilm with a sterile injection needle [29]. The plants (-bottle complexes) were placed in an incu-tissue box (SPL Life Sciences Co. Ltd., Pochen, Korea). After these steps were completed, the plants were grown in a growth chamber at 23°C with a photoperiod of 8 h light/16 h dark. To obtain the root exudates of aphid BTH-, and SDW-treated plants, the newly developed leaves of 5-week-old seedlings were inoculated with 20 aphids for aphid treatment and infiltrated with 0.5 mM BTH or SDW using a 1 ml syringe without needle for BTH and SDW treatments [29] (Fig. 4A). A week after treatment, the root exudates were collected and spread on TSA medium to check for contamination. The root exudates were filtrated with an injection filter (0.45 μm pore; Sartorius Stedim Biotech GmbH, Goettingen, Germany) and stored at −20°C before use.

Assessment of the Growth Rate of *P. polymyxa* with the Amendment of Root Exudate

To study the growth rate of *P. polymyxa* E681 on root exudate, the bacteria were grown in TSB medium that contained 12.5% and 50% root exudate in the wells of 96-well plates. The 12.5% root exudate treatment was composed of 50 μl of TSB, 37.5 μl of half-strength BSMS broth, and 12.5 μl of root exudate for a total of 100 μl. The 50% root exudate treatment was composed of 50 μl of TSB and 50 μl of root exudate. The optical density of *P. polymyxa* E681 was measured using a 96-well plate spectrophotometer (Infinite 200 PRO NanoQuant; Techan, Männedorf, Switzerland) at λ = 600 nm. The heat-treated root exudates were autoclaved at 121°C for 15 min. The experiment had a completely randomized design with four replications and was independently repeated four times.

Effect of Root Colonization with *P. polymyxa* E681 on the Aphid Infestation

The *P. polymyxa* E681 was grown in TSA medium at 30°C for 24 h. The bacterial cells were collected, suspended in SDW, and adjusted to OD_{600} = 3 and OD_{600} = 0.1. The bacterial cell suspension (OD_{600} = 3), 1 mM BTH, and SDW were drenched in 50 ml on the roots of 3-week-old pepper plants. Simultaneously, the plant leaf was infiltrated with the bacterial cell suspension (OD_{600} = 0.1), 0.5 mM BTH, and SDW. The experiment was conducted in the KRIBB greenhouse facility in Daejeon, South Korea. A week after treatment with E681, BTH, and SDW, the plants were inoculated with 10 aphids on a plant leaf. The number of aphids per plant was counted 2, 4, 6, 8, 10, and 12 days after aphid inoculation. The experiment had a completely randomized design with eight replications and was independently repeated two times.

Statistical Analyses

Analysis of variance of the experimental data was performed using JMP software ver. 5.0 (SAS Institute, Cary, NC, USA). The

significant effects of the treatments were determined by the magnitude of the F-value (p = 0.05). When a significant F-value was obtained, the means were separated by Fisher's protected least significant difference (LSD) tests at p = 0.05 [11, 38].

Results

Effect of Aphid Infestation on the Number of Rhizobacteria Under Greenhouse Conditions

The populations of gram-positive bacteria on the roots of aphid- and BTH-treated peppers were significantly higher, by 1.04- and 1.03-fold, respectively, than those on the roots of the water control at 2 weeks after treatment (Fig. 2A). Among the gram-positive bacteria, we detected many colonies with a unique morphology on the aphid-treated pepper roots. To verify the morphology-based counting assay, *Paenibacillus* spp. were confirmed by 16S rRNA sequencing (data not shown). In the aphid treatment, the numbers of *Paenibacillus* spp. were higher by 1.26- and 1.4-fold than those in the control after 1 and 2 weeks, respectively. In the BTH treatment, the numbers of *Paenibacillus* spp. were also higher than those of the control at 1 and 2 weeks after treatment (Fig. 2B). Physical and chemical properties of the field soil were evaluated. The soil pH, electrical conductivity, cation-exchange capacity, organic compound, and total nitrogen contents were 6.34, 0.29 ds/m, 7.67 cmol$^+$/kg, 1.88%, and 0.07%, respectively.

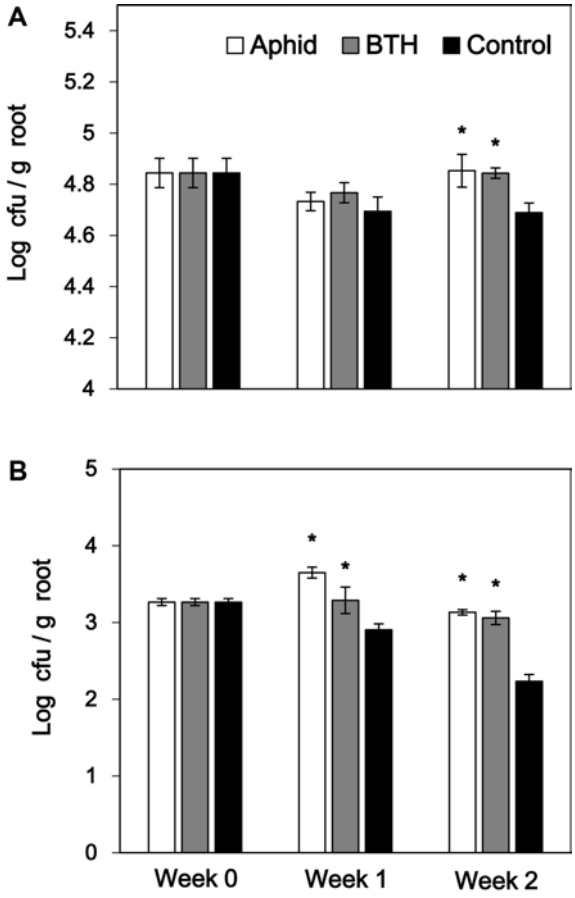

Fig. 2. Effect of foliar aphid infestation on the number of gram-positive rhizobacteria on roots grown in field soil under greenhouse conditions.

The populations of rhizobacteria were analyzed at 0, 1, and 2 weeks after treatment with 10 aphids, 1 mM BTH, and water as a control. (**A**) Populations of gram-positive bacteria were determined by counting bacterial colonies cultured from roots. (**B**) Populations of *Paenibacillus* spp. among the colonies of gram-positive bacteria were determined by counting the colonies with the *Paenibacillus* spp. morphology. An asterisk (*) indicates a significant difference between the treatments and the control according to the least significant difference at p = 0.05. The error bars indicate the SEM.

Effect of Aphid Infestation on the Number of *P. polymyxa*

Two weeks after treatment with aphids and BTH, the numbers of *P. polymyxa* E681 (used as a model for *Paenibacillus* spp.) were higher than those of the control (Fig. 3). These results confirmed that the numbers of *Paenibacillus* spp. on roots increased with the aphid infestation on leaves.

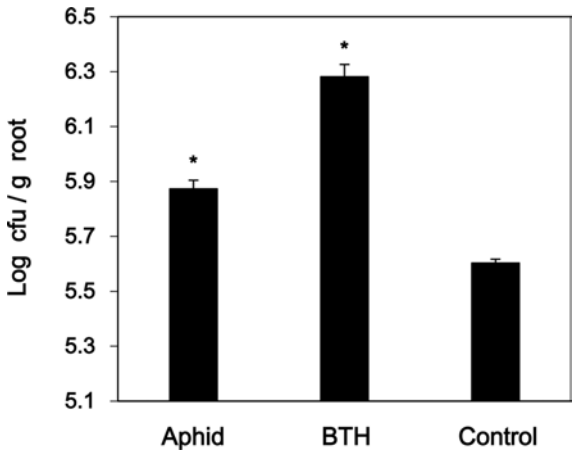

Fig. 3. Effect of foliar aphid infestation on the number of *P. polymyxa* E681 on roots grown in field soil under greenhouse conditions.

Populations of *P. polymyxa* E681 were determined by counting colonies cultured on medium that contained rifampicin. Pepper roots were pretreated with *P. polymyxa* E681 before the treatments with 10 aphids, 1 mM BTH, and water treatment were applied. An asterisk (*) indicates a significant difference between the treatment and the control according to the least significant difference at p = 0.05. The error bars indicate the SEM.

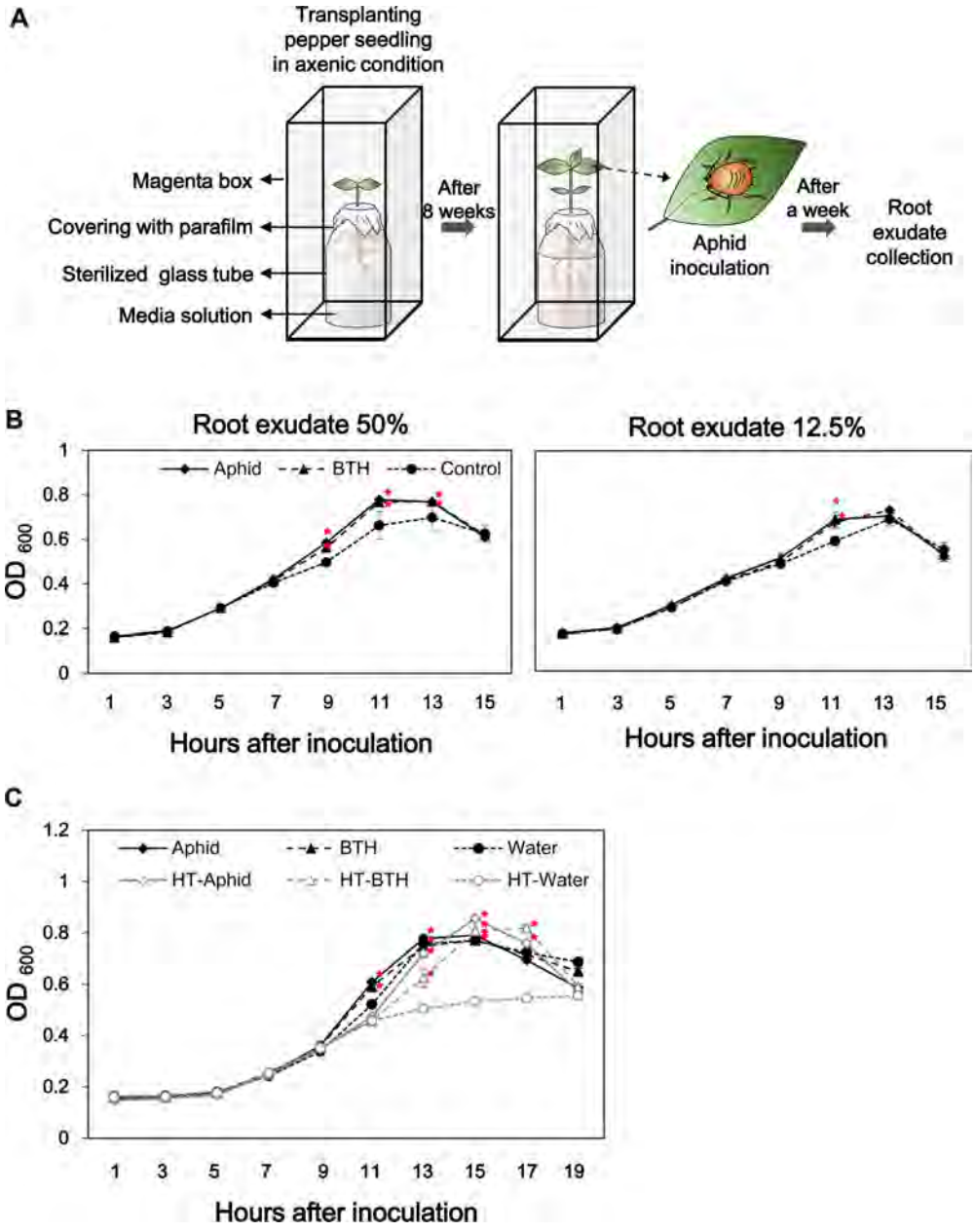

Fig. 4. Effect of root exudates from pepper seedlings infested with aphids on the growth rate of *P. polymyxa* E681.
(**A**) Schematic representation of the harvest of root exudates under axenic conditions. Pepper seedlings were transplanted into sterilized glass tubes that contained plant media solution and were grown for 6 weeks before treatment with 20 aphids, 0.5 mM BTH, and water. Root exudates were harvested 1 week after the treatments were applied. (**B**) Growth curves of *P. polymyxa* E681 in tryptic soy broth medium with the addition of 50% and 12.5% root exudates. (**C**) Growth with the 50% root exudates compared with the heat-treated (HT) root exudates. An asterisk (*) indicates a significant difference between the treatments and the control according to the least-significant difference at $p = 0.05$. The error bars indicate the SEM.

Effects of Root Exudate from Aphid-Infested Plants on the Growth of *P. polymyxa* E681

The growth of the model species *P. polymyxa* E681 was evaluated with the additions of root exudate to the bacterial media. The growth of cells in the exponential phase from both aphid- and BTH-treated plant roots was promoted by the 50% and 12.5% root exudate treatments (Fig. 4B). With the addition of 50% root exudate to the media, the following linear equations and R^2 values for growth were obtained for the period from 7 to 11 h: y =

0.1788x + 0.2371 and R^2 = 0.9979 for the aphid treatment, y = 0.177x + 0.2272 and R^2 = 0.9923 for the BTH treatment, and y = 0.129x + 0.2633 and R^2 = 0.9731 for the control. For the addition of 12.5% root exudate, the linear equations and R^2 values were as follows: y = 0.1339x + 0.2715 and R^2 = 0.9701 for the aphid treatment, y = 0.132x + 0.263 and R^2 = 0.9567 for the BTH treatment, and y = 0.0913x + 0.3108 and R^2 = 0.9931 for the control (Fig. 4B).

We assessed whether heat-treated (121°C for 15 min) root exudates maintained a positive effect on bacterial growth. Based on the results, the heat-treated root exudates from both aphid and BTH treatments promoted cell growth as well as the root exudates that were not heat-treated, although the peak in growth was delayed 2 h compared with the root exudates that were not heat-treated. However, the heat-treated root exudates of the controls lost the function to promote cell growth. During the period from 9 to 15 h, the linear equations and R^2 values for growth were as follows: y = 0.1285x + 0.2686 and R^2 = 0.9937 for the heat-treated aphid treatment, y = 0.1301x + 0.2394 and R^2 = 0.9961 for the heat-treated BTH treatment, and y = 0.0507x + 0.3487 and R^2 = 0.8495 for the control (Fig. 4C).

Increase in the Aphid Population with Root Colonization by *P. polymyxa* E681

Three-week-old pepper plants were drenched with E681, BTH, and water and then inoculated with 10 aphids a week after treatment. After 2, 6, and 12 days, in the E681 treatment, the aphid populations were 1.5-fold, 1.4-fold, and 1.44-fold larger, respectively, than those of the control (Fig. 5). However, after 2, 6, and 12 days in the BTH treatment, the numbers of aphids were 1.4-fold, 1.4-fold, and 1.62-fold lower, respectively, than those of the control. For all time points, the effects of the treatments with E681 and BTH were statistically significant (Fig. 5).

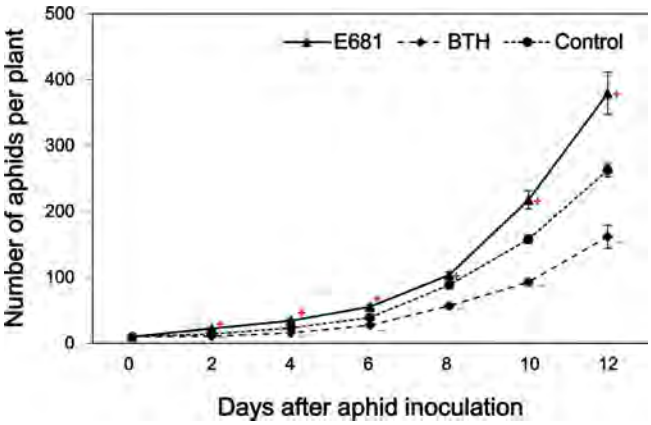

Fig. 5. Increase in the aphid population following the pretreatment of pepper roots with *P. polymyxa* E681.
Roots were pretreated with an E681 cell suspension (OD_{600} = 3), 1 mM BTH, and water as a control before inoculation with 10 aphids. The number of aphids was counted after the inoculation. Plus (+) and minus (-) indicate significant positive and negative differences, respectively, between treatments and control according to the least-significant difference at p = 0.05. The error bars indicate the SEM.

Discussion

Although the effects of foliar insects on soil microflora through the mediation of belowground plant physiology have been evaluated in many studies, the effects of foliar insects on the recruitment of specific species of bacteria and the role of these bacteria in affecting the susceptibility of plants to further insect attack are largely unknown. In the current study, with the aboveground aphid infestation, *Paenibacillus* spp. were selected, which are in a unique genus of gram-positive bacteria with the capacity to attenuate plant defenses against aphid infestation. To summarize, our results provided new information that the bacilli-mediated modulation of plant defense mechanisms was dependent on the foliar phloem-sucking insect species.

As noted previously, the number of *Paenibacillus* spp. increased in the aphid treatment (Fig. 2). Based on previous studies on interactions with plants in which the *Paenibacillus* spp. were applied to seeds and seedlings under greenhouse and field conditions, the roles of *Paenibacillus* spp. are to promote plant growth and to induce defense responses, in addition to demonstrating direct antagonism against plant pathogens via antibiotic production [8, 10, 12, 16, 26]. The primary question is to explain the occurrence from the large pool of the *Paenibacillus* spp. that were adapted to roots under foliar aphid infestation. Our hypothesis was that a specific component of root exudates had a positive effect on the growth rate of *Paenibacillus* spp. To validate this hypothesis, a newly developed procedure to collect root exudate was used (Fig. 4A). Notably, the application of root exudates from the aphid-infested plants promoted the growth rate of *P. polymyxa* E681, a model bacterium for *Paenibacllus* spp. (Fig. 4B), which indicated that the aphid infestation modulated plant physiology and led to the secretion of an unknown component in root exudates that specifically increased the growth of *Paenibacillus* spp. Currently, we do not know which component increases the populations of *Paenibacillus* spp., and not those of gram-negative bacteria. With further study, the heat-stable component of root exudates from plants infested by aphids promoted the growth of bacteria (Fig. 4C). In the heat-

treated control, cell growth decreased significantly compared with that of the control that was not heat-treated; whereas in the heat-treated exudates from aphid and BTH treatments, cell growth was delayed but then increased compared with the aphid and BTH treatments that were not heat-treated (Fig. 4C). These results suggest that plants release an unknown component in root exudates that promotes E681 cell growth, but the component is not heat stable. Additionally, the increase in growth in the heat-treated aphid and BTH treatments indicated a heat-stable component that was different compared with the control treatment. In previous studies, organic acids such as malic acid and citric acid in root exudates promoted the growth of rhizobacteria [1, 4, 13, 25]. Another candidate group of compounds that promote growth are the benzoxazinoids, which are found in the root exudates of maize and attract *P. putida* [18]. SA is another candidate because it increases in the root exudates of a sucking insect-infested plants [29]. However, the organic acids and phenolic compounds such as SA and benzoxazinoid may not be heat stable and therefore cannot be candidates in the current study as determinants of root exudates. To date, no studies report on the use of heat-stable root exudate components to manipulate bacterial growth, and for the root exudates affected by the phloem-sucking of aphids in an infestation, further study is required to identify the heat-stable compounds that caused an increase in the growth of bacteria.

Our results demonstrated clearly that the aboveground aphid infestation recruited *Paenibacillus* spp. in the field soil, which was validated by the data for a model rhizobacterium, *P. polymyxa* E681 (Fig. 3). The effect of the increased number of belowground *Paenibacillus* spp. was demonstrated with a soil drench of *P. polymyxa* E681 that attenuated the basal resistance of pepper against aphid infestation (Fig. 5). Similarly, the pre-application of gram-negative *P. fluorescens* WCS417r to the roots of *Arabidopsis* increases the weight gain and the intrinsic growth rate of the aphid *M. persicae* by modifying the defenses related to plant hormone signaling [22]. Following treatment with strain WCS417 on the roots of aphid-infested plants, the activity of the JA-regulated defense pathway that involves the gene *LOX2* increases. Additionally, the abscisic acid (ABA) signaling pathway that involves the gene *ABA1* and the transcriptional factor of the JA-signaling factor *MYC2*, which is positively modulated by ABA, is suppressed. These results suggest that the effect of WCS417 on aphids occurred because of the suppression of ABA signaling, whereas JA signaling increased in *Arabidopsis* [22]. Therefore, subsequent genetic studies must determine whether the effects observed in our data were because of ABA or JA signaling.

In addition to the putative signaling effects of the ABA pathway, the plant hormone indole-3-acetic acid (IAA) can modulate the susceptibility of plants [30]. A general phytohormone, IAA is produced by plants and several bacteria, including plant growth-promoting rhizobacteria and pathogens [30]. Based on a previous report, auxin signaling is involved in plant resistance against bacterial pathogens [17]. Based on our data, the root exudates of pepper plants treated with aphids and BTH increased the transcriptional expression of the *ipdC* gene and production of IAA (data not shown), which modulates IAA production in bacteria via the indole-3-pyruvate (IPA)-dependent biosynthesis pathway [30]. In *Arabidopsis*, the plant defense response to recognition of pathogen-associated molecular patterns is to down-regulate auxin signaling by targeting auxin receptor transcripts, which suggests that low auxin signaling increases the resistance to bacterial pathogens [17]. The exogenous application of IAA and IAA analogs provides further evidence that auxins can increase plant susceptibility [2, 17, 39]. The auxin analogs 2,4-dichlorophenoxyacetic acid and 1-naphthaleneacetic acid increase the symptoms of disease in *Arabidopsis* infected by *P. syringae* pv. *tomato* DC3000 and *P. syringae* pv. *maculicola* [2, 17, 39]. In our study, the large number of aphids on plants following application of *P. polymyxa* might be caused by the activation of plant IAA signaling mediated by the exogenously produced IAA of *P. polymyxa.* However, we did not confirm the *in situ* secretion of IAA by *P. polymyxa* in the greenhouse. Additionally, the biosynthesis of bacterial IAA promotes the root colonization of plant roots [14, 20, 31]. For example, IAA production plays an important role in the colonization of roots by bacteria in the roots of bent grass, and the root population of an IAA null mutant HP72LI of *P. fluorescens* was reduced compared with the wild-type *P. fluorescens* HP72 [31]. Similarly, the mutation of the key enzyme *ipdC* for IAA biosynthesis in *P. putida* resulted in reduced bacterial populations on canola roots [20]. Collectively, these results lead to the hypothesis that an increase in the gene expression of *ipdC* mediated by root exudates from aphid-infested plants helped *P. polymyxa* E681 colonize roots.

When our results were combined, a series of processes that influenced the effect of aphids on rhizobacteria and the effect of rhizobacteria on aphids were identified. For these processes, we provided new evidence that foliar insects increase rhizobacteria through the modulation of root exudates, which leads to an increase in the susceptibility of

plants to insects. The results of this study provide new insights and will increase our understanding of insect-plant-microbe interactions in ecological systems.

Acknowledgments

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