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Humic and Fulvic Acids Promote Growth and Flowering in Petunias at Low and Optimal Fertility

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Abstract. **Humic substances are components of soil organic matter that influence soil structure and fertility. Humic and fulvic acids can be extracted from soil and other organic sources, and are used as biostimulants to promote plant growth and increase nutrient availability and uptake. The goal of this study was to determine whether selected humic and fulvic acid–based commercial products would promote growth and flowering of petunia (*Petunia ×hybrida*) 'Picobella Blue' grown in soilless media with low or optimal fertilizer rates. Plants were grown in 11.4-cm pots filled with peat-based media [80:20 peat:perlite (v/v); pH 5.4]. Three biostimulant products were evaluated at different rates and application frequencies: Huma Pro, a liquid humic acid biostimulant; Fulvi Pro, a liquid fulvic acid biostimulant; and Micromate, a powder containing both humic and fulvic acids. In Expt. 1, Huma Pro and Fulvi Pro were drenched weekly onto the growing media at a rate of 5, 10, or 20 mL·L^{-1}; Micromate was drenched weekly at a rate of 5, 10, 20, or 40 g·L^{-1}. Plants were fertilized with either 50 mg·L^{-1} nitrogen (N) (low fertility) or 100 mg·L^{-1} N (optimal fertility) from Jack's Professional 20N–1.3P–15.7K Petunia FeED each irrigation. Control plants received fertilizer but no biostimulant treatments. In Expt. 2, biostimulant treatments were drenched once at transplant, biweekly, or weekly at a rate of 1.25, 2.5, 5, or 10 mL·L^{-1} for Huma Pro and Fulvi Pro; and at 5, 10, 20, or 40 g·L^{-1} for Micromate. All plants received constant liquid feed at the lower fertilizer rate of 50 mg·L^{-1} N. In Expt. 1, plants fertilized with 100 mg·L^{-1} N and treated with 20 g·L^{-1} Micromate had the best performance. The average shoot dry weight was 32% greater than that of the control plants. Micromate (20 g·L^{-1})-treated plants had an average of five more flowers per plant, and they flowered 4 days earlier than untreated control plants. In Expt. 2, plants treated with 40 g·L^{-1} of Micromate weekly had the greatest shoot dry weight compared with the other treatments. Weekly Micromate treatments (40 g·L^{-1}) resulted in plants with an average of 13 more flowers per plant, which flowered 7 days earlier than control plants. Plants treated with Fulvi Pro and Huma Pro at 20 mL·L^{-1} had a significantly greater concentration of potassium in shoot tissue, whereas Micromate treatments at 20 and 40 g·L^{-1} resulted in a greater concentration of phosphorous in the shoots. The humic and fulvic acids in Micromate improved petunia crop quality by promoting vegetative growth, increasing flower numbers, and reducing the time to flower.**

The greenhouse industry is looking for alternatives to chemical fertilizers, pesticides, and growth regulators to maintain plant quality while reducing environmental impacts. Biostimulants have been highlighted recently as tools to help reduce chemical inputs. Biostimulants contain microorganisms or other substances that stimulate various natural processes in the plant that lead to improved nutrient-use efficiency and abiotic stress tolerance (Colla and Rouphael 2015). The use of biostimulants in agriculture aims to promote growth while improving crop quality, increasing yield, and enhancing tolerance to various environmental stresses (Colla and Rouphael 2015).

Nonmicrobial biostimulants include plant extracts, humic substances, phytohormones, protein hydrolysates, chitosan, phosphite, and silicon (du Jardin 2015; Rouphael and Colla 2020). Humic substances are chemically complex organic compounds that have been categorized into humins, humic acids, and fulvic acids based on molecular weight and solubility (Canellas et al. 2015; du Jardin 2015). Humic acids are high-molecular weight compounds that are soluble at alkaline conditions, whereas fulvic acids are comparably lower molecular weight compounds that are soluble at both acidic and alkaline conditions (Calvo et al. 2014; Stevenson 1994). Because of their smaller size, fulvic acids can be absorbed more easily by the roots, stems, and leaves through cation exchange, diffusion, and active transport, thereby facilitating the uptake of minerals and nutrients into plants and enhancing plant growth (Mylonas and McCants 1980; Zhang et al. 2021).

Humic acids and fulvic acids are used in commercial biostimulant products because of their beneficial effects on soils and plants (Canellas et al. 2015). The application of humic substances can improve soil characteristics by increasing porosity, cation exchange capacity, and water-holding capacity (Ampong et al. 2022). Humic substances promote plant growth by causing structural and physiological changes in the roots and shoots, which improve nutrient-use efficiency, and by modulating primary and secondary metabolism related to enhancing abiotic stress tolerance (Canellas et al. 2015).

The scientific literature has documented many instances of plant growth promotion or biostimulation by humic substances, but plant responses can be inconsistent and unpredictable (Canellas and Olivares 2014; Olanrewaju et al. 2017; Rose et al. 2014). Plant responses to humic substances are highly influenced by the source of the humic or fulvic acids, the plant species and stage of development, application rates and methods, growing media, and environmental conditions (Canellas et al. 2015). A random-effects meta-analysis of the results from the plant biostimulant literature revealed that increases in shoot dry weights of 22% and root dry weights of 21% resulted from the application of humic substances (Rose et al. 2014). The meta-analysis by Rose et al. (2014) also reported that shoot growth promotion was more likely to increase under highly stressful environmental conditions (28%) than under nonstressful conditions (18%).

Humic substances can have a positive impact on the quality and yield of horticultural crops by improving shoot and root growth, chlorophyll content and photosynthesis, flowering time, and fruit and flower number and size (Drobek et al. 2019). The foliar application of humic acid, fulvic acid, and calcium, both individually and in combinations, promoted growth, and increased shoot fresh and dry weight, fruit firmness, and yield in field-grown tomatoes (*Lycopersicon esculentum*) (Husein et al. 2015). The greatest improvements were observed in tomato plants treated with a foliar application that included humic acid, fulvic acid, and calcium (Husein et al. 2015). When treated with foliar humic acid fertilizer, chrysanthemums (*Chrysanthemum indicum*) in soilless media had improved growth and flowering, and significant increases in photosynthetic activity (Fan et al. 2014). Humic acid applications to gladiolus (*Gladiolus* sp.) bulbs before planting, enhanced growth, flower number, and plant quality, and reduced the time to flowering (Baldotto and Baldotto 2013). In contrast, humic acid drenches at different rates did not increase dry weights (roots or shoots) or yield of blueberries (*Vaccinium corymbosum*) grown in soilless substrate (Nunez et al. 2023).

The majority of biostimulants have better performance when plants are under abiotic stress conditions such as drought; nutrient deficiencies; extreme temperatures, salinity, and radiation; and high or low soil pH (Drobek et al. 2019; Rouphael and Colla 2020). For producers of horticultural crops, one of the greatest benefits of humic substances would be the potential increases in nutrient-use efficiency, which allows for the production of high-quality crops with fewer inorganic fertilizer inputs (Parađiković et al. 2018). Humic acids extracted from shale ore improved

tomato plant growth, fruit yield, and fruit quality, but the greatest increases were observed when plants were grown under nutrient-stress conditions (Monda et al. 2021). Monda et al. (2021) found that humic acid treatment increased tomato numbers by 19% and 16% at low fertility with quarter- and half-strength nutrient solutions, respectively, whereas humic acid applications at full fertility decreased the number of tomatoes by 13% compared with controls.

Several studies have shown the potential benefit of humic substances in soil-based production systems, but less is known about the application of products derived from humic or fulvic acids in soilless culture. The adoption of developing technologies by growers requires research-based information about the efficacy, correct management, and applicability of these products in floriculture crops grown in soilless media. The goal of our study was to determine whether selected humic and fulvic acid–based commercial products would promote growth and flowering of petunia (*Petunia ×hybrida*) grown in soilless media with low or optimal fertilizer rates. Objective 1 was to evaluate growth and flowering in petunia plants treated weekly with drench applications of the products at different rates at low and optimal fertility. We hypothesized that all the products containing humic and/or fulvic acids would increase petunia growth, increase flower number, and decrease the time to flowering, and that these improvements would be greatest at low fertility. Objective 2 was to determine the frequency of the application needed to obtain the greatest growth promotion in petunia. We hypothesized that at the greater application rates, one-time applications would be as effective at promoting growth as weekly applications. Petunia was used as a plant model in these experiments because of its economic importance to the floriculture industry.

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Materials and Methods

Seed growth and environmental conditions. *Petunia ×hybrida* 'Picobella Blue' seeds were started in a 288-plug tray filled with Pro-Mix PGX (Premier Tech Horticulture, Quakertown, PA, USA). Seeds were germinated under fluorescent lights with a 12-h photoperiod. High humidity was maintained using a propagation dome. Seedlings were moved to the greenhouse after 3 weeks to acclimate to the greenhouse environment. Seedlings were fertilized with 50 mg·L^{-1} nitrogen (N) from Jack's Professional 20N–1.3P–15.7K Petunia FeED (J.R. Peters, Allentown, PA, USA) until transplant. The greenhouse air temperature at canopy height was 21.1 to 24.4 °C during the day and 15.5 to 18.3 °C at night, with 70% relative humidity. A photoperiod of 14 h was maintained, and supplemental light was provided by a 1:1 mix of metal halide and high-pressure sodium lamps when the photosynthetically active radiation (PAR) was less than 250 μmol·m^{-2}·s^{-1} at bench height. Shade was provided when the PAR was more than 400 μmol·m^{-2}·s^{-1} .

Four weeks after sowing, the seedlings were transplanted into 11.4-cm standard round pots filled with a peat-based medium. The medium was mixed in-house with 80% peat (Premier Tech Horticulture) and 20% coarse perlite (PVP Industries Inc., North Bloomfield, OH, USA) by volume. A wetting agent (AquaGrowL; Aquatrols, Paulsboro, NJ, USA) was added at 7.7 mL·100 L^{-1} of media, and dolomitic limestone (Oldcastle Lawn & Garden, Atlanta, GA, USA) was added to adjust media to a final pH of 5.4.

Biostimulant treatments. Three commercial biostimulant products and one control were included as treatments. The products that contained humic substances were Huma Pro (HP), a liquid humic acid biostimulant (Bio Huma Netics Inc., Gilbert, AZ, USA); Fulvi Pro (FP), a liquid fulvic acid biostimulant (Bio Huma Netics Inc.); and Micromate (MM), a powder containing both humic and fulvic acids (Mesa Verde Humates, San Ysidro, NM, USA). According to the labels, HP contains 16% humic acid and FP contains 20% fulvic acid plus 6% organic matter. MM is composed of 24% humic and fulvic acids. All the product's a.i. (humic substances) originate from oxidized leonardite.

In Expt. 1, different concentrations of each product were tested at two fertilizer rates of 50 and 100 mg·L^{-1} N from a commercial water-soluble fertilizer that contained 20N–1.3P–15.7K and micronutrients (Jack's Professional Petunia FeED 20-3-19, J.R. Peters). These rates are referred to as low and optimal fertility. Micronutrients in the fertilizer included 1.34% magnesium (Mg), 0.01% copper (Cu), 0.2% iron (Fe), 0.05% manganese (Mn), and 0.05% zinc (Zn). Petunia plants were irrigated daily, or as needed, with one of the two fertilizer treatments. All biostimulant products were diluted in city water, and 150 mL was drenched on each pot weekly starting at transplant. Rates for HP and FP included 5, 10, and 20 mL·L^{-1}, whereas MM was drenched at 5, 10, 20, and 40 g·L^{-1}. Control plants did not receive any biostimulants and were drenched with water on treatment days. This experiment was conducted from Jul to Sep 2022. The petunias were harvested 6 weeks after transplant when most of the plants were at a marketable stage with at least three open flowers. The experiment was a randomized complete block design (RCBD) with a total of 22 treatments and 10 individual plant replicates.

In Expt. 2, biostimulant treatments were applied once at transplant, biweekly (once every 2 weeks), or weekly (once a week), and all plants were grown with a single fertilizer rate of 50 mg·L^{-1} N from Jack's Professional 20N–1.3P–15.7K Petunia FeED (J.R. Peters). The low rate of 50 mg·L^{-1} N was chosen because the greatest differences in growth between the biostimulant and the control treatments were observed with the lower rate of fertilizer in Expt. 1. Treatments started at transplant, and biostimulants were diluted in city water and applied as a media drench as described in Expt. 1. HP and FP rates were reduced based on the results from Expt. 1 and included 1.25, 2.5, 5, and 10 mL·L^{-1}, whereas rates for MM included 5, 10, 20, and 40 g·L^{-1}. Control plants did not receive any biostimulant treatment. Plants were fertigated as needed. The experiment was conducted from Aug to Sep 2022 and plants were harvested 5 weeks after transplant. The experiment was an RCBD that included 37 treatments and 10 individual plant replicates.

Growth and flowering parameters measurement. In both experiments, nondestructive plant growth measurements were conducted every 2 weeks. Leaf greenness was measured with a SPAD-502 chlorophyll meter (Konica Minolta Sensing Americas, Inc., Ramsey, NJ, USA) on the second fully expanded leaf from the meristem. A ruler was used to measure the plant height (H) and the two perpendicular widths (W) to calculate the growth index (GI) using the formula GI = [($W1$ + $W2$)/2 + H]/2 (Niu et al. 2010). Photographs, media pH using a pH probe (Hanna Instruments, Inc., Smithfield, RI, USA), and electrical conductivity (EC) using an EC probe (ProCheck version 7; Decagon Devices, Inc., Pullman, WA, USA) were also taken biweekly. The pH and EC of the media were measured 1 h after irrigation to ensure consistent media moisture content.

The date of the first open flower on each plant was recorded, and the flowering time from transplant was calculated. At the end of each experiment, shoots were harvested, and open flowers and buds showing color were removed after counting. Roots were collected only for Expt. 2. Bulk medium was shaken off the roots, and the root systems were washed using a root elutriator (UGA Instrument Shop, Athens, GA, USA). Roots, shoots, buds, and open flowers were collected separately; placed in paper bags; dried in a forced-air drying oven at 60 °C for 5 d; and weighed to determine the dry weight (DW).

Shoot nutrient analysis. In Expt. 1, dried shoot tissue was sent for nutrient analysis at the Service Testing and Research Laboratory

(STAR) Laboratory, The Ohio State University (Wooster, OH, USA). Plant tissue of two experimental blocks were pooled for analysis, giving three replicates of each of the following treatments: MM at 5, 10, 20, 40 g·L^{-1} and FP and HP at 20 mL·L^{-1}. Plant tissue was ground and then digested using an automated microwave digestion system (Discover SP-D; CEM, Matthews, NC, USA). The total concentration of the nutrients—phosphorus (P), potassium (K), aluminum (Al), boron (B), calcium (Ca), Cu, Fe, Mg, Mn, molybdenum (Mo), sodium (Na), sulfur (S), and Zn—was measured by inductively coupled plasma (ICP) emission spectrometry (Agilent 5110 ICP-OES; Agilent Technologies, Santa Clara, CA, USA) (Isaac and Johnson 1985). The concentration of total N was measured by combustion using a Vario Max Cube Carbon-Nitrogen Analyzer (Elementar Americas, Mt. Laurel, NJ, USA).

Biostimulant product nutrient analysis. The nutrient concentrations in the biostimulant products were analyzed by ICP at the STAR Laboratory as described earlier. For total N, samples were sent to the US Department of Agriculture Agricultural Research Service Application Technology Research Unit Analytical Laboratory (Wooster, OH, USA) and analyzed via combustion. The samples were filtered and then analyzed using a TNM-L Total Nitrogen Module (Shimadzu Scientific Instruments, Columbia, MD, USA). The total amount of the individual nutrients supplied to plants by each biostimulant treatment and throughout the course of the experiment was calculated for the 20-g·L^{-1} MM and 20-mL·L^{-1} HP and FP treatments.

Statistical analysis. For both experiments, the statistical analysis was conducted using an analysis of variance (ANOVA) in RStudio (ver. 4.2.2; RStudio, Boston, MA, USA) using the model y = Treatment + Block. If ANOVA identified significant differences, the means were separated using Tukey's honestly significant difference test at $\alpha = 0.05$ ($P < 0.05$). The normality of the residuals was checked visually and using the Shapiro-Wilk's test. Days to first flower, flower number, and bud number were analyzed using the Kruskal-Wallis rank-sum test, followed by the Dunn's test for multiple comparisons against the control with the R package FSA Version 0.9.5 (R Foundation for Statistical Computing, Vienna, Austria) ($P < 0$ 0.05).

Results

MM treatment promoted growth in petunias grown at both low and optimal fertility conditions. MM, which includes humic and fulvic acids, was the only biostimulant that increased vegetative growth and flowering in petunia compared with untreated control plants in Expt. 1 (Fig. 1A and B). For plants grown at low fertility (50 mg·L^{-1} N), the GI at 2 weeks after transplant was significantly greater in plants that received weekly drenches with 10, 20, and 40 g·L^{-1} MM (MM 10, MM 20, and MM 40) compared with control plants that were not treated with humic substances (Fig. 2A). MM 10-, MM 20-, and MM 40-treated plants were 25%, 48%, and 42% larger than untreated controls. For plants grown at optimal fertility (100 mg·L^{-1} N), the MM 20 and MM 40 treatments also resulted in significantly greater GIs (28% and 24%, respectively) at week 2.

Plants treated with MM were still larger than control plants at week 4, but the differences in GIs were not significant at either low or optimal fertility (Fig. 2B). However, we observed a significant effect of MM on the shoot DW. Compared with the control, MM 40-treated plants showed a 53% increase in shoot DW at low fertility (Fig. 2C), and MM 20- and MM 40-treated plants showed a 31% and 30% increase in DW, respectively, at optimal fertility. Growth promotion was not observed in petunias treated with the lowest rate of MM (5 g·L^{-1}) (Fig. 1). There were no significant effects of the HP or FP applications on GI in petunias grown at low or optimal fertility (Fig. 2). Petunia plants treated with the greatest rate of FP (FP 20) had a lower GI at both low and optimal fertility, but they were not statistically different from control plants (Fig. 2A and B). Only the shoot DW of plants grown at low fertility and treated with FP 20 was significantly lower than the control (Fig. 2C).

Overall, in our study, petunias grown at low fertility (50 mg·L^{-1} N from 20N–1.3P–15.7K Petunia FeED) were smaller than plants grown with twice the concentration of nutrients (optimal fertility at 100 mg·L^{-1} N from 20N–1.3P–15.7K Petunia FeED), confirming that the low fertility treatment was not optimal for plant growth (Fig. 1). Control plants at the low fertility rate were also showing symptoms of nutrient deficiency, including chlorosis and accelerated leaf senescence. These deficiency symptoms were observed in all HP treatments (HP 5, HP 10, and HP 20) and on FP 10-, FP 20-, and MM 5-treated plants at low fertility (Fig. 1A). For plants grown with the optimal fertility rate, lower leaf chlorosis was observed only with the HP 10, HP 20, FP 10, FP 20, and MM 5 treatments (Fig. 1B). Despite these visual deficiency symptoms on older leaves, soil plant analysis development (SPAD) readings, which were taken on young leaves, did not show any significant differences in leaf greenness between treated and control plants (data not shown). Measurements of media pH and EC were taken on week 4 after transplant, but there was no effect of biostimulant treatment or fertilizer rate on media pH or EC (data not shown).

MM treatment reduced flowering time and increased the number of flowers per plant in petunias grown at low fertility. Flowering time (i.e., time to the first open flower), flower numbers, and flower DW were also improved with the MM treatment (Table 1). Control

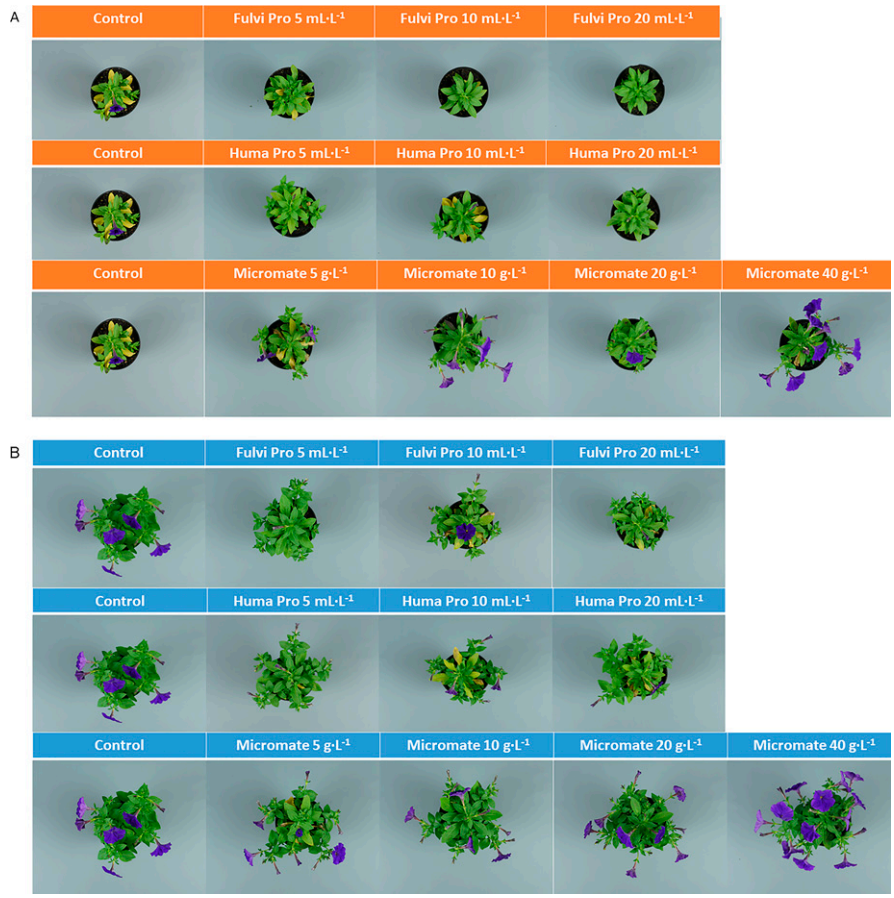

Fig. 1. Expt. 1. *Petunia ×hybrida* 'Picobella Blue' treated weekly via drench with Fulvi Pro (fulvic acid), Huma Pro (humic acid), and Micromate (humic + fulvic acid) (**A**) with a low fertilizer rate [50 mg·L^{-1} nitrogen (N)] on week 4 after transplant and (**B**) with optimal fertilizer rate (100 mg·L^{-1} N) on week 4 after transplant.

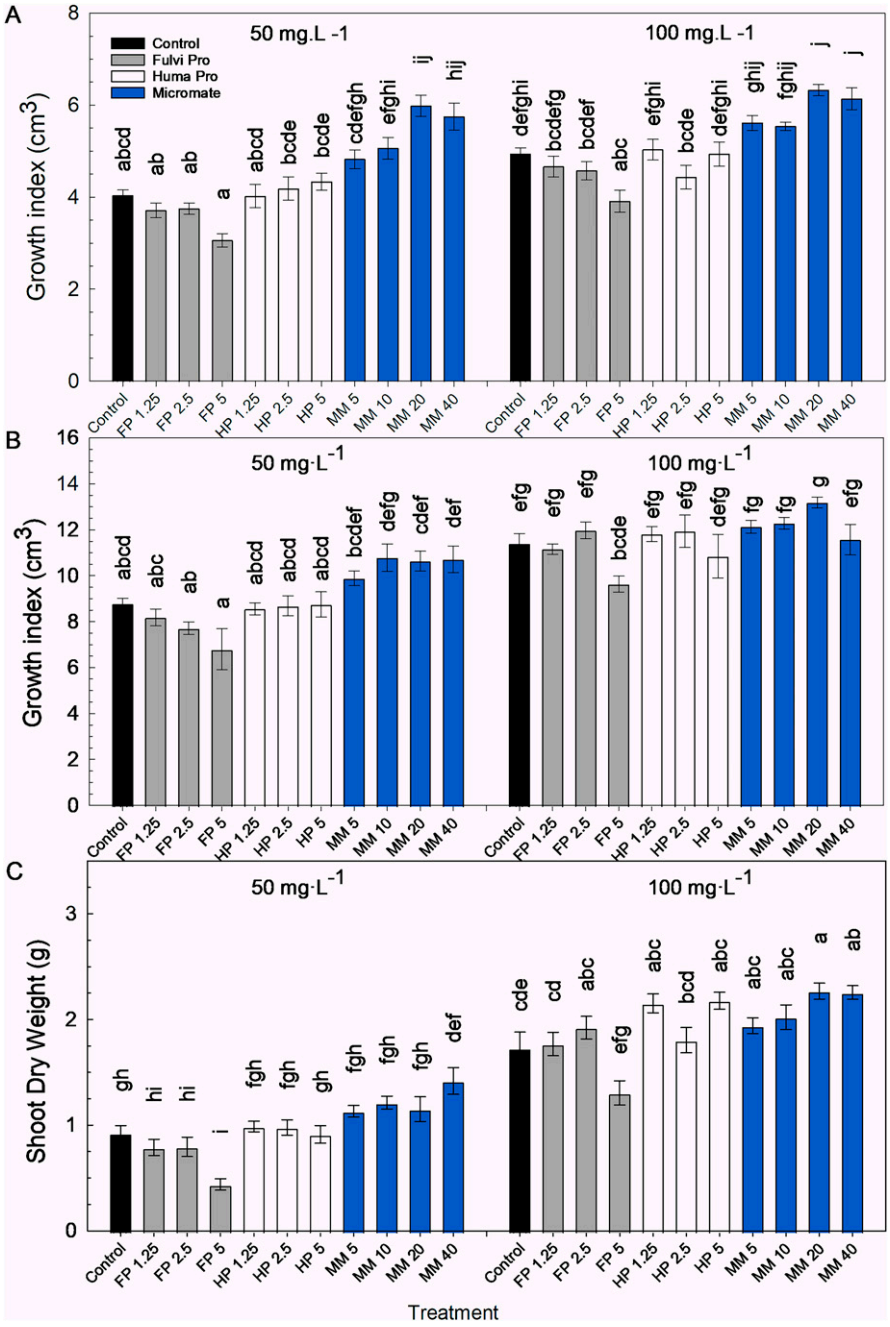

Fig. 2. Expt. 1. (**A**) Growth index of *Petunia* ×*hybrida* 'Picobella Blue' at week 2 and (**B**) week 4 after transplant. Fulvi Pro (FP), Huma Pro (HP), and Micromate (MM) treatments were applied weekly via drench. The number in the treatment name refers to the application dose. (**C**) Final shoot dry weight of petunias. Bars represent the mean ± standard error for each treatment (n = 10). Different letters represent statistically significant differences between the means ($P \leq 0.05$). Plants were fertilized with either 50 mg·L^{-1} nitrogen (N) (left) or 100 mg·L^{-1} N (right).

petunias at optimal fertility flowered an average of 2 d earlier than controls at low fertility, but this difference was not significant. Flowering time was consistently reduced by MM treatments. All four rates of MM at low fertility accelerated flowering, with flowering time reduced by day 7 in the MM 40-treated petunias (Table 1). At optimal fertility, the MM 20 and MM 40 treatments resulted in petunias that flowered 3.5 and 3.9 d earlier, respectively. Flower timing was unaffected by HP or FP applications at low or optimal fertility, except for the greatest rate of FP (FP 20), at which petunias flowered an average of 4.5 d later than control petunias.

At low fertility, the control petunias had an average of seven open flowers per plant at harvest (6 weeks after transplant) compared with control plants at optimal fertility, which had an average of 16.8 open flowers per plant (Table 1). MM treatments increased flower numbers at harvest, but these differences were only significant at low fertility. MM 40-treated petunias had 9.5 more flowers per plant than control petunias. All MM treatments at low fertility also increased the DW of open flowers. In contrast, the greatest FP rate (FP 20) had a negative impact on the number of flowers per plant and flower DW only at optimal fertility (Table 1). FP 20-treated plants produced fewer flowers and accumulated lower flower DW than control plants. Similarly, total flower DW was increased by MM at low but not optimal fertility. In addition to open flowers, we also counted, collected, and dried any flower buds that were showing color. Flower bud number and DW were unaffected by any of the treatments (Table 1).

Humic and fulvic acids influenced the mineral nutrient concentration in the shoots of petunias grown at low fertility and optimal fertility. The N concentration in untreated control petunias grown at low and optimal fertility was not different (Table 2). Although the tissue N concentration in MM-treated plants was less than in control plants at both low and high fertility, only MM 20 at low fertility showed a significant decrease. MM 20-treated plants had 24% less N than the untreated control (Table 2).

The greatest and most consistent treatment effects were seen with P (Table 2). The shoot P concentration in untreated control plants grown at low and optimal fertility was not different. MM treatment increased the P concentration in the shoots at both low and optimal fertility. Compared with the control, MM 20 and MM 40 treatments increased P content by 36% and 85%, respectively, at optimal fertility, and by 73% and 127%, respectively, at low fertility. Petunias grown at low fertility and treated with MM 20 or MM 40 had a greater shoot P concentration than the untreated control plants grown at optimum fertility. Interestingly, HP and FP had the opposite effect on tissue P concentration, but this effect was significant only at optimal fertility. Under optimal fertility, the HP 20 treatment reduced shoot P content by 52%, and the FP 20 treatment reduced P by 44%.

The K concentration in untreated control plants grown at low and optimal fertility was not significantly different (Table 2). Shoot K concentration was reduced by the MM treatment at both low and optimal fertility, although these differences were only significant at low fertility. FP (FP 20) increased K concentration significantly at both low and optimal fertility (43.9% and 44.3%, respectively), whereas HP (HP 20) increased K concentration significantly only at low fertility (29.8%) (Table 2).

The Ca, Mg, and S concentrations in the shoots of untreated control plants grown at low and optimal fertility were also not different (Table 2). For both Ca and Mg, the average shoot concentrations were generally lower in the HP, FP, and MM treatments at both fertility levels, but not all these differences were significant. At low fertility, the Ca concentration of MM 40-treated petunias was decreased by 26% compared with control petunias (Table 2). The Mg concentration was significantly less in the MM 10, MM 20, and MM 40 treatments at low fertility, with decreases of 22.1%, 22.4%, and 24%, respectively

Table 1. Effect of weekly applications of humic substance drenches on flower characteristics of *Petunia ×hybrida* 'Picobella Blue' (Expt. 1).

Treatment[i]	Fertilizer rate (mg·L^{-1} N)[ii]	Time to first flower (d)[iii]	No. of flowers	No. of buds	Flower dry wt (g)	Bud dry wt (g)
Control	50	30.9 f–h[iv]	7.0 a–c	3.8 a–c	0.195 ef	0.050 de
HP 5	50	31.7 gh	6.2 ab	4.1 a–d	0.162 ef	0.066 c–e
HP 10	50	30.7 f–h	5.4 ab	4.0 a–d	0.156 ef	0.057 de
HP 20	50	30.0 e–h	6.1 ab	2.9 ab	0.165 ef	0.049 de
FP 5	50	31.4 gh	8.6 a–d	4.0 a–e	0.157 ef	0.053 de
FP 10	50	32.4 hi	4.4 a	5.0 a–f	0.125 f	0.081 a–e
FP 20	50	35.4 i	4.7 ab	1.7 a	0.032 f	0.012 e
MM 5	50	27.7 c–e	13.5 c–f	5.6 b–f	0.370 cd	0.087 a–d
MM 10	50	27.7 c–e	14.7 d–h	6.1 c–f	0.392 cd	0.095 a–d
MM 20	50	24.4 ab	11.8 b–e	5.1 a–f	0.367 cd	0.074 b–e
MM 40	50	23.9 a	16.5 e–i	5.8 b–f	0.468 a–c	0.087 a–d
Control	100	28.4 d–f	16.8 e–i	7.2 d–f	0.509 a–c	0.122 a–c
HP 5	100	29.1 d–g	19.0 f–i	8.0 f	0.610 ab	0.129 ab
HP 10	100	29.8 d–h	14.3 d–g	6.9 c–f	0.456 b–d	0.118 a–c
HP 20	100	28.6 d–g	17.1 e–i	7.4 ef	0.521 a–c	0.127 a–c
FP 5	100	28.9 d–g	15.0 d–h	6.0 b–f	0.494 a–c	0.104 a–d
FP 10	100	28.9 d–g	18.6 f–i	7.3 f	0.574 ab	0.129 ab
FP 20	100	31.1 f–h	9.8 a–d	6.0 b–f	0.299 de	0.100 a–d
MM 5	100	27.4 b–e	21.1 hi	7.8 f	0.609 ab	0.137 a
MM 10	100	26.9 b–d	20.7 g–i	7.6 f	0.592 ab	0.120 a–c
MM 20	100	24.9 a–c	21.3 hi	7.3 ef	0.624 a	0.125 a–c
MM 40	100	24.5 a–c	21.9 i	6.3 b–f	0.627 a	0.122 a–c

[i] The number in the treatment name stands for the application doses: Huma Pro (HP) and Fulvi Pro (FP) at 5, 10, and 20 mL·L^{-1}; and Micromate (MM) at 5, 10, 20, and 40 g·L^{-1}.
[ii] N = nitrogen.
[iii] Time to first flower is the number of days from transplant to the first open flower.
[iv] The results are the means of 10 replicates. Different letters represent statistically significant differences among the means within a column ($P \leq 0.05$).

(Table 2). Last, the average shoot S concentration was greater in some of the MM-treated petunias, but none of the treatments were significantly different from control plants at either low or optimal fertility.

None of the micronutrients were significantly different in the shoots when comparing control petunias at low or optimal fertility (Table 3). The Fe, Cu, Mn, and Mo concentrations in the shoots were not significantly affected by HP, FP, or MM treatments (Table 3). The average tissue Al concentration increased in MM-treated plants in a dose-dependent manner. However, only the effect of MM 40 at optimal fertility was significant. FP 20- and HP 20-treated plants had an average tissue Al concentration that was less than untreated control plants at both low and optimal fertility, but the observed differences were not statistically significant (Table 3). The average B concentration in tissue was less in MM-, HP-, and FP-treated plants than in untreated controls at both low and optimal fertility. However, only the effects of treatments FP 20, MM 5, MM 10, and MM 40 at low fertility were statistically significant (Table 3). The tissue Na concentrations were significantly less than the control in plants treated with HP 20 and FP 20 (Table 3). The tissue Zn concentration was only significantly less than control plants treated with MM 40. The Zn concentration in MM 40-treated petunias was 42.7% less than control Zn concentrations at lower fertility.

One-time and biweekly applications of MM are equally effective at promoting growth and flowering compared with weekly applications. In Expt. 2, we evaluated the effect of application frequency and rate on the efficacy of MM, FP, and HP at promoting growth and flowering. Four weeks after transplant, the GI was greater in most of the MM treatments compared with the control (Fig. 3). A one-time application of MM at 5 or 10 g·L^{-1} and a biweekly MM application at 5 g·L^{-1} did not result in significantly increased GIs. In contrast, these lower application rates did increase the GI when MM was applied weekly. The greatest increases in the GI were observed in plants treated with the greatest MM rates (20 or 40 g·L^{-1}). The GIs of plants treated with the greatest rates (MM 20 and MM 40) were unaffected by application frequency. HP applications did not have any significant effect on the GI regardless of the rate or application frequency. Plants treated with FP at high doses had lower GIs than the untreated controls. Biweekly or weekly applications of FP 5 and FP 10, and a one-time application of FP 10 resulted in significant reductions in the plant GI. Leaf greenness as indicated by SPAD readings was unaffected by the HP or MM treatments, but lower SPAD readings were obtained from plants treated with FP 10 either biweekly or weekly (Fig. 4).

Shoot DWs at the end of the experiment showed similar trends to those observed with the GIs. MM application increased shoot DW significantly (Fig. 5A). One-time MM application at 20 and 40 g·L^{-1} increased shoot DW by 65% and 107%, respectively, compared with the control. Biweekly MM application at 10, 20, and 40 g·L^{-1} increased shoot DW by 82%, 128%, and 175%, respectively. All four MM rates increased shoot DW when applied weekly. The shoot DWs of MM 5-, MM 10-, MM 20-, and MM 40-treated plants were 74%, 134%, 152%, and 186% greater, respectively, than control plants. Similarly, the majority of MM treatments increased root DW (Fig. 5B). The greatest root DWs were obtained when plants were drenched with MM 40 either one time, biweekly, or weekly. Only the one-time MM applications at 5 and 10 g·L^{-1} were not significantly different from the untreated control. One-time, biweekly, or weekly application of 40 g·L^{-1} MM resulted in similar root DW. Compared with the untreated control, one-time, biweekly, and weekly applications of MM at 40 g·L^{-1} resulted in 160%, 189%, and 181%, greater root DW, respectively.

HP applications did not have any significant effects on shoot or root DW regardless of the rate or application frequency (Fig. 5). Plants treated with FP at high doses showed

Table 2. Mineral macronutrient concentrations in *Petunia ×hybrida* 'Picobella Blue' dry shoot tissue (Expt. 1).

Treatment[i]	Fertilizer rate (mg·L^{-1} N)[ii]	Macronutrients (mg·g^{-1})[iii]					
		N	P	K	Ca	Mg	S
Control	50	29.5 a–d[iv]	1.67 a–d	30.42 de	18.57 b	13.11 b	3.05 a–f
FP 20	50	34.9 a	1.18 a	41.54 f	14.36 ab	10.50 ab	2.71 a–c
HP 20	50	35.0 a	1.11 a	41.64 f	16.05 ab	11.47 ab	2.76 a–c
MM 5	50	26.1 b–e	1.31 ab	23.05 a–d	14.28 ab	10.49 ab	2.42 a
MM 10	50	24.3 c–e	1.83 b–d	19.37 ab	13.95 ab	10.21 a	2.49 ab
MM 20	50	22.5 e	2.88 f	20.85 a–c	13.91 ab	10.17 a	3.20 b–f
MM 40	50	23.0 de	3.78 g	17.32 a	13.67 a	9.95 a	3.53 d–f
Control	100	35.0 a	2.21 de	28.86 c–e	16.01 ab	12.10 ab	3.32 c–f
FP 20	100	35.8 a	1.53 a–c	39.48 f	14.15 ab	10.29 a	2.72 a–c
HP 20	100	34.4 a	1.46 a–c	37.08 ef	15.37 ab	11.43 ab	2.87 a–e
MM 5	100	31.6 ab	1.96 cd	24.29 a–d	13.43 a	10.71 ab	2.84 a–d
MM 10	100	33.1 a	2.59 ef	25.49 a–d	12.24 a	9.96 a	3.12 a–f
MM 20	100	33.2 a	3.00 f	23.37 a–d	13.74 a	10.55 ab	3.63 ef
MM 40	100	30.0 a–c	4.09 g	26.28 b–d	12.67 a	10.05 a	3.68 f

[i] The number in the treatment name stands for the application doses: Huma Pro (HP) and Fulvi Pro (FP) at 5, 10, and 20 mL·L^{-1}; and Micromate (MM) at 5, 10, 20, and 40 g·L^{-1}.
[ii] N = nitrogen.
[iii] Ca = calcium; K = potassium; Mg = magnesium; P = phosphorus; S = sulfur.
[iv] The results are the means of 10 replicates. Different letters represent statistically significant differences among the means within a column ($P \leq 0.05$).

Table 3. Mineral micronutrient concentrations in *Petunia ×hybrida* 'Picobella Blue' dry shoot tissue (Expt. 1).

Treatment[i]	Fertilizer rate ($mg·L^{-1}$ N)[ii]	Micronutrients ($\mu g·g^{-1}$)[iii]							
		Al	Fe	B	Cu	Mn	Mo	Na	Zn
Control	50	518.3 a–d[iv]	752.5 a	49.1 b	2.2 ab	327.1 b	0.5 a	12,540 cd	106.3 d
FP 20	50	140.6 a	662.9 a	37.6 a	2.3 ab	249.7 ab	0.8 a	9,644 a	93.9 b–d
HP 20	50	66.0 a	610.2 a	41.6 ab	2.3 ab	249.9 ab	0.7 a	9,862.3 ab	93.1 b–d
MM 5	50	198.1 a	603.4 a	36.1 a	2.0 ab	304.0 ab	0.5 a	11,850 b–d	98.4 cd
MM 10	50	326.1 ab	585.9 a	36.2 a	2.2 ab	291.7 ab	0.6 a	12,376.7 cd	87.0 a–d
MM 20	50	829.4 b–d	634.6 a	39.3 ab	2.2 ab	273.4 ab	0.3 a	12,923.3 d	84.3 a–d
MM 40	50	964.0 cd	645.9 a	38.7 a	2.6 b	257.1 ab	0.4 a	12,880 d	60.9 a
Control	100	364.6 ab	677.0 a	43.0 ab	1.9 ab	257.2 ab	0.6 a	11,936.7 b–d	88.3 a–d
FP 20	100	68.3 a	700.4 a	36.9 a	1.7 a	237.9 ab	0.4 a	10,503.3 a–c	91.7 a–d
HP 20	100	65.4 a	785.4 a	38.2 a	1.7 a	291.8 ab	0.4 a	10,563.3 a–c	94.7 b–d
MM 5	100	231.0 a	632.9 a	35.6 a	1.8 ab	259.2 ab	0.7 a	12,186.7 cd	80.8 a–d
MM 10	100	427.5 a–c	498.2 a	36.4 a	2.0 ab	201.5 a	0.4 a	11,966.7 cd	68.4 a–c
MM 20	100	570.7 a–d	616.7 a	41.7 ab	1.9 ab	252.6 ab	0.6 a	12,580 cd	67.9 a–c
MM 40	100	1039.4 d	637.6 a	36.4 a	2.3 ab	228.1 ab	0.6 a	13,160 d	64.4 ab

[i] The number in the treatment name stands for the application doses: Huma Pro (HP) and Fulvi Pro (FP) at 20 $mL·L^{-1}$; and Micromate (MM) at 5, 10, 20, and 40 $g·L^{-1}$.
[ii] N = nitrogen.
[iii] Al = aluminum; B = boron; Cu = copper; Fe = iron; Mn = manganese; Mo = molybdenum; Na = sodium; Zn = zinc.
[iv] The results are the means of 10 replicates. Different letters represent statistically significant differences among the means within a column ($P \leq 0.05$).

lower shoot DW than the untreated controls (Fig. 5A). Biweekly FP 10 applications or weekly FP 5 and FP 10 applications resulted in significant reductions in shoot DW. Greater concentrations of FP reduced average root DW, but mean differences were not statistically significant (Fig. 5B).

In Expt. 2, the control plants first flowered 28.8 d after transplant (Table 4). MM treatments had a positive influence on flowering by reducing the time from transplant to the first open flower. All the MM 20 and MM 40 treatments (one-time, biweekly, and weekly applications) flowered earlier than control plants. MM at 5 $g·L^{-1}$ reduced flowering time only when applied weekly, whereas the rate of 10 $g·L^{-1}$ MM reduced flowering time when applied biweekly or weekly. The HP and FP treatments did not affect the time to first open flower (Table 4).

At harvest (6 weeks after transplant), the control plants had an average of one open flower and 0.7 bud per plant. All the HP and FP treatments had similar open flower and bud numbers. Similarly, the total flower and bud DWs were not different from control plants. Flower number, bud number, flower DW, and bud DW were increased by the MM 20 and MM 40 treatments. MM 10 had significantly more flowers and buds only when treated biweekly and weekly. MM-treated plants had from 2 to 13 more flowers per plant than control plants. The largest flower counts and flower DWs were observed with the biweekly and weekly MM 40 applications.

The biostimulant products contained different concentrations of macro and micronutrients. The N content of FP was the greatest of the three products, at 113.06 $\mu g·mL^{-1}$ compared with only 2.23 and 13.09 $\mu g·mL^{-1}$ in HP and MM, respectively (Tables 5–7). The P concentration in FP (0.093 $\mu g·mL^{-1}$) and HP (0.644 $\mu g·mL^{-1}$) was lower than that of MM (2.920 $\mu g·mL^{-1}$). In contrast, K concentrations were 1738% and 3913% greater with FP and HP, respectively, compared with MM. The S in the three products was similar, and S concentration ranged from 40 to 49 $\mu g·mL^{-1}$. FP and HP contained greater levels of Mg and Ca than MM. All three products contained micronutrients except for Mo, which was undetectable in FP and MM. The most notable differences included greater levels of Zn and Cu in FP and HP compared with MM, and much greater Al (3.348 $\mu g·mL^{-1}$) in HP. The Fe concentration in HP (2.424 $\mu g·mL^{-1}$) was higher than in FP (0.039 $\mu g·mL^{-1}$) and MM (0.015 $\mu g·mL^{-1}$).

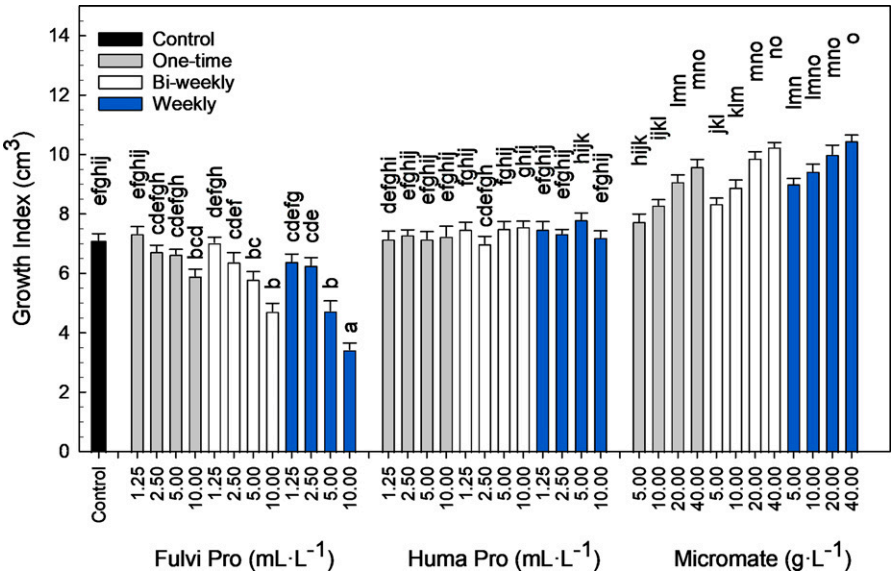

Fig. 3. Expt. 2. Growth index of *Petunia ×hybrida* 'Picobella Blue' at week 4 after transplant. Fulvi Pro, Huma Pro and Micromate were applied once, biweekly, or weekly. The black bar represents the control, gray bars represent the one-time application at transplant, white bars are the plants treated biweekly, and blue bars are the plants treated weekly with the application dose shown on the *x*-axis. The results are the means of 10 replicates ± the standard error ($n = 10$). Different letters represent statistically significant differences among the means ($P \leq 0.05$).

Discussion

Growers of ornamental plants are interested in identifying sustainable alternatives to reduce fertilizer applications and increase plant quality because of the increasing cost and environmental impact of chemical fertilizer use in greenhouse production. Although the use of biostimulants has increased in agriculture, specific evaluations are still needed to identify the best products for greenhouse ornamentals and to optimize rates and application frequency for production in soilless media. Humic substances promote plant growth via multiple modes of action, influencing both the primary and secondary metabolism of plants, including root exudation (Canellas and Olivares 2014). Growth promotion is the result of increased photosynthetic rate and chlorophyll content, increased production of auxins and

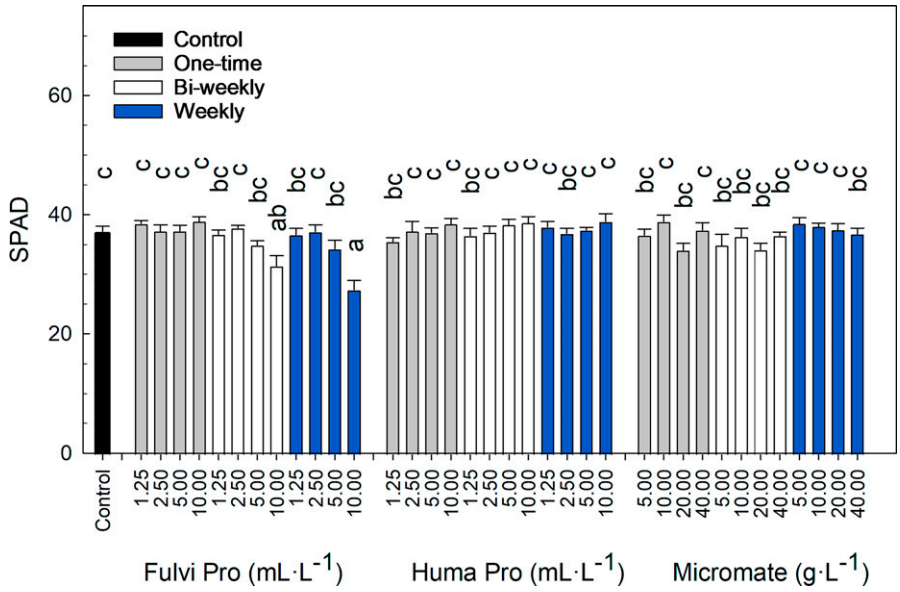

Fig. 4. Expt. 2. Soil plant analysis development (SPAD) (plant greenness) index for *Petunia ×hybrida* 'Picobella Blue' leaves at week 4 after transplant. Fulvi Pro, Huma Pro, and Micromate were applied once, biweekly, or weekly. The black bar represents the control, gray bars represent the one-time application at transplant, white bars are the plants treated biweekly, and blue bars are the plants treated weekly with the application dose shown on the *x*-axis. The results are the means of 10 replicates ± the standard error ($n = 10$). Different letters represent statistically significant differences among the means ($P \leq 0.05$).

cytokinins, and changes in root architecture and microorganism interactions that improve water and nutrient-use efficiency and uptake (Canellas and Olivares 2014). In our study, three biostimulants that contain humic substances were evaluated on petunias grown in peat-based media. We found that the biostimulant that contained both humic and fulvic acids (MM) had the best performance in comparison with the other humic and fulvic acid products.

Only MM, a product containing humic and fulvic acids, promoted petunia growth consistently. MM, the product containing both humic and fulvic acids, resulted in the rapid promotion of plant growth, with an increased GI observed at 2 weeks after transplant (Fig. 2). Growth promotion was observed at both low and optimal fertility, but plants treated with 40 g·L^{-1} MM had a greater increase in shoot DW at low fertility (53% increase) compared with the increase observed in plants at optimal fertility (30% increase). Although increases in shoot DW were observed in tomato plants treated with humic acid and fulvic acid individually, the greatest increases were observed when humic acid, fulvic acid, and Ca were applied in combination (Husein et al. 2015). Yield results from tomatoes showed an increase in tomato numbers with humic acid treatments at 25% of the optimal fertility (19% increase) and 50% of the optimal fertility (16% increase), but humic acid applications at optimal fertility had a detrimental effect on yield parameters (13% decrease in tomato numbers) (Monda et al. 2021). In our experiment with petunia, the extent of the growth promotion by MM started to decline at the greater rates (especially at the optimal fertility rate), but we did not observe any negative effects of the greater application rates (Fig. 1).

Growth promotion by humic substances is greatly influenced by rates and application methods (Canellas et al. 2015). The effects of multiple rates (5, 10, 15, and 20 kg·ha^{-1}) of humic acid and fulvic acid on various parameters of growth promotion were tested on yarrow (*Achillea millefolium*) grown in sandy loam soil in the greenhouse (Bayat et al. 2021). All treatments increased shoot fresh weight (FW) and DW significantly compared with untreated control plants. The greatest increases were seen with the greatest rates, which resulted in a 60% and a 50% increase in the shoot DW compared with control plants from the fulvic acid (20 kg·ha^{-1}) treatment and the humic acid (20 kg·ha^{-1}) treatment, respectively. Multiple experiments have reported that lower rates of fulvic or humic acids are not effective at promoting growth and that the greatest rates can have reduced or detrimental effects (Lulakis and Petsas 1995; Mylonas and McCants 1980; Suh et al. 2014). Although all three products we tested had humic substances originating from oxidized leonardite, the amount of a.i. differed. HP was a liquid formulation with 16% humic acid, and FP was a liquid formulation with 20% fulvic acid, whereas MM was a powdered product composed of 24% humic and fulvic acids. It is possible that the lack of benefits and reduced growth and quality observed with HP and FP could be because the rates that we used were either too high or too low for optimal responses. Growth promotion has been reported with media drenches of fulvic and humic acids (Bayat et al. 2021; Turan et al. 2021), but both have also been effective as foliar applications (Fan et al. 2014; Ibrahim et al. 2019). Future experiments will test these products using additional methods of application.

MM improved flowering in petunias. Ornamental plants such as petunias are grown for the beauty of their flowers; therefore, flower numbers and size are important characteristics determining ornamental crop quality. For the floriculture industry, the goal is to produce high-quality flowering plants as quickly as possible to reduce the costs associated with growing the plants in the greenhouse. In our study, MM at 40 g·L^{-1} increased flower number and DW with weekly drench applications at both low and optimal fertility (Table 1), and when drenched biweekly at the same rate (Table 4). When gerbera (*Gerbera jamesonii* L.) plants were irrigated with fertilizer solutions containing different concentrations of humic acid, weight loss and incidence of bent neck in the cut flowers was reduced, and vase life was increased (Nikbakht et al. 2008). At 1000 mg·L^{-1} humic acid, the vase life was extended an average of 3.7 d compared with flowers from untreated plants. Humic acid treatment at 500 mg·L^{-1} increased the number of cut flowers per plant by 52%, but this increase was not as great with the 1000-mg·L^{-1} treatment. Humic acid drenches also improved flower number, flower diameter, flower FW, and vase life in zinnias (*Zinnia elegans*) (Khan et al. 2021). At the greatest rate (10 kg·ha^{-1}) of humic acid, the days to flowering were also reduced by 11 d. Foliar applications of humic acid improved stem and flower diameter, shoot and root DW, and leaf area of chrysanthemums (Fan et al. 2014). These improvements were associated with increased net photosynthetic rate, greater chlorophyll content, and improved chloroplast structure.

Biweekly applications of MM were as effective as weekly applications at promoting growth and improving flowering characteristics. In Expt. 2, we investigated application timing to determine whether we could get better responses from HP and FP, and to determine whether we could obtain similar growth promotion with less-frequent MM applications. Weekly biostimulant applications are costly in terms of product costs as well as the labor needed to drench the product on containers in a large production greenhouse. A one-time drench application at transplant would be a more efficient way to provide a biostimulant treatment. Sometimes a one-time application at a greater rate is as effective as multiple lower doses over time, but this is not always the case with biostimulants, which are highly affected by interactions with the plants, microbes, and the environment (Canellas et al. 2015). Considering the increases in the shoot and root DW, and improvements to flowering characteristics observed in our study, we recommend that a biweekly application of 40 g·L^{-1} MM is the most effective for growth promotion while recognizing some cost savings compared with weekly applications.

Humic substances affected shoot tissue mineral nutrient concentrations. It is well documented that humic substances increase

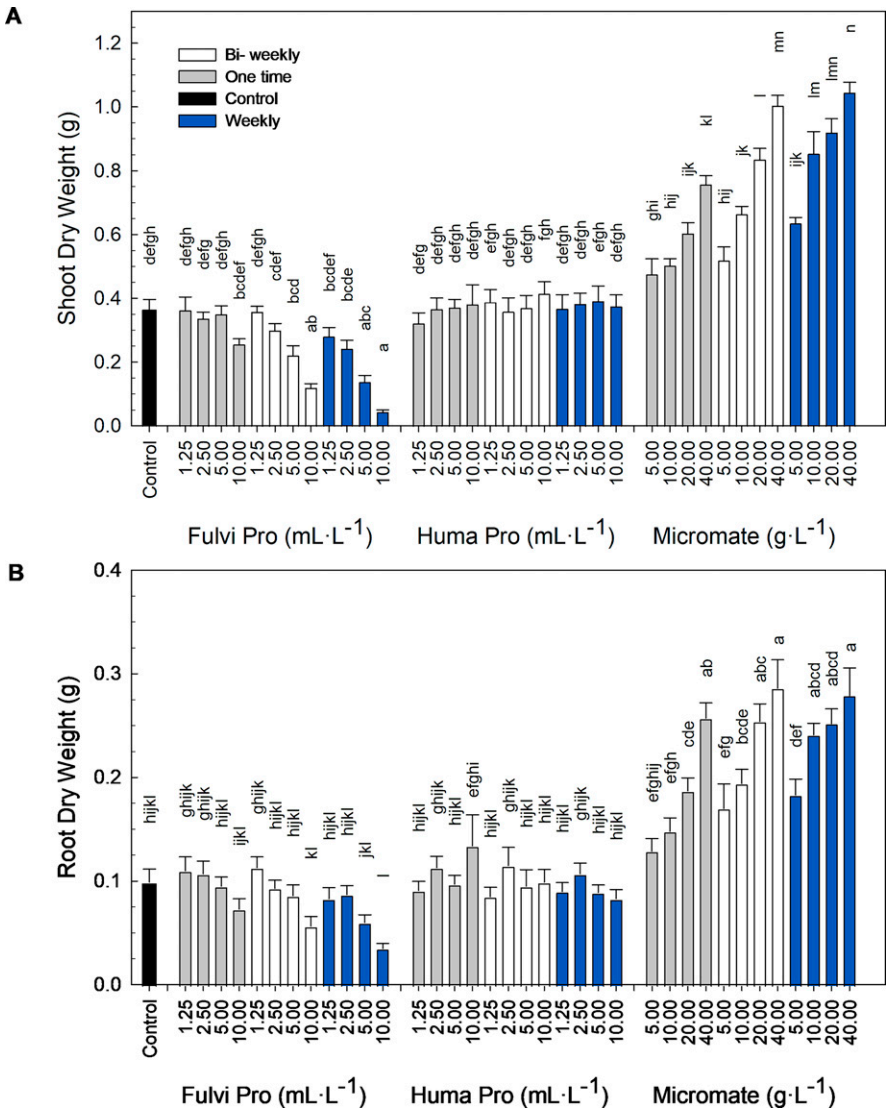

Fig. 5. Expt. 2. Shoot (**A**) and root (**B**) dry weight of *Petunia* ×*hybrida* 'Picobella Blue' at week 4 after transplant. Fulvi Pro, Huma Pro, and Micromate were applied once, biweekly, or weekly. The black bar represents the control, gray bars represent the one-time application at transplant, white bars are the plants treated biweekly, and blue bars are the plants treated weekly with the application dose shown on the *x*-axis. The results are the means of 10 replicates ± the standard error (n = 10). Different letters represent statistically significant differences among the means ($P \leq 0.05$).

nutrient availability and plant uptake. General increases in nutrient uptake are the result of hormonal-type biostimulation of lateral root induction and root hair initiation (Canellas et al. 2015). MM treatments at the greater rates (20 and 40 g·L^{-1}) resulted in significant increases in shoot P concentration (from 36% to 137% greater than controls), with greater increases at low vs. optimal fertility (Table 2). Humic and fulvic acids increase P solubilization, and plant availability and uptake by increasing the phosphatase activity of soil microorganisms, reducing sorption of phosphate ions to soil particles, increasing the cation exchange capacity of the soil, interfering with the precipitation of insoluble calcium phosphate, and inducing high-affinity phosphate transporters in plant roots (Ampong et al. 2022; Canellas et al. 2015; du Jardin 2015; Rose et al. 2014). Other reports have suggested that humic acids may also interfere with the formation of insoluble precipitates of iron phosphate, resulting in increased uptake of both Fe and P (David et al. 1994). Although MM resulted in large increases in P concentration in the shoots, the concentration of Fe and Ca were not increased. High concentrations of P can interfere with the uptake and translocation of Zn, and treatments with the greatest tissue concentrations of P (MM 20 and MM 40) had the lowest concentrations of Zn (Tables 2 and 3) (Sanchez-Rodriguez et al. 2021). Interestingly, petunia shoots from the FP and HP treatments had lower P concentrations compared with controls.

In tomato, the greatest rate of humic acid (1280 mg·L^{-1}) increased P, K, Ca, Mg, Fe, Mn, and Zn concentrations in tomato leaves, whereas the lower rate (640 mg·L^{-1}) only increased K concentration. Shoot N concentration was not increased by either humic acid treatment (David et al. 1994). In contrast, tissue N concentration was increased by both humic acid and fulvic acid applications in yarrow, and the effects on P and K were more inconsistent (Bayat et al. 2021). Enhanced nutrient uptake is promoted by humic substance–stimulated H^+ adenosine triphosphatase activity and the induction of ion transporters; increased nitrate uptake and assimilation can also result from the upregulation of glutamine synthetase and glutamine synthase activities (Canellas et al. 2002; Ertani et al. 2011). There were few significant effects of the humic substances on N concentration in petunia shoots in our experiment (Table 2). Under low fertility, the MM-treated plants had a lower N concentration than controls, and FP- and HP-treated plants had slightly greater N concentrations. Nitrogen concentration was more similar between treatments at the optimal fertility, suggesting that humic substances did not increase N uptake when conditions were not deficient. Although humic substances can also increase the availability of micronutrients by chelating and cotransporting metal cations to the plants (Yang et al. 2021), in our study there was no evidence of humic substance–enhanced uptake of micronutrients. Only Al was significantly greater with the MM 40 (optimal fertility) treatment (Table 3).

Another interesting observation in our study was that K concentrations in the FP 20 and HP 20 treatments were increased by 29% to 36% (in both the low and optimal fertility conditions), whereas the K concentration in leaves with the MM treatments was reduced. Shoots from the MM 20 treatments were 23% and 31% less, respectively, at optimal and low fertility. In gerbera, the leaves from the greatest humic acid treatment (1000 mg·L^{-1}) had increased accumulation of N, P, Mg, and Ca, whereas the K concentration was less than control leaves (Nikbakht et al. 2008). This pattern with greater P and lower K was similar to what we found with petunia leaves from MM treatments. The gerbera plants were also grown in peat-based media and treated with humic acid prepared from leonardite (containing 61.2% carbon, 3.13 g·kg^{-1} N dry matter, and 2.89 g·kg^{-1} P dry matter) (Nikbakht et al. 2008). Because of their slow rate of mineralization, humic substances do not provide a direct source of macronutrients to plants and do not serve as a replacement for N or P fertilization (Canellas et al. 2015). The differences in nutrient accumulation with the different experiments within the scientific literature can be influenced by the source of humic substances, which may form complexes and chelate metals differently based on different chemical structures. Nutrient analysis of the products in our experiment showed that HP and FP contain high levels of K, whereas MM does not (Tables 5–7). This likely contributes to the high concentrations of K in the tissues of FP- and HP-treated petunias (Table 2).

Table 4. Effect of application frequency on flower characteristics of *Petunia ×hybrida* 'Picobella Blue' (Expt. 2).

Treatment[i]	Application frequency	Time to first flower (d)[ii]	No. of flowers	No. of buds	Flower dry weight (g)	Bud dry weight (g)
Control	None	28.8 h–l[iii]	1.0 ab	0.7 ab	0.035 a–c	0.010 a
FP 1.25	Once	28.1 g–l	0.9 ab	0.6 ab	0.028 ab	0.009 a
FP 1.25	Biweekly	28.4 h–l	0.5 a	0.7 ab	0.013 a	0.010 a
FP 1.25	Weekly	28.8 h–l	1.1 ab	0.6 ab	0.026 ab	0.004 a
FP 2.5	Once	29.1 j–l	0.9 ab	0.6 ab	0.049 a–c	0.011 a
FP 2.5	Biweekly	31.0 l	0.8 ab	0.1 ab	0.017 a	0.004 a
FP 2.5	Weekly	29.6 j–l	0.9 ab	0.3 ab	0.026 ab	0.003 a
FP 5	Once	29.4 j–l	0.7 ab	0.4 ab	0.016 a	0.009 a
FP 5	Biweekly	28.7 f–l	0.4 a	0.5 ab	0.011 a	0.006 a
FP 5	Weekly	28.0 d–l	0.7 ab	1.2 ab	0.002 a	0.004 a
FP 10	Once	30.2 kl	0.5 a	0.5 ab	0.011 a	0.005 a
FP 10	Biweekly	32.0 g–l	0.1 a	0.2 ab	0.001 a	0.002 a
FP 10	Weekly	ND[iv]	0.0 a	0.0 a	0.000 a	0.000 a
HP 1.25	Once	28.8 i–l	1.0 ab	0.4 ab	0.023 ab	0.006 a
HP 1.25	Biweekly	27.3 f–k	0.9 ab	0.6 ab	0.026 ab	0.008 a
HP 1.25	Weekly	27.9 h–l	1.0 ab	0.6 ab	0.028 ab	0.006 a
HP 2.5	Once	28.1 h–l	1.1 ab	0.7 ab	0.026 ab	0.009 a
HP 2.5	Biweekly	28.0 h–l	1.3 a–c	0.7 ab	0.037 a–c	0.010 a
HP 2.5	Weekly	27.9 g–l	0.7 ab	0.8 ab	0.020 ab	0.016 a
HP 5	Once	28.8 i–l	1.1 ab	0.7 ab	0.025 ab	0.008 a
HP 5	Biweekly	28.9 i–l	0.8 ab	1.0 ab	0.019 ab	0.013 a
HP 5	Weekly	27.0 f–k	1.0 ab	0.7 ab	0.025 ab	0.007 a
HP 10	Once	28.1 g–k	0.7 ab	0.8 ab	0.017 a	0.011 a
HP 10	Biweekly	27.7 g–k	1.0 ab	0.3 ab	0.025 ab	0.004 a
HP 10	Weekly	27.0 e–k	0.9 ab	0.7 ab	0.022 ab	0.012 a
MM 5	Once	26.9 e–j	1.2 ab	1.2 ab	0.032 ab	0.015 a
MM 5	Biweekly	25.5 b–h	1.3 a–c	1.9 bc	0.037 a–c	0.028 ab
MM 5	Weekly	25.2 a–g	2.9 bc	4.1 de	0.080 bc	0.062 cd
MM 10	Once	25.8 c–i	1.4 a–c	1.4 ab	0.043 a–c	0.022 ab
MM 10	Biweekly	24.0 a–e	3.5 cd	4.7 d–f	0.094 cd	0.064 cd
MM 10	Weekly	24.3 a–f	6.2 e	5.5 ef	0.198 ef	0.101 ef
MM 20	Once	23.9 a–f	5.2 de	3.4 cd	0.142 de	0.047 bc
MM 20	Biweekly	22.6 ab	9.9 fg	5.6 ef	0.289 gh	0.068 cd
MM 20	Weekly	23.2 a–c	11.8 gh	6.5 f	0.349 hi	0.100 ef
MM 40	Once	23.7 a–d	9.4 f	5.1 d–f	0.259 fg	0.072 c–e
MM 40	Biweekly	21.8 a	13.4 hi	5.3 ef	0.380 ij	0.085 d–f
MM 40	Weekly	22.4 a	14.2 i	6.0 f	0.414 j	0.102 f

[i] The number in the treatment name stands for the application doses: Huma Pro (HP) and Fulvi Pro (FP) at 1.25, 2.5, 5, and 10 mL·L^{-1}; and Micromate (MM) at 5, 10, 20, and 40 g·L^{-1}.
[ii] Time to first flower is the number of days from transplant to the first open flower.
[iii] The results are the means of 10 replicates. Different letters represent statistically significant differences among the means within a column ($P \leq 0.05$).
[iv] ND = no data. the plant did not flower during the experiment.

Table 5. Mineral nutrient concentrations in the commercial biostimulant Huma Pro.

Nutrient	Concn (μg·mL^{-1})[i]	Amount per application (g)[ii]	Total applied (g)[iii]
Nitrogen	2.23	0.335	2.01
Phosphorus	0.644	0.096	0.579
Potassium	249.6	37.44	224.64
Calcium	43.94	6.59	39.54
Magnesium	18.17	2.72	16.35
Sulfur	48.94	7.34	44.04
Iron	2.42	0.363	2.18
Aluminum	3.34	0.502	3.01
Boron	0.168	0.025	0.151
Copper	0.092	0.013	0.082
Manganese	0.030	0.004	0.027
Molybdenum	0.003	0.0005	0.003
Sodium	105.46	15.81	94.91
Zinc	0.220	0.033	0.198

[i] 1 μg·mL^{-1} = 1 ppm. Mineral nutrient content (measured in micrograms) in 1 mL of product solution.
[ii] Amount of nutrient applied to the plant with a single application of 150 mL of solution at the rate of 20 μg·mL^{-1}.
[iii] Total amount of nutrient applied based on the six applications throughout the entire experiment.

Conclusion

Humic substances can improve plant growth and flowering, but results can be highly inconsistent and largely influenced by plant species, growing media, source of the humic substances and formulations of the biostimulants, rates and methods of application, and environmental conditions (Canellas et al. 2015). Although the meta-analysis conducted by Rose et al. (2014) indicated that growing media did not have as much influence on plant growth-promoting effects of humic substances as the source of the humic substances, the type of plant, the growing environment, and the manner of application, there are fewer published reports investigating plants growing in soilless media. Our results indicate that humic substances (the combination of humic acid and fulvic acid in the product MM, specifically) can promote petunia growth significantly and improve flowering, thereby producing high-quality plants with lower fertilizer rates. Considering the differences that we observed with the three humic substance–containing products in petunia, it will be important to test these products on other crops and determine how greenhouse environmental conditions and production practices influence this efficacy. The synergy of humic substances and plant growth-promoting bacteria in greenhouse production systems also should be explored as a way to improve the growth-promoting effects of humic and fulvic acid under nutrient-deficient conditions. Humic substances should be included in greenhouse growers' biostimulant toolbox, but more testing on specific herbaceous and woody crops is still needed.

Table 6. Mineral nutrient concentrations in the commercial biostimulant Fulvi Pro.

Nutrient	Concn (μg·mL^{-1})[i]	Amount per application (g)[ii]	Total applied (g)[iii]
Nitrogen	133.06	19.94	119.75
Phosphorus	0.093	0.013	0.083
Potassium	114.32	17.14	102.88
Calcium	52.50	7.87	47.25
Magnesium	23.76	3.56	21.38
Sulfur	40.6	6.09	36.54
Iron	0.039	0.005	0.035
Aluminum	0.035	0.005	0.031
Boron	0.143	0.021	0.128
Copper	0.129	0.019	0.115
Manganese	0.004	0.0006	0.003
Molybdenum	ND[iv]	ND	ND
Sodium	93.12	13.96	83.8
Zinc	0.340	0.05	0.305

[i] 1 μg·mL^{-1} = 1 ppm. Mineral nutrient content (measured in micrograms) in 1 mL of product solution.
[ii] Amount of nutrient applied to the plant with a single application of 150 mL of solution at the rate of 20 μg·mL^{-1}.
[iii] Total amount of nutrient applied based on the six applications throughout the entire experiment.
[iv] ND = not detected or under the detection limit.

Table 7. Mineral nutrient concentrations in the commercial biostimulant Micromate.

Nutrient	Concn (μg·mL^{-1})[i]	Amount per application (g)[ii]	Total applied (g)[iii]
Nitrogen	13.09	1.96	11.78
Phosphorus	2.92	0.438	2.62
Potassium	6.22	0.933	5.59
Calcium	24.53	3.67	22.07
Magnesium	11.19	1.67	10.07
Sulfur	48.31	7.24	43.47
Iron	0.015	0.002	0.013
Aluminum	0.173	0.025	0.155
Boron	0.102	0.015	0.092
Copper	0.002	0.0002	0.001
Manganese	0.073	0.01	0.065
Molybdenum	ND[iv]	ND	ND
Sodium	18.36	2.75	16.52
Zinc	0.018	0.002	0.015

[i] 1 μg·mL^{-1} = 1 ppm. Mineral nutrient content (measured in micrograms) in 1 mL of product solution.
[ii] Amount of nutrient applied to the plant with a single application of 150 mL of solution at the rate of 20 μg·mL^{-1}.
[iii] Total amount of nutrient applied based on the six applications throughout the entire experiment.
[iv] ND = not detected or under the detection limit.

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