Evaluating the Growth-promoting Effects of Microbial Biostimulants on Greenhouse Floriculture Crops

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Additional index words. *Bacillus*, beneficial microorganisms, flowers, herbaceous ornamentals, mycorrhizae, petunia, zinnia

Abstract. Microbial biostimulants can promote ornamental plant growth during production and improve crop performance under abiotic stresses. Even though biostimulants have shown potential in many agricultural applications, the effectiveness and specificity of many products are not well understood. The objective of this study was to analyze the growth-promoting effects of microbial biostimulants during the greenhouse production of floriculture crops. We evaluated 13 biostimulant products in greenhouse-grown zinnia (*Zinnia elegans* ‘Magellan Ivory’) and petunia (*Petunia ×hybrida* ‘Carpet White’) at low fertility (one-third of the optimal fertilizer concentration). Biostimulant products 1 and 2 containing multiple species of beneficial bacteria and fungi, and product 10 containing *Bacillus subtilis* QST 713, were found to increase various aspects of plant growth, including the growth index, leaf chlorophyll content (SPAD index), and shoot biomass. Both flower biomass and numbers were greater in petunia treated with product 1, and leaf size increased in zinnia treated with products 1, 2, and 10. Plants treated with these effective biostimulants at low fertility had similar or better growth and quality than untreated plants grown under optimal fertility. The concentration of various nutrient elements in leaves was higher in zinnia plants treated with biostimulant products 1, 2, or 10 compared with the negative control. Some putative mechanisms for biostimulant effectiveness, the possible reasons for biostimulant ineffectiveness, and the potential for using biostimulants as a sustainable cultural strategy are discussed. This study provides useful information about microbial biostimulant effectiveness, which is important for the development and utilization of biostimulants in the greenhouse production of floriculture plants.

Plant biostimulants can be applied to greenhouse-grown crops to increase quality in a sustainable way by improving resource use efficiency and reducing the application of other chemicals (Rouphael and Colla, 2020a). Biostimulants are substances and/or microorganisms that can promote plant

growth and development and enhance plant tolerance to abiotic stress (Drobek et al., 2019). The value of biostimulants has been demonstrated in different areas of agriculture, including hydroponic and soil-based systems, field- and greenhouse-grown crops, and traditional and organic farming (Rouphael and Colla, 2020b). The global market value of biostimulants was \$2.6 billion in 2019 and is predicted to exceed \$4.9 billion by 2025 (marketsandmarkets.com, 2020). Unfortunately, many biostimulants continue to produce inconsistent, ineffective, or even negative effects (Yakhin et al., 2017). Science-based recommendations for the proper utilization of biostimulants are needed for biostimulant product development, marketing, and implementation. Therefore, it is important to conduct comprehensive evaluations of biostimulant products under relevant production environments.

Biostimulants can be categorized as microbial biostimulants and non-microbial biostimulants. Microbial biostimulants include plant growth-promoting bacteria (PGPB) and beneficial fungi (Du Jardin, 2015). Common genera of PGPB include *Bacillus*, *Pseudomonas*, and *Azospirillum* (Ruzzi and Aroca, 2015), with *Bacillus* being one of the most predominant (Hashem et al., 2019). The most well-

characterized beneficial fungi are endomycorrhizae [arbuscular mycorrhizal fungi (AMF)] and *Trichoderma* (López-Bucio et al., 2015; Rouphael et al., 2015). Non-microbial biostimulants include seaweed extracts, humic and fulvic acids, protein hydrolysates, and chitosan (Rouphael and Colla, 2020b). Beneficial bacteria and fungi that colonize the plants can provide lasting benefits to plant growth and health throughout greenhouse production, increase shelf life during retailing, and improve landscape performance for the final consumers (Paradičković et al., 2019). Formulated biostimulant products often combine multiple active ingredients and may include microbial and non-microbial components to synergistically improve plant growth and development. However, the formulations are often established based on experience instead of solid scientific evidence to support the effectiveness of the products (Rouphael and Colla, 2018).

Beneficial microbes can promote plant growth via diverse mechanisms, which include producing or regulating phytohormone levels, increasing plant nutrient availability and uptake, and producing secondary metabolites (Dong et al., 2019; Ruzzi and Aroca, 2015; White et al., 2019). Microbial mechanisms for improving nutrient bioavailability and uptake include nitrogen (N) fixation (e.g., *Rhizobium*-legume symbioses), nutrient solubilization, and expansion of root surface area (Courtney et al., 2015; Halpern et al., 2015). Indole-3-acetic acid (IAA) is a form of auxin commonly produced by beneficial microbes (e.g., *Bacillus* and *Pseudomonas*) to stimulate plant growth and lateral root formation, which increases root surface area and enhances the uptake of plant nutrients (Lim and Kim, 2009; Ortiz-Castro et al., 2020). The production of organic acids and acid phosphatases by beneficial microbes, such as *Bacillus* and *Pseudomonas*, can solubilize immobilized phosphorus (P) in soil or soilless media so that it becomes more available for plants to take up (Cabello et al., 2005; Pii et al., 2015; Sharon et al., 2016). AMF facilitate the increased uptake and nutrient transport to the plants through extensive networks of hyphae colonizing the roots (Genre et al., 2020). Beneficial microbes can also produce secondary metabolites such as volatile organic compounds (VOCs) to promote plant growth (Bitas et al., 2013). These modes of action form a complex regulatory network that eventually leads to plant morphological changes, such as increased shoot or root size, expanded leaf area, higher chlorophyll content, earlier and larger flowers, and improved yield and quality (Mine et al., 2014; Paradičković et al., 2019; Ruzzi and Aroca, 2015).

The efficacy of biostimulants can be affected by the host plants, environmental conditions, and other production inputs. Depending on the plant species, certain microbes may not be able to colonize and benefit the plant hosts due to a lack of response to root exudate signaling (Drogue et al., 2012). The level of host specificity varies among different strains of microbes (Kloepper, 1996). For example, the symbiotic relationship between rhizobia and legumes is highly specific (Hirsch et al.,

Received for publication 20 July 2021.
Accepted for publication 6 Oct. 2021.
Published online 17 December 2021.

Salaries and research support were provided in part by State and Federal funds appropriated to OARDC/CFAES, The Ohio State University. Journal Article Number HCS 20-07. This work was financially supported by the OSU D.C. Kiplinger Floriculture Endowment. Support was also provided by The Ohio State University Graduate Fellowship, The CFAES Environmental Graduate Research Fellowship, and the Altman Family Scholarship. We thank Laura Chapin for technical support and Kaylee South for statistical advice.

Mention of trade names does not imply a guarantee or warranty of the products named or the others not named.

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2001), whereas the colonization and services of AMF and *Bacillus* are relatively common in various plant species (Klironomos et al., 2000; Lee et al., 2013; Radhakrishnan et al., 2017). Environmental variables, such as extreme temperatures, low humidity, and high salt levels in the growing media, can negatively impact the colonization and functions of the beneficial microbes (Pineda et al., 2013). In addition, production inputs, such as fertilizer concentrations and composition, also play an important role in determining the efficacy of biostimulants in field soil or greenhouse soilless systems (Bulluck et al., 2002; Xia et al., 2019). The over-application of fertilizer when using a biostimulant may inhibit the growth and colonization of beneficial microbes. It is well-established that high levels of P suppress AMF colonization and effectiveness, as shown in tomato (*Solanum lycopersicum*), petunia, pepper (*Capsicum annuum*), and clover (*Trifolium alexandrinum*) (Breuillin et al., 2010; Davies et al., 2000; Nagy et al., 2009; Raiesi and Ghollara, 2006). Growing maize (*Zea mays*) under nutrient-deficient conditions encourages the colonization of N-fixing bacteria and AMF (Wu et al., 2005). The efficacy of beneficial microbes can also be affected by plant-growing medium composition as shown in lettuce (*Lactuca sativa*), where six different bacterial inoculants were tested for plant growth promotion in 10 different growing media that contained peat, coconut or wood fibers, compost, or inorganic materials (Van Gerrewey et al., 2020). Since the ability of microbial biostimulants to promote plant growth and development can be altered by the plant hosts, growing environment, and various cultural inputs, it is important to consider these interacting factors when conducting biostimulant trials.

The greenhouse production of floriculture crops requires high levels of fertilizer application (Bulgari et al., 2015). However, the excessive use of fertilizer can cause environmental pollution (Wani et al., 2018). Biostimulants provide a sustainable strategy for greenhouse producers to reduce fertilizer inputs while maintaining or improving crop growth and quality (Backer et al., 2018; Roupael and Colla, 2018). To better understand the specific benefits of biostimulants on greenhouse-grown floriculture crops, the objective of this study was to evaluate the growth-promoting effects of various microbial biostimulant products using zinnia (*Zinnia elegans*) and petunia (*Petunia ×hybrida*). It was hypothesized that biostimulant treatments would allow for the production of high-quality floriculture crops with reduced fertilizer applications.

Materials and Methods

Expt. 1: Evaluating biostimulant efficacy in zinnia

Zinnia elegans 'Magellan Ivory' seeds were sown into peat-based media (Promix PGX; Premier Tech Horticulture, Quebec, Canada) in plug trays, covered with a propagation dome, and grown under fluorescent lighting with a 14 h photoperiod. Seedlings were transferred to a greenhouse after 20 d, acclimated to the environment for 7 d, and transplanted into 11.4 cm-standard round pots

with a peat-based growing media (Promix BX; Premier Tech Horticulture). The plants were grown in a greenhouse located in Wooster, OH (The Ohio State University, Wooster Campus) with set temperatures of 21/18 °C day/night and a 14-h photoperiod supplemented with metal halide and high-pressure sodium lights (GLX/GLS e-systems GROW lights; PAR-Source, Petaluma, CA) when light intensity was lower than 350 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Expt. 1 was conducted from Sept. to Nov. 2017 with an average day/night temperature of $24.5 \pm 2.4/17.5 \pm 1.9$ °C and a daily light integral of 12.1 ± 5.9 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$. Starting at transplanting, plants were treated with one of the 13 different commercial biostimulant products based on manufacturers' recommendations, whereas different application frequencies of a few products were included as different treatments (Table 1). Plants not treated with any product were used as a negative control (NC). Plants treated with biostimulants and the NC plants were fertilized at each irrigation using a constant liquid feed at a lower-than-optimal fertilizer concentration of 50 $\text{mg}\cdot\text{L}^{-1}$ N from 15N–2.2P–12.5K–2.9Ca–1.2Mg (Jack's Professional LX Water-Soluble Fertilizer; JR Peters, Allentown, PA). Positive control (PC) plants were not treated with any biostimulant product and were fertilized with an optimal fertilizer concentration of 150 $\text{mg}\cdot\text{L}^{-1}$ N from 15N–2.2P–12.5K–2.9Ca–1.2Mg at each irrigation. The experiment was ended when plants had flowers, and were considered to be at a marketable stage, 6 weeks after transplanting. This experiment was a randomized complete block design (RCBD) with 24 replicates for each treatment.

Plant growth and performance assessments for Expt. 1 included plant growth index (GI), SPAD index, shoot and root biomass, root:shoot ratio, leaf size, and leaf mineral nutrient analysis. Plant GI was calculated weekly by taking the average of the two perpendicular width measurements (W) and the height (H) using the equation: $\text{GI} = [(W1 + W2)/2 + H]/2$ (Niu et al., 2010). SPAD measurements were taken weekly on the second newly, fully expanded leaf of each plant (SPAD-502 chlorophyll meter; Kinoca Minolta, Ramsey, NJ). Six weeks after transplanting, plant biomass, leaf size, and tissue mineral nutrient concentrations were measured. The shoot and roots of each plant were collected separately, dried in a forced air-drying oven set at 60 °C for at least 4 d, and weighed to determine dry biomass. Root and shoot biomass measurements were used to calculate the root:shoot ratio for each plant. The leaf size was measured based on the second newly, fully expanded leaf of each plant at the end of week 6. The leaves were removed, scanned with a desktop scanner, and leaf size was calculated using image analysis software (ImageJ; National Institutes of Health, Bethesda, MD). These leaves were used for tissue-nutrient analyses. Leaves were dried in a 60 °C forced air oven for at least 3 d and ground to pass a 2-mm sieve. Nutrient analyses were conducted by the Service Testing and Research Laboratory (STAR Laboratory, The Ohio State University/OARDC, Wooster, OH). Total N content was

analyzed using a Vario Max combustion analyzer (Elementar Americas, Ronkonkoma, NY) with the Dumas combustion method (Sweeney, 1989). Plant tissue was digested using a microwave system (Discover SP-D; CEM Corporation, Matthews, NC). The concentrations of other mineral elements were determined using an inductively coupled plasma spectrometer (Prodigy Dual View ICP spectrometer; Teledyne Leeman Laboratories, Hudson, NH) (Isaac and Johnson, 1985).

Expt. 2: Validating biostimulant efficacy in petunia

Petunia ×hybrida 'Carpet White' seeds were germinated, and the 20-day-old seedlings were moved to the greenhouse to acclimate to the environment for 7 d before they were transplanted into a peat-based media as described in Experiment I. The plants were grown under the same set of greenhouse conditions and fertilized with lower-than-optimal concentrations of fertilizer as described in Expt. 1. Expt. 2 was conducted from Nov. 2017 to Jan. 2018 with an average day/night temperature of $22.4 \pm 1.5/15.6 \pm 0.5$ °C and a daily light integral of 11.5 ± 4.2 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$. Based on the results of Expt. 1, 11 commercial biostimulant products were tested in Expt. 2 following manufacturers' recommendations, and different application frequencies were added as additional treatments to better understand the efficacy of the products (Table 1). As previously described, plants not treated with any biostimulant were used as a NC and plants not treated with any biostimulant but fertilized with the optimal concentrations of fertilizer were used as a PC. The experiment was ended at 5 weeks after transplant when the plants had flowers and were at the marketable stage. The experiment was an RCBD with 24 replicates for each treatment.

Growth and performance assessments for plants in Expt. 2 included plant GI, SPAD index, and plant biomass as described in Expt. 1. In addition, the total number of open flowers plus buds with color on each plant was counted as flower number at the termination of the experiment at 5 weeks after transplant. These flowers and buds were removed from the plant, dried in a 60 °C oven for at least 4 d, and weighed to determine dry flower biomass. The dry biomass was also measured for the vegetative shoots (leaves and stems) and the roots. Root and total shoot biomass (including flowers, buds, and vegetative parts) measurements were used to calculate the root:shoot ratio for each plant. Electrical conductivity (EC) and pH values of the growing medium were measured 5 weeks after transplant with handheld meters (LAQUATwin meter; Horiba, Irvine, CA) using the Pour-Thru extraction method (Cavins et al., 2008).

Nutrient analyses of effective biostimulant products. Mineral-nutrient analyses of biostimulant products 1, 2, and 10, which were found effective in promoting plant growth in this study, were conducted by the STAR Laboratory (The Ohio State University/OARDC). Each sample was digested using a microwave-assisted acid digestion method (EPA 3051S method). The total C and N contents were measured using a

Table 1. Trade names, application rate, and active ingredient of the biostimulant products tested in Expts. 1 (zinnia) and 2 (petunia).

Product	Trade name	Application ^z	Active ingredients
1	MycoApply All Purpose Granular	In zinnia and petunia Mix with potting media at transplant (10 mL per liter potting media)	<i>Bacillus subtilis</i> , <i>B. licheniformis</i> , <i>B. pumulus</i> , <i>B. amyloliquefaciens</i> , <i>B. megatarium</i> , <i>Glomus intraradices</i> , <i>G. mosseae</i> , <i>G. aggregatum</i> , <i>G. etunicatum</i> , <i>G. clarum</i> , <i>G. deserticola</i> , <i>G. monosporum</i> , <i>Paraglomus brasilianum</i> , <i>Gigaspora margarita</i> , <i>Rhizopogon villosulus</i> , <i>R. luteolus</i> , <i>R. amylopogon</i> , <i>R. fulvigleba</i> , <i>Pisolithus tinctorius</i> , <i>Scleroderma cepa</i> , <i>S. citrinum</i> , slow-release organic fertilizer 3–1–1
2	Diehard Complete	In zinnia and petunia Mix with potting media at transplant (31 mL per liter potting media)	<i>Bacillus megaterium</i> , <i>B. subtilis</i> , <i>B. amyloliquefaciens</i> , <i>B. azotoformans</i> , <i>B. licheniformis</i> , <i>B. pumulus</i> , <i>B. Firmus</i> , <i>B. coagulans</i> , <i>B. stearothermophilus</i> , <i>B. polymyxa</i> , <i>Pseudomonas putida</i> , <i>P. fluorescence</i> , <i>P. aureofaciens</i> , <i>P. durum</i> , <i>Streptomyces lydicus</i> , <i>S. griseus</i> , <i>S. coelicolor</i> , <i>Glomus mosseae</i> , <i>G. intraradices</i> , <i>G. claroideum</i> , <i>G. etunicatum</i> , <i>G. clarum</i> , <i>G. deserticola</i> , <i>G. microaggregatum</i> , <i>Pisolithus tinctorius</i> , <i>Rhizopogon</i> , <i>Trichoderma hamatum</i> , <i>T. harzianum</i> , <i>T. viride</i> , <i>T. reesei</i> , humic acids, sea kelp extract, yucca plant extract, root promoting vitamin, amino acids
3	BioGenesis I NP	In zinnia and petunia Mix with potting media at transplant (13 mg per liter potting media)	<i>Arthrobacter globiformis</i> , <i>Azospirillum brasilense</i> , <i>A. lipoferum</i> , <i>Azotobacter chroococcum</i> , <i>A. paspali</i> , <i>A. vinelandii</i> , <i>Bacillus amyloliquefaciens</i> , <i>B. atrophaeus</i> , <i>B. licheniformis</i> , <i>B. megaterium</i> , <i>B. pumilus</i> , <i>B. subtilis</i> , <i>B. thuringiensis</i> , <i>Brevibacillus brevis</i> , <i>Micrococcus luteus</i> , <i>Pseudomonas fluorescens</i> , <i>P. putida</i> , <i>Rhodopseudomonas palustris</i> , <i>Rhodospirillum rubrum</i> , <i>Streptomyces griseus</i>
4	Vivi	In zinnia and petunia Mix with potting media at transplant (2.5 g per liter potting media)	Grape seed meal and cocoa shell meal
5	MycoApply EndoMaxx	In zinnia and petunia 100 mL soil drench one time at transplant (4 mg per liter water)	<i>Glomus aggregatum</i> , <i>Glomus intraradices</i> , <i>Glomus etunicatum</i> , <i>Glomus mosseae</i>
6a	Nature's source	In zinnia and petunia 100 mL soil drench biweekly starting at transplant (0.6 g per liter water)	<i>Bacillus subtilis</i> , <i>Brevibacillus brevis</i> , <i>Trichoderma harzianum</i> , <i>B. megaterium</i> , <i>B. methylotrophicus</i> , <i>B. vallismortis</i> , <i>B. licheniformis</i> , <i>B. stratosphericus</i> , <i>Paenibacillus dendritiformis</i> , <i>Streptomyces diastaticus</i> , <i>Trichoderma harzianum</i>
6b		In petunia 100 mL soil drench weekly starting at transplant (0.6 g per liter water)	
7a	EcoFlora	In zinnia and petunia 100 mL soil drench monthly starting at transplant (1 g per liter water)	<i>Bacillus subtilis</i> , <i>B. polymyxa</i> , <i>B. pumilus</i> and <i>Paenibacillus azotofixans</i> , <i>Pseudomonas aureofaciens</i> , <i>Streptomyces lybicus</i> , <i>Trichoderma harzianum</i>
7b		In petunia 100 mL soil drench weekly starting at transplant (1 g per liter water)	

(Continued on next page)

Table 1. (Continued)

Product	Trade name	Application ^z	Active ingredients
8a	Environoc 401	In zinnia and petunia 100 mL soil drench monthly starting at transplant (31 mL per liter water)	<i>Azospirillum brasilense</i> , <i>Bacillus altitudinis</i> , <i>B. amyloliquefaciens</i> , <i>B. licheniformis</i> , <i>Cellulomonas cellasea</i> , <i>Pseudomonas fluorescens</i> , <i>P. putida</i> , <i>P. stutzeri</i> , <i>Streptomyces albidoflavus</i>
8b		In petunia 100 mL soil drench weekly starting at transplant	
9a	N-Texx soil inoculant with humic acid	(31 mL per liter water) In zinnia and petunia 100 mL soil drench biweekly starting at transplant	<i>Bacillus subtilis</i> , <i>Pseudomonas putida</i> , humic acid
9b		(1.3 mL per liter water) In petunia 100 mL soil drench weekly starting at transplant	
10	Cease	(1.3 mL per liter water) In zinnia and petunia 100 mL soil drench weekly starting at transplant	<i>Bacillus subtilis</i> QST 713
11	NA ^y	(15 mL per liter water) In zinnia and petunia 100 mL soil drench weekly starting at transplant	<i>Pseudomonas chlororaphis</i> 48G9
12	NA ^y	(2.1 mL per liter water) In zinnia 100 mL soil drench weekly starting at transplant	<i>Yersinia frederiksenii</i> AP-187
13	Bio-YIELD	(2.1 mL per liter water) In zinnia 100 mL soil drench weekly starting at transplant (2.1 mL per liter water)	<i>Pseudomonas brassicacearum</i> Wood3

^z1 L = 1000 mL = 33.8 fl oz, 1 g = 1000 mg = 0.0353 oz.

^yProducts 11 and 12 included single strains of bacteria that are currently not commercially available.

VARIO Max Cube Carbon—Nitrogen Analyzer (Elementar Americas, Ronkonkoma, NY) with high-temperature combustion (Nelson and Sommers, 1996). The concentrations of other mineral elements were measured using an inductively coupled plasma spectrometer (Agilent 5110 ICP-OES; Agilent Technologies, Santa Clara, CA) (Isaac and Johnson, 1985). The total applied nutrient levels over the experiment were calculated for each product by multiplying the nutrient concentrations by the amount used at each application in the experiments.

Statistical analyses. All statistical analyses were conducted in R 3.3.1 (R Core Team, 2013). Plant GI, SPAD index, biomass, leaf size, leaf mineral nutrient content, pH, and EC data were analyzed using analysis of variance followed by protected mean separation via Tukey's honestly significant difference. The model used was $y = \mu + \text{Treatment} + \text{Block} + e$. Plant GI measurements from week 0 were used as the covariate for the GI data following the model $y = \mu + \text{Covariate} + \text{Treatment} + \text{Block} + e$. Flower number analysis was performed using the model $y = \mu + \text{Treatment} + \text{Block} + e$ in the Poisson test or Quasi-Poisson test when the residual deviance was higher than the df.

Results

Expt. 1: Biostimulant products promote zinnia growth in greenhouse production

Plant size and leaf chlorophyll content. To validate the efficacy of different biostimulant

products in greenhouse production systems with reduced fertilizer input, a greenhouse trial testing 13 different biostimulant products was conducted on zinnia plants under low fertility (50 mg·L⁻¹ N). The size of zinnia plants was evaluated based on the plant GI. Plants treated with product 10 had a higher GI than the NC plants that received no biostimulant treatment starting from week 2 (data not shown) and stayed consistently larger from week 2 to week 6 (Fig. 1A–C). Product 1-treated plants were larger than the NC 5 to 6 weeks after treatment (Fig. 1B and C). The application of product 2 decreased plant size in zinnia (Fig. 1A–C). At week 4, the application of product 2 resulted in a smaller plant size than the NC, but this negative effect on growth was no longer observed at week 5 (Fig. 1A and B). None of the other biostimulant treatments resulted in significant differences in plant size compared with the NC (Fig. 1A–C). After 6 weeks of treatments, zinnias treated with product 1 were 12% larger than the NC plants, and plants treated with product 10 were 15% larger than the NC. Neither of these treatments resulted in plants that were the same size or larger than PC plants fertilized with 150 mg·L⁻¹ N (Fig. 1C).

The SPAD index of zinnia leaves from plants treated with products 1, 2, or 10 was higher than NC plants from week 4 through week 6 (Fig. 1D–F). SPAD index is often used to assess the chlorophyll content in plant leaves (Jiang et al., 2017). Six weeks after treatment initiation, the SPAD index indicated that

product 1-treated plants had a 24% higher chlorophyll content, product 2-treated plants had a 27% higher chlorophyll content, and product 10-treated plants showed a 14% higher chlorophyll content compared with the NC plants (Fig. 1F). Product 1- and product 10-treated plants had leaf chlorophyll contents that were at the same level or higher than the PC, whereas the leaf chlorophyll content of plants treated with product 2 was consistently higher than the PC (Fig. 1D–F). None of the other products showed a positive effect on leaf chlorophyll content in zinnia (Fig. 1D–F). At the time of harvest (week 6), zinnias treated with products 1, 2, or 10 were visibly greener, and zinnias treated with products 1 or 2 looked larger than the untreated NC plants as seen in the photos in Fig. 1G.

Biomass and leaf size. At the final harvest, zinnia plants treated with biostimulant products 1, 2, and 10 had greater shoot biomass than the NC (Fig. 2A). Shoot biomass was 45% higher in plants treated with either product 1 or product 10 compared with the NC (Fig. 2A). Interestingly, even though product 2 did not show a promoting effect on GI in zinnia, the application of product 2 resulted in a 20% increase in shoot biomass compared with the NC (Fig. 2A). None of these biostimulants caused an increase in final shoot biomass that was equivalent to that measured in the PC plants (Fig. 2A). Plants treated with product 2 had smaller root biomass than the NC plants, but no other biostimulant treatment affected root biomass compared with the

Fig. 1. Plant size (Growth index) and leaf chlorophyll content (SPAD index) evaluations of zinnia treated with biostimulant products, negative control (NC), and positive control (PC). (A–C) Growth index from week 4 to week 6. (D–F) SPAD index from week 4 to week 6. (G) Pictures representing zinnia (PC, NC, plants treated with products 1, 2, and 10 from left to right) 6 weeks after treatments were initiated. Bars represent the mean ($n = 24$) $\pm$ SE, and bars with different letters indicate a significant difference ($P \leq 0.05$).

NC (Fig. 2B). Because of the increased shoot biomass and unchanged or decreased root biomass, the root:shoot ratio was lower with the application of products 1, 2, or 10 when compared with the NC plants, and this ratio was similar to or less than that of the PC plants (Fig. 2C). Root:shoot biomass ratio decreased by 44% in plants treated with product 1, 58% in plants treated with product 2, and 28% in plants treated with product 10 (Fig. 2C).

The leaf size at week 6 was 57% larger in product 1-treated plants, 55% higher in product 2-treated plants, and 47% higher in

product 10-treated plants compared with the NC (Fig. 2D). The leaves of plants treated with products 1, 2, or 10 grown under limited fertility were as large as the leaves of the PC plants grown under the optimal level of fertility (Fig. 2D). No other biostimulant treatment resulted in growth-promoting effects on shoot biomass or leaf size.

Mineral nutrient content of zinnia leaves. To determine the effect of biostimulant products on plant nutrition when grown at a lower fertility level, the concentrations of mineral nutrients in the leaves of zinnia treated with

products 1, 2, or 10 were analyzed along with the PC and NCs. The application of product 1 resulted in higher levels of N, potassium (K), sulfur (S), iron (Fe), boron (B), copper (Cu), manganese (Mn), and zinc (Zn) than the NC, and these elements were found to be at similar or higher levels in product 1-treated zinnias compared with the PC (Table 2). Plants treated with product 2 showed increased levels of N, K, S, B, Cu, Mn, and Zn compared with the NC, and similar or higher levels of these elements were found with product 2 treatment in comparison with the PC (Table 2). Product 10

Fig. 2. Final harvest plant production measurements of petunia treated with biostimulant products, negative control (NC), and positive control (PC). (A) Shoot dry biomass. (B) Root dry biomass. (C) Root:shoot biomass ratio. (D) Leaf size. Bars represent the mean ($n = 24$) $\pm$ SE, and bars with different letters indicate a significant difference ($P \leq 0.05$). 1 g = 0.0353 oz, 1 mm² = 0.00155 inch².

treatment increased the concentrations of N, P, magnesium (Mg), B, Cu, and Zn in plant leaves compared with those of the NC plants, and the levels of these elements were also similar or higher than those in the PC (Table 2). No effects on calcium (Ca) or aluminum (Al) content were observed with the biostimulant treatments or optimal fertilization (PC) compared with the NC (Table 2).

Expt. 2: Biostimulant products promote petunia growth in greenhouse production

Plant size and leaf chlorophyll content. To validate the growth-promoting effect of biostimulants, another greenhouse trial using petunia was conducted with 11 of the biostimulant products from Expt. 1. Different application frequencies were included for products 6, 7, 8, and 9 to help understand the influence of application frequency on the efficacy of the products. Similar to Expt. 1, plants treated with biostimulant products 1 or 10 had a greater GI from week 3 through week 5 compared with the NC (Fig. 3A–C). Starting at week 4, plants treated with product 2 were larger than the NC (Fig. 3B and C). At week 5, plants treated with biostimulant products 1, 2, or 10 and grown with the low fertilizer concentration of 50 mg·L⁻¹ N were 18% to 19% larger than the NC (50 mg·L⁻¹ N with no biostimulant) and were as big as the PC plants grown with the optimal fertilizer concentration of 150 mg·L⁻¹

N (Fig. 3C). Plants treated with product 4 showed decreased plant size when compared with the NC from week 3 through week 5 (Fig. 3A–C). No additional products resulted in differences in plant size compared with the NC (Fig. 3A–C).

The SPAD index indicated that the application of biostimulant products 1 or 2 resulted in higher leaf chlorophyll content than the NC and was at the same level or higher than the PC from week 3 through week 5 (Fig. 3D–F). At week 5, plants treated with products 1 or 2 showed 19% and 28% higher chlorophyll content than the NC, respectively (Fig. 3F). None of the other products, including product 10, showed differences in leaf chlorophyll content compared with the NC (Fig. 3D–F). At the week of final harvest (week 5), visual differences were observed in plant size and leaf greenness (chlorophyll content) between the plants treated with biostimulant products 1 or 2 and the NC, and product 10-treated plants also looked larger in comparison with the NC plants in Expt. 2 (Fig. 3G).

Leachate pH and EC. The leachate pH and EC of the plant media in Expt. 2 were measured 5 weeks after the biostimulant treatment initiation. No difference was found in media pH between the PC and NC, or any biostimulant-treated plants, except for the plants treated with products 1 or 2 (Fig. 4A). Product 1- and product 2-treated plants had a leachate pH of 6.3–6.4, which was lower compared with the pH (6.7–6.9) of the NC and PC (Fig. 4A).

There was no difference in EC between the NC and PC (1.2–1.6 mS·cm⁻¹) (Fig. 4B). Plants treated with products 1 or 2 had a higher EC of 2.6 mS·cm⁻¹ and 4.5 mS·cm⁻¹, respectively (Fig. 4B). No difference in EC was found with other treatments compared with the controls (Fig. 4B).

Shoot, root, and flower biomass. Consistent with plant size (GI), vegetative shoot biomass was 70% higher in plants treated with products 1 or 2 and 48% higher in plants treated with product 10 when compared with the NC (Fig. 5A). In particular, the vegetative shoot biomass of product 1- and product 2-treated plants were similar to the PC (Fig. 5A). In contrast, the product 4 or product 6b treatment caused a reduction in vegetative shoot biomass (Fig. 5A). The root biomass was lower in plants treated with product 2 or 4 than the NC (Fig. 5B). Because of the increased shoot biomass and reduced or similar-sized root systems, the root:shoot ratio of petunia plants treated with products 1, 2, or 10 was lower than the NC (Fig. 5C). The root:shoot biomass ratio was 44% lower in plants treated with product 1, 56% lower in plants treated with product 2, and 33% lower in plants treated with product 10 compared with the NC (Fig. 5C). All three treatments resulted in root:shoot ratios that were equivalent to or lower than the PC (Fig. 5C). There was no difference in root:shoot ratio between plants treated with other biostimulant products and the NC (Fig. 5C).

Table 2. Mineral nutrient concentrations in the leaves of zinnias treated with effective growth promoting biostimulant products in Expt. 1.

Nutrient element ^z	Positive control ^y	Negative control ^x	Product 1-treated plants	Product 2-treated plants	Product 10-treated plants
N (mg·g ⁻¹)	24.9 b ^w	17.1 c	28.1 ab	30.4 a	24.0 b
P (mg·g ⁻¹)	5.1 c	6.2 b	3.3 d	4.5 c	10.9 a
K (mg·g ⁻¹)	50.5 c	45.9 c	75.8 b	93.1 a	48.7 c
S (mg·g ⁻¹)	2.3 b	1.7 b	4.4 a	4.1 a	2.6 b
Mg (mg·g ⁻¹)	20.8 a	13.0 cd	16.2 bc	9.6 d	19.6 ab
Fe (μg·g ⁻¹)	56.8 ab	45.2 b	80.4 a	65.9 ab	65.6 ab
B (μg·g ⁻¹)	152.1 b	159.2 b	220.8 a	202.5 a	207.2 a
Cu (μg·g ⁻¹)	2.9 ab	2.1 b	3.2 a	3.3 a	3.3 a
Mn (μg·g ⁻¹)	323.0 c	323.5 c	519.4 b	739.8 a	429.2 bc
Zn (μg·g ⁻¹)	94.1 b	53.8 c	167.2 a	113.3 b	99.0 b
Ca (mg·g ⁻¹)	25.4 a	24.1 a	22.5 a	15.7 b	26.5 a
Al (μg·g ⁻¹)	19.9 a	18.1 a	20.0 a	13.6 a	22.7 a

^z1 mg·g⁻¹ = 1000 μg·g⁻¹ = 1,000,000 ppm.^yPositive control plants were grown with the optimal concentration of fertilizer (150 mg·L⁻¹ N) with no biostimulant treatment.^xNegative control plants (no biostimulant treatment) and biostimulant treated plants were grown with a low concentration of fertilizer (50 mg·L⁻¹ N).^wValues followed by different letters in each row are significantly different at $P \leq 0.05$ level, according to Tukey's honestly significant difference test.

To determine the effect of biostimulants on petunia quality, flower biomass and flower number were measured. Plants treated with biostimulant product 1 had 46% more flower biomass than the NC and plants treated with product 2 had 31% more flower biomass than the NC (Fig. 5D). Plants treated with product 1 produced on average 3 more flowers per plant than the NC (Fig. 5E). Flower biomass was increased in product 2-treated petunia, whereas no difference was found in the numbers of flowers produced by these plants (Fig. 5D and E). Although product 10 treatment did not improve flower production, no negative effect was found in flower number or biomass in plants treated with product 10 compared with the NC (Fig. 5D and E).

Nutrient composition of the effective commercial products

Biostimulants with growth-promoting effects in this study (products 1, 2, and 10) were analyzed to measure their nutrient composition and determine the concentration of each nutrient that would have been applied with each application and in total throughout the experiment. To provide a general comparison between the nutrients from the biostimulant products and the fertilizer, we calculated the amount of applied fertilizer N, P, and K based on an approximate application of 200-mL at each fertilization. As shown in Table 3, product 1 provided over 100 mg N per application, product 2 contained over 300 mg N per application, and this was also equivalent to the total amount of nutrients supplied by these products over the life of the experiment because they were only applied once at transplanting. Product 10 contained less than 0.02 mg N per application, and it was applied weekly, giving a total N application of 0.12 mg for zinnia and 0.1 mg for petunia throughout the experiments (Table 3). Plants grown at the low fertility level (50 mg·L⁻¹ N), including biostimulant-treated plants and NC plants, received the equivalent of 10 mg N per irrigation, whereas plants grown at the optimal fertility level (150 mg·L⁻¹ N) received ≈30 mg N per irrigation. Similarly, about 23 mg of P were applied with product

1, about 75 mg of P with product 2, and only 0.005 mg of P with product 10 application (Table 3). The total applied P from product 10 was 0.03 mg on zinnia and 0.025 mg on petunia (Table 3). Plants also received about 1.46 mg and 4.38 mg P with the low and the optimal fertility levels, respectively, at each irrigation. Over 80 mg K was applied with product 1 treatment and over 300 mg K was applied with product 2 treatment (Table 3). The amount of K applied with product 10 was only 0.004 mg per application, and in total 0.024 mg for zinnia and 0.02 mg for petunia (Table 3). The low concentration of fertilizer provided ≈8.3 mg K and the optimal concentration of fertilizer provided plants with about 25 mg K at each irrigation. Other mineral nutrients such as S, Mg, Ca, and Fe were also applied at a much higher level with product 1 or 2 treatment compared with product 10 treatment (Table 3).

Discussion

Some biostimulant products resulted in plant growth promotion

Growth promotion was observed in both zinnia and petunia in this study with only a few of the tested products. MycoApply All Purpose Granular (product 1) and Diehard Complete (product 2), which contained multiple species of beneficial bacteria and fungi, and Cease (product 10), which contained *Bacillus subtilis* QST 713, promoted plant growth in zinnia and petunia under low fertility growing conditions (Table 1, Fig. 1, 2, 3, and 5). The growth-promoting effects from biostimulant treatment in this study are consistent with previous research in petunia, impatiens (*Impatiens walleriana*), pansy (*Viola ×wittrockiana*), China aster (*Callistephus chinensis*), balsam (*Impatiens balsamina*), and tomato, where microbial inoculants, including *Pseudomonas* spp., *Bacillus* spp., or AMF are able to improve plant growth under nutrient stress (Adesemoye et al., 2009; Gaur et al., 2000; Hoda and Mona, 2014; Nordstedt et al., 2020). Our results also demonstrated that under these growing conditions the majority of the biostimulants did not result in observable growth promotion that would enhance crop

quality or allow for floriculture crop production with the reduced application of fertilizers.

When grown under stressful growing conditions like nutrient deficiency, plants have an increased root:shoot ratio (Harris, 1992; Nielsen et al., 2001). Similarly, in this study, the NC plants produced with limiting nutrient levels (50 mg·L⁻¹ N) had a larger root:shoot ratio than PC plants (150 mg·L⁻¹ N) (Figs. 2C and 5C). The plants treated with biostimulant products 1, 2, and 10 had a root:shoot ratio that was lower than the NC and more similar to the PC (Figs. 2C and 5C). Although the increase of root:shoot ratio from beneficial microbe treatment has been considered as an indicator of higher nutrient uptake (Alizadeh and Parsaemehr, 2011; Davies et al., 2002; He et al., 2019; Nguyen et al., 2019), when the plants are grown in limited space (i.e., containers), a lower root:shoot ratio may indicate higher nutrient uptake efficiency of the plant due to the increased shoot biomass with the same root biomass (Nordstedt et al., 2020). Therefore, the lower root:shoot ratio with the application of biostimulant products under low fertility suggested improvement of nutrient uptake efficiency, which resulted in increased tissue nutrient concentrations that were more similar to those of the PC plants.

Leaf size has been used as an indicator of plant growth when trialing biostimulants (Elarroussia et al., 2016; Saa et al., 2015), and biostimulant treatments with products 1, 2, and 10 resulted in increased leaf size in zinnia (Fig. 2D). Similar positive effects on leaf size or leaf area have been observed in multiple ornamental plants such as zinnia, carnation, snapdragon, vinca, and multiflora rose (*Rosa multiflora*) inoculated with AMF alone (Asrar et al., 2012; Cartmill et al., 2007, 2008; Navarro et al., 2012) or with beneficial bacteria *Pseudomonas fluorescens* (Kumar et al., 2018; Saini et al., 2017). *Bacillus* spp. increase leaf area in tomato, pepper, clover (*Trifolium repens*), and alder (*Alnus glutinosa*) (García et al., 2004; Gutiérrez-Mañero et al., 2001; Han et al., 2014). All three effective products (biostimulant 1, 2, and 10) contain *Bacillus*, and products 1 and 2 both contain multiple species of AMF (Table 1).

Fig. 3. Plant size (Growth index) and leaf chlorophyll content (SPAD index) evaluations of petunia treated with biostimulant products, negative control (NC), and positive control (PC). (A–C) Growth index from week 3 to week 5. (D–F) SPAD index from week 3 to week 5. (G) Pictures representing petunia (PC, NC, plants treated products 1, 2, and 10 from left to right) 5 weeks after treatments were initiated. Bars represent the mean ($n = 24$) $\pm$ SE, and bars with different letters indicate a significant difference ($P \leq 0.05$).

Plant health and quality improvements with biostimulant treatments

Improvements in plant health and quality were also observed in zinnia and petunia treated with products 1, 2, or 10. SPAD index, a measurement positively correlated with leaf chlorophyll content, is used as an indicator of plant health (Jiang et al., 2017). Similar to our results, increased chlorophyll levels have been previously observed in zinnia and vinca (*Catharanthus roseus*) inoculated with both PGPB (e.g., *P. fluorescens*) and beneficial fungi (e.g., AMF) (Hsu and Micallef, 2017; Kumar et al., 2018; Qasim et al., 2014; Saini et al., 2017).

Leaf nutrient concentration can also be used as an indicator of plant health. In pansy and impatiens treated with *Pseudomonas poae* or *P. fluorescens*, there was a significant increase in the N, P, K, Ca, and S concentration of the leaf tissue compared with untreated control plants when they were produced under low fertility (Nordstedt et al., 2020). Snapdragon (*Antirrhinum majus*) treated with AMF had higher levels of N, P, K, Ca, and Mg (Asrar et al., 2012). Higher concentrations of N, P, K, Ca, Mg, Fe, Mn, Zn, and B were observed in lettuce, melon (*Cucumis melo*), pepper, tomato, and zucchini (*Cucurbita pepo*)

with the cotreatment of AMF and *Trichoderma* (Colla et al., 2015). In this study, the application of products 1, 2, or 10 increased the leaf N content to levels comparable to zinnias fertilized with 150 mg L^{-1} N (Table 2). This is especially notable for product 10, which unlike products 1 or 2, did not contain large amounts of N.

Flower timing, size, and number are important characteristics for most floriculture crops. In our experiment with petunia, product 1 improved both flower numbers and biomass, whereas product 2 only increased total flower biomass (Fig. 5D and E). Treatment with

Fig. 4. Leachate pH (A) and electrical conductivity (EC) (B) of petunia treated with biostimulant products, negative control (NC), and positive control (PC). Bars represent the mean (n = 24) ± SE, and bars with different letters indicate a significant difference ($P \leq 0.05$). $1 \text{ mS}\cdot\text{cm}^{-1} = 1 \text{ mmho}\cdot\text{cm}^{-1}$.

various microbial inoculants increases flower production in greenhouse floriculture crops including zinnia, petunia, impatiens, pansy, chrysanthemum (*Chrysanthemum × morifolium*), geranium (*Pelargonium peltatum*), carnation (*Dianthus caryophyllus*), and snapdragon (*Antirrhinum majus*) (Asrar et al., 2012; Göre and Altin, 2006; Navarro et al., 2012; Nordstedt et al., 2020; South et al., 2021; Sukeerthi et al., 2020). Increased flower size has been reported

in zinnia, carnation, and marigold treated with beneficial microbes (Asrar and Elhindi, 2011; Navarro et al., 2012; Saini et al., 2017). Although we did not observe any difference in flowering time in this study (data not shown), reduced flowering time has been observed in petunia, China aster, and balsam treated with AMF (Gaur et al., 2000). Decreasing the time to first flower can reduce the time and cost for crop production.

Potential modes of action of growth-promoting biostimulants in this study

Our results from Expts. 1 and 2 showed that the applications of products 1, 2, and 10 demonstrated significant growth-promoting effects in zinnia and petunia. As indicated by the manufacturers' labels, both products 1 and 2 contain a diverse selection of beneficial bacteria and fungi, whereas product 10 only contains *Bacillus subtilis* strain QST 713 as the sole active ingredient (Table 1). Although different beneficial microbes are included in products 1 and 2, both products contain multiple species of AMF and *Bacillus* (Table 1). AMF are the most well-characterized beneficial fungi for plant growth promotion (Giovannini et al., 2020). AMF can colonize plant roots and promote nutrient uptake and transport in plants through extended hyphae (Genre et al., 2020). Inoculation with AMF is also beneficial under abiotic stresses such as drought, salinity, and nutrient deficiency (Rouphael et al., 2015). *Bacillus* is widely studied as a genus of PGPB (Kumar et al., 2011). Strains of *B. subtilis* can synthesize auxin, produce ACC deaminase, and improve plant nutrient uptake through N fixation and P solubilization (Awasthi et al., 2011; Latif Khan et al., 2016; Swain et al., 2012).

Even though *B. subtilis* was included in multiple biostimulant products in this study, not all the products containing *B. subtilis* showed growth-promoting effects, although the strains of *B. subtilis* cannot be determined from most of the labels (Table 1). This indicated that even with the same plant species grown under similar environments, the effectiveness of microbial biostimulants was not solely determined by the individual beneficial microbial ingredients, but also impacted by the compatibility among the microbes, the concentrations and viability of the microbes, the additive ingredients, the application rates and methods, and the stability of the products (Kumar and Aloke, 2020).

The media pH differences also revealed potential modes of action of effective products. The lower media pH levels (6.3–6.4) of product 1- and product 2-treated plants were closer to the optimal growing pH for petunia (pH = 5.5–6.2) than the positive and NCs (pH = 6.7–6.9). Nutrients, including Fe, B, Cu, Mn, and Zn, are less bioavailable for plant uptake at media pH above 6.5 (Gibson, 2007). The lower media pH may also improve the nutrient uptake efficiency by enhancing the growth-promoting effects of AMF (Fageria and Zimmermann, 1998; Zhu et al., 2007).

Many biostimulant labels are incomplete, and products contain nutrients and other components that are not included on the label. Biostimulants often include various plant extracts, vitamins, or amino acids, which may stimulate plant growth by various mechanisms including providing additional sources of macro or micronutrients for the plant. Nutrient analysis of the effective products showed that products 1 and 2 treatments provided high levels of mineral nutrients,

Fig. 5. Final harvest plant production measurements of petunia treated with biostimulant products, negative control (NC), and positive control (PC). (A) Vegetative shoot dry biomass. (B) Root dry biomass. (C) Root:shoot biomass ratio. (D) Flower dry biomass. (E) Flower number. Bars represent the mean (n = 24) ± SE. Bars with different letters indicate a significant difference ($P \leq 0.05$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. $1 \text{ g} = 0.0353 \text{ oz}$.

Table 3. The mineral nutrient composition of the effective plant growth promoting biostimulant products 1, 2, and 10. Nutrient concentrations in each product were measured, and then the amount of each nutrient that was applied to the plant in a single application was calculated.

Nutrient element	Product 1		Product 2		Product 10	
	Product ^z (mg·g ⁻¹) ^y	Applied ^x (mg) ^y	Product (mg·g ⁻¹)	Applied (mg)	Product (mg·g ⁻¹)	Applied (mg)
N	26.900	101.676	30.235	346.675	12.860	0.019
P	6.202	23.466	6.536	74.943	3.401	0.005
K	21.215	80.277	27.830	319.099	2.739	0.004
S	28.655	108.430	24.300	278.624	0.299	0.000
Mg	20.655	78.158	10.280	117.870	0.921	0.001
Fe	10.530	39.845	5.801	66.514	0.333	0.001
B	0.072	0.273	0.040	0.461	0.000	0.000
Cu	0.020	0.075	0.009	0.109	0.001	0.000
Mn	0.093	0.350	0.278	3.185	0.007	0.000
Zn	0.076	0.288	0.052	0.599	0.007	0.000
Ca	32.765	123.983	24.795	284.299	1.720	0.003
Al	12.480	47.224	12.945	148.427	0.668	0.001

^zConcentration of mineral nutrients in the original product (mg·g⁻¹).

^y1 mg = 0.0000353 oz, 1 mg·g⁻¹ = 1,000,000 ppm.

^xThe amount of a nutrient that was applied to the plant with a single application of the product. This was also equal to the total amount of nutrients applied throughout the experiment for Products 1 and 2 because they were only applied once at transplanting by mixing into the potting media. Product 10 was applied weekly via soil drench. To determine the total amount of each nutrient that would be contributed by Product 10 over the course of the experiment, the applied value must be multiplied by six applications for zinnia (Expt. 1) and five applications for petunia (Expt. 2).

whereas product 10 had much lower levels of nutrients for each application (Table 3). The higher media EC of product 1- and product 2- treated plants supports the finding that these products had high nutrient additives (Fig. 4B).

These unspecified nutrient components may be directly responsible for improved plant growth and will impact meaningful comparisons between the effects of different biostimulant products. For example, the increase of N in zinnia leaf tissue from product 1 or 2 treatment was likely influenced by the amount of N in the products. Product 10 was therefore most effective, enhancing plant growth with low levels of additive nutrients, suggesting that the increased nutrient levels in the plant tissue were a direct result of *Bacillus subtilis* in product 10. Interestingly, even with the amount of P that was applied with product 1 and product 2 treatments, the concentration of P was reduced in zinnia leaves compared with the NC. While product 10 only contained a low level of P, it was the only treatment that promoted the uptake of P by plants (Tables 2 and 3). We also observed a reduced P level in the PC (Table 2). This could be because high levels of fertilizer nutrients disrupted the colonization and P solubilization abilities of the native P-solubilizing microbes in the growing media (Breuillin et al., 2010; Davies et al., 2000; Nagy et al., 2009). Another possible reason is that AMF could also have been impacted by peat-based growing media, as shown in silver grass (*Miscanthus sinensis*) and onion (*Allium cepa*), where AMF colonization and effectiveness vary in different types of peat-mixed media (Linderman and Davis, 2003; Ma et al., 2007). Even though S is not a common component of professional greenhouse fertilizers, both product 1 and product 2 treatments resulted in increased S concentration in the leaf tissue (Table 2). The increase of S uptake in plants might be due to the increased availability of S in media by the S-oxidizing bacteria (e.g., *Bacillus*), the enhanced transport

through AMF, and the additional S applied by the products (Behera et al., 2014; Gahan and Schmalenberger, 2014; Meldau et al., 2013).

Biostimulant products with no growth-promoting effects in this study

Based on the results from Expts. 1 and 2, the majority of the biostimulant products tested in this study did not show any growth-promoting effects when applied to greenhouse-grown petunia or zinnia under our experimental conditions. Additionally, negative effects have been found with some biostimulant treatments. Plant size and vegetative shoot growth were suppressed in petunias treated with product 4, the only non-microbial biostimulant product in the trial (Figs. 3A, 3C, and 5A). Additionally, treatment 6b, which was product 6 applied at twice the recommended frequency as in treatment 6a, also reduced the vegetative shoot biomass in petunia (Fig. 5A). This was expected considering that the efficacy of microbial biostimulants can be highly variable even with the same microbial ingredients. For example, product 5 contained four different species of mycorrhizae applied as a one-time soil drench at transplant. While we did not observe growth promotion in this study, we have seen consistent growth promotion with a similar product containing the same mycorrhizae that is incorporated into the media at transplant (unpublished data). The lack of positive effects could be due to non-optimized application rates, non-balanced ingredients, or the lack of viability of the microbes. In addition, plant species and cultivars, growing media, and fertilization can also impact the function of biostimulants (Cakmakçi et al., 2006; Van Gerrewey et al., 2020; Vestberg et al., 2005). Therefore, testing the biostimulant products with different plants, diverse types of growing media, and various forms and concentrations of fertilizers would provide pivotal information to determine the range of applicability of the biostimulants. Through the testing, it is also important to optimize the

application rates (i.e., concentration and frequency) and the methods (e.g., soil drench or foliar spray) for specific plants under different production conditions.

Biostimulant applications can increase the sustainability of greenhouse production systems

Biostimulant products 1, 2, and 10 not only improved plant growth when compared with the NC, but even led to the same or sometimes better quality and increased size and leaf nutrient concentrations compared with PC plants. This confirmed that high-quality greenhouse floriculture crops can be produced with lower fertilizer inputs when effective biostimulants are used. A media drench with *Caballeronia zhejiangensis* strain C7B12 promotes growth in greenhouse petunias, resulting in plants with larger shoot sizes and greater flower numbers than plants produced with twice the fertilizer concentrations (South et al., 2021). Similar effects have been found in strawberry (*Fragaria × ananassa*), lettuce, arugula (*Eruca sativa*), broccoli (*Brassica oleracea*), potato (*Solanum tuberosum*), maize, wheat (*Triticum aestivum*), and grain amaranth (*Amaranthus cruentus* and *A. hypochondriacus*), showing that the applications of microbial biostimulants can enhance crop growth and quality to the same or higher level than plants produced with optimal fertility (Fiorentino et al., 2018; Lingua et al., 2013; Parra-Cota et al., 2014; Tanwar et al., 2014; Velivelli et al., 2015; Wahid et al., 2020). A study on spinach (*Spinacia oleracea*) and zucchini shows that the application of mycorrhizal biostimulants during greenhouse production reduces CO₂ emissions per ton of marketable spinach leaves or zucchini fruit compared with the untreated control by enhancing yield and produce quality under low fertility (Hamedani et al., 2020). Therefore, augmenting fertilizer applications with biostimulants cannot only improve plant growth and quality, but also provide for a more sustainable production environment.

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