

Effects of overwatering and fertilizer formulation on growth, nutrient content, and substrate
redox status of *Petunia* × *hybrida* during greenhouse production

by

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Abstract

Overwatered spring crops such as *Petunia x hybrida* are subject to a range of biotic and abiotic disorders including hypoxia, nutrient deficiencies, and increased susceptibility to root rot diseases. Overwatering often results in symptoms of interveinal chlorosis of youngest foliage leading to the assumption that it causes iron (Fe) deficiency. Five greenhouse experiments were conducted to evaluate effects of substrate water status and fertilizer formulation on four petunia cultivars produced in a glass-glazed greenhouse during spring seasons of 2024, 2025, and 2026.

Petunia ‘Cascadias Indian Summer’ (IS) and ‘Headliner Strawberry Sky’ (SS) plants were grown under three different watering regimes of overwatered (Over; rewatered when weight of sentinel pots dropped to 90 to 95+5% of container capacity, CC), optimally watered (Opt; 60+5% of CC), and underwatered (Under; 35 to 45+5% of CC) and two different fertilizer sources of Jack’s Professional General Purpose (Gen) and Jack’s Professional FeED Plus Mg (Pet). The following experiment had only Over and Opt as well as cultivars SS, ‘SlingShot Black Magic’ (BM), and ‘SlingShot Phantom’ (PH). The optimal watering regime yielded larger or similar plant growth based on width, fresh and dry weights. SPAD readings of youngest foliage were different based on fertilizer source with Pet resulting in greener young foliage. While IS, BM, and PH petunia did not develop yellowing of youngest foliage in any treatment, SS petunia developed distinctive symptoms of interveinal chlorosis in youngest foliage of overwatered plants fertilized with Gen fertilizer, suggesting a genetic dimension to the disorder. However, tissue analysis of SS petunia revealed no difference in Fe between watering regimes in the first study and greater Fe in Over in the second study, suggesting that Fe deficiency was not likely the cause of the disorder. Other tissue differences occurred across watering treatments for P, Ca, Mg, S, and Mn and between fertilizer formulations in P, Mg, and S. Notably, Mn was reduced in

young tissue of Over plants in both studies. Symptomology is effectively mitigated by using Pet fertilizer. Tissue Mn content was reduced by overwatering, but Fe was not deficient.

Waterlogged or hypoxic substrate conditions cause microbially mediated reduction of Mn and Fe that changes their plant bioavailability and leaching potential. Iron-inefficient crops such as petunia may be more vulnerable to waterlogged conditions as the substrate availability of these micronutrients change. In a third experiment with Petunia ‘Headliner Strawberry Sky’ (SS), plants were grown with two different watering regimes of Over and Opt and four different fertilizer sources: Jack’s Professional General Purpose (JPGP, Gen), JPGP with additional Mn EDTA (Gen+Mn), JPGP with additional Fe EDTA (Gen+Fe), and Jack’s Classic Petunia Feed (Pet), all at 150 ppm N. The following spring, two experiments were performed to assess the effect of plant maturity on overwatering stress using SS and the additional petunia cultivar ‘SureShot Dark Blue’ (DB). This fourth experiment used young plants that were newly transplanted when experimental watering treatments of Over and Opt started compared to the fifth experiment where plants were allowed to become well established before the same watering treatments of Over and Opt began. Water status was crossed with three different fertilizers: Gen, Gen+Mn, and Control (Con; no micronutrients). Both Fe and Mn Indicator of Reduction In Soils (IRIS) films were used to quantify the amount of Fe and Mn reduction in each experimental treatment via microbial activity. Plant weights, leaf greenness, substrate solution nutrient levels from pour through extraction, and tissue nutrient content of youngest leaves were measured. Tissue Fe levels were greater in Over compared to Opt in all three experiments, and tissue Mn was greater in Opt compared to Over in the third experiment, but the same in the fourth and fifth experiments. Across all three of these experiments, total Mn removal from IRIS films was greater in Over compared to Opt, ranging between 10.9% and 61.4%. Total Fe removal was

greater in Over treatments as well; however, the percentages of removal across treatments were much less than for Mn, between 0.4% and 1.6%. While SPAD differences occurred based on fertilizer formulation and water status, very little overwatered chlorotic foliage occurred, suggesting that more mature plants are less likely to develop symptomology in waterlogged conditions. Changes in leaf greenness in response to Over varied by plant maturity: Young plants in the fourth experiment had greener leaves as the experiment progressed and older plants in the fifth experiment had less green leaves.

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Chapter 1 - Petunia Cultivar Study

Influence of Overwatering on Foliage Chlorosis and Nutrient Deficiency of Four Petunia Cultivars

Keywords: greenhouse production, irrigation, iron deficiency, manganese deficiency, soilless substrate

Abstract.

Growth and nutrient content of *Petunia xhybrida* ‘Cascadias Indian Summer’ (IS) and ‘Headliner Strawberry Sky’ (SS; Expt. 1) plants were grown under three different watering regimes of overwatered (Over; rewatered when weight of sentinel pots dropped to 90 to 95+5% of container capacity, CC), optimally watered (Opt; 60+5% of CC), and underwatered (Under; 35 to 45+5% of CC) and two different fertilizer sources of Jack’s Professional General Purpose and Jack’s Professional FeED Plus Mg. Expt. 2 had only Over and Opt as well as culitvars SS, ‘SlingShot Black Magic’ (BM), and ‘SlingShot Phantom’ (PH). The optimal watering regime generally yielded the largest plants based on width, fresh and dry weights. SPAD readings (index of relative chlorophyll content) of youngest foliage were different based on fertilizer source. While IS, BM, and PH petunia did not develop yellowing of youngest foliage in any treatment, SS petunia developed distinctive symptoms of interveinal chlorosis in youngest foliage of overwatered plants fertilized with general purpose fertilizer. However, tissue analysis of SS petunia revealed no difference in Fe between watering regimes in Expt 1 and greater Fe in Over in Expt. 2. Differences did occur across watering regimes in tissue P, Ca, Mg, S, and Mn and between fertilizer formulations in P, Mg, and S. Overwatering induced visual symptomology of chlorotic young foliage in SS petunia, but not IS, BM, and PH petunia, which suggests a genetic

component to the disorder. Symptomology is effectively mitigated by using petunia feed. Tissue nutrient content is affected by overwatering, but Fe is not significantly different.

Introduction

Overwatering spring greenhouse crops often results in abiotic disorders including hypoxia (oxygen deficiency), nutrient deficiencies across substrate pH levels, and predisposition of crops to biotic disorders such as root rot diseases. Extension specialists (e.g. Getter 2014) and commercial growers (e.g. Crum 2011) have discussed the issue of overwatering and its associated problems. An early mention of the effects of overwatering in reference to five different floriculture crops indicated that “yellowing of the foliage around the margin of the leaf and between the veins while the veins remain a dark green is a symptom caused by either (a) an insufficient root system, (b) overwatering, or (c) an alkaline soil and iron deficiency” (Poesch 1937). Recently, Veazie and Whipker (2022) listed possible causes as insufficient Fe fertility, high substrate pH, root rot, or overwatering. Because the symptomology of the disorder matches that of iron and/or manganese deficiency (Gibson et al.), nutrition is frequently associated as a cause. However, there is a dearth of research related to the multi-negative abiotic interactions associated with overwatering in the greenhouse production and soilless substrate environment, though more research has been conducted in field production and soil-based systems (Habibi et al. 2023; Jiménez et al. 2024).

The effects of overwatering are pronounced in vegetatively propagated petunias and other iron-inefficient crops, which are spring crops that have inhibited absorption of micronutrients at greater substrate pH (>6.0; (Williams and Scoggins 2018). This predisposition to inhibited nutrient uptake could also be at play when this group of crops is overwatered, especially if chemical reactions or microbial activity in the substrate affect rhizosphere pH subsequently

affecting micronutrient uptake in these crops. Fertilizers formulated specifically for petunia and other iron-inefficient crops have been developed that provide greater rates of Fe, Mn, and Mg. Products such as Jack's Professional 20-3-19 Petunia FeED Plus Mg (Jack's Professional, JR Peters Inc., Allentown, PA, USA) and Masterblend 20-3-19 Petunia Formula (Masterblend International-Tyler Enterprises, Morris, IL) have been developed to deliver micronutrients, especially iron, at high enough rates to overcome high pH nutrient absorption problems. The amount of nutrients associated with interveinal chlorosis, notably Fe, Mn, and Mg, in Jack's Professional Petunia FeED exceeds that of Jack's Professional 20-10-20 General Purpose by 400, 200, and 838%, respectively. In addition, petunia special fertilizers typically contain chelated forms of micronutrients to maintain availability across a range of pH (Runkle and Fisher 2014).

Following is a brief review of different mechanisms associated with plant decline in waterlogged conditions. Low availability of Fe in the substrate is directly related to chemical and biological activities in the rhizosphere, which has a high iron requirement due to plant nutrient absorption and microorganism density. For example, iron deficiency in Florida vegetable crops (tomato, pepper, and bean) was associated with waterlogged/hypoxic conditions that can increase carbon dioxide production leading to bicarbonate formation and iron fixation, which contributes to iron deficiency (Ozores-Hampton 2013). Therefore, a key way to avoid iron deficiency is to avoid overwatered soils. Microbially mediated reduction of nitrate, Fe, and Mn can also occur under waterlogged conditions as microbes switch from aerobic to anaerobic metabolic pathways (McBride 1994).

Iron often exists in an oxidized state (Fe(III)) that is bioavailable to plants. Strategy I plant species, such as petunia, uptake iron through active acidification of the rhizosphere as well as

enzymic reduction of iron both aimed at reducing Fe from Fe(III) to more soluble Fe (II) to circumvent bioavailability issues (Ning et al. 2023).

Yafuso and Fisher (2017) studied oxygenation of irrigation water during container production of bedding plants, including the iron-inefficient calibrachoa; it did not result in improved plant growth. The authors concluded that to increase oxygen supply to roots in container production, growers should focus on having adequate substrate air porosity and avoid overwatering. In fact, commercial greenhouse producers will mitigate conditions of overwatering by altering physical properties of soilless media to be more porous (Andrew Melton, personal communication).

The objectives of this research were to 1) compare effects of over- and underwatering on petunia growth across a wide range of cultivars; 2) evaluate whether plant water status influences tissue nutrient content from a general purpose versus petunia special fertilizer; and 3) determine if chlorotic young leaves associated with overwatering petunias is related to nutrient deficiency.

Materials and Methods

Overview of experiments. Two experiments were conducted in a glass-glazed greenhouse of Kansas State University's Throckmorton Plant Sciences Center in Manhattan, KS, USA. The first experiment (Expt. 1) was conducted during Spring 2024 from 1 Mar to 5 Apr. *Petunia x hybrida* 'Headliner Strawberry Sky' (SS1) and 'Cascadias Indian Summer' (IS) were grown with three watering regimes of underwatered (Under), optimally watered (Opt), and overwatered (Over) crossed with two fertilizers, 20-10-20 Peat-Lite (Gen) and 20-3-19 Petunia FeED + Mg (Pet), both from Jack's Professional, JR Peters. There were two pots per experimental unit (e.u.) that were arranged in a completely random design (CRD). Two cultivars by three watering

regimes by two fertilizers with five replications and two pots per e.u. totaled 120 pots in this experiment.

The second experiment (Expt. 2) was conducted during Spring 2025 from 6 Mar to 23 Apr. Petunia ‘Headliner Strawberry Sky’ (SS2), ‘SlingShot Black Magic’ (BM), and ‘SlingShot Phantom’ (PH) were grown with two watering regimes of overwatered (Over) and optimally watered (Opt) crossed with the same two fertilizers used in Expt. 1 (Gen and Pet). Again, two pots per e.u. were arranged in a CRD. Three cultivars by two watering regimes by two fertilizers with five replications and two pots per e.u. totaled 120 pots in this experiment.

For both experiments, rooted liners were potted into BM6 All-Purpose Mix (Berger, Saint-Modeste, QC, CA) in 5 in (770 mL volume) round green azalea pots (HC Companies, Twinsburg, OH, USA). Pots were filled with substrate, brushed to level off, and then lightly tapped three times to settle substrate. Additional substrate was added and pots were leveled off. Bulk density was $0.07 \text{ g}\cdot\text{cm}^{-3}$, air-filled porosity was 27.0%, and total porosity was 83.2% based on five replications.

Cultivar treatments. Vegetative hybrid petunia cultivars used in this research were Headliner™ series ‘Strawberry Sky’, bred by Selecta North America (West Chicago, IL, USA); a heat tolerant, early flowering, medium-vigor, mounded, semi-trailing petunia with large red flowers and a white- speckled pattern and Cascadias™ series ‘Indian Summer’ bred by Danziger (HaShiv'a, Israel) (‘DCAS303’). In addition, two cultivars from the SlingShot™ novelty petunia collection developed by Ball FloraPlant were trialed: ‘Black Magic’, a mounded, trailing variety with nearly black, non-stripping flowers; and ‘Phantom’ (‘Balpephan’; PP22440) with black flowers with a yellow star pattern.

For Expt. 1, rooted liners of SS1 and IS were received from Welby Gardens (Arvada, CO, USA) during week 9 and potted on 1 Mar 2024. Plants were grown for 12 days to establish a healthy root system prior to implementing the water status and fertilizer treatments (Fig. 1a) on 13 Mar 2024 0 days after treatment (DAT). For Expt. 2, rooted liners of SS2, BM, and PH were received from Tagawa Greenhouse Enterprises (Brighton, CO, USA) during week 10 and potted on 6 Mar 2025. Plants were grown for 14 days prior to implementing treatments on 20 Mar 2025 (0 DAT). Prior to implementation of water and fertilizer treatments, all potted plants were grown with about 200 ppm N from the Gen fertilizer.

Water status treatments. Over, Opt, (Expts. 1 and 2) and Und (Expt. 1 only) water status treatments were established by weighing sentinel pots once to twice daily (Jonas and Williams 2000; Yafuso and Fisher 2017). Gravimetric moisture content was used to establish container capacity. Pots were fertilized based on treatment and then allowed to drain until no more water leached, approximately 30 min. Designated sentinel pots (one per treatment combination) were then weighed to establish weight at container capacity. In the following day(s), sentinel pots were reweighed and if the average of all sentinel pot weights of a given water status treatment fell below its defined threshold, all pots of that water status treatment would be fertigated. Under, Opt, and Over water status treatments were established by rewatering when sentinel pots averaged 45, 60%, or 95% $\pm$ 5% of weight at container capacity (CC), respectively. Once Over pots needed to be rewatered twice a day [26 Mar (13 DAT) in Expt. 1 and 3 Apr 25 (14 DAT) in Expt. 2], plastic saucers (Lallisa, Atlanta, GA) were set under pots in the Over treatment to maintain waterlogged conditions. This method resulted in pots being fertigated 12 or 18 times for Under and Opt, respectively, in Expt. 1 and 12 times for Opt in Expt. 2.

Fertilizer treatments. Two different fertilizer treatments were used in both experiments, Gen (20-10-20 Peat-Lite (Jack's Professional, JR Peters Inc., Allentown, PA, USA) and Pet (20-3-19 Jack's Professional Petunia FeED plus Mg), applied at 150 ppm N. Comparing Pet and Gen, respectively, at 150 mg N/liter solution was 1.5 versus 0.375 mg·liter⁻¹ Fe; Mn: 0.375 versus 0.188 mg·liter⁻¹ Mn; and 10 versus 1.2 mg·liter⁻¹ Mg. Nitrogen was provide as 60% nitrate and 40% ammonium.

Data collection and analyses. In Expt. 1, growth data of width, fresh and dry weights, and SPAD was collected on 5 Apr 2024. In Expt. 2, these measures as well as root system ratings were collected for SS on 18 Apr, for PH on 21 Apr, and for BM on 23 Apr 2025. Leaf greenness was determined by averaging three readings per e.u. taken on youngest leaves using SPAD-502 meter (Konica Minolta, Ramsey, NJ, USA). These youngest leaves were harvested for tissue analyses on by removing enough youngest leaf blade tissue from both pots in an e.u. 5 to 10 g was recovered for analysis. Fresh weight of whole above-ground plant shoots was measured and then placed in a 70°C forced-air oven for several days, then dry weights were measured. Dried tissue was ground into powder for tissue analyses using a mortar and pestle that was wiped clean between samples to avoid contamination. In Expt. 2 only, root system health was evaluated by rating overall root system and indicated surface roots (Fig. 3 & 4, respectively).

Solution nutrient sampling and analyses. PourThru solution sampling was conducted on 4 Apr in Expt. 1 and 18, 21, and 23 Apr for SS, PH, and BM, respectively, in Expt. 2 (Cavins and Williams 2018). Plants were fertigated as normal, allowed to drain for 30 min, then ~30 mL distilled water was added to pots and allowed to drain into saucer under each pot. Samples were then stored in refrigerator until analyses occurred. Solution sample pH and EC were measured from both Expts. 1 and 2 using a pH meter (Bluelab Corp., Tauranga, NZ) and EC meter

(Accumet Excel XL20, Fisher Scientific, Waltham, MA, USA). Probes were rinsed and dried between samples to avoid contamination. Plant water samples were assayed by Kansas State University's Soil Testing Lab (Manhattan, KS, USA). Leachate was tested for Zn, NO_3^- -N, NH_4^+ -N, P, Ca, Mg, K, Cu, Fe, Mn, and S. Exp. 2 additionally tested for Na. Elements in leachate samples (Zn, P, Ca, Mg, Na, K, Cu, Fe, Mn, and S) were determined by ICP-OES (Model 5800, Agilent Technologies, CA, USA). NH_4^+ -N determined via indophenol colorimetric reaction and NO_3^- -N determined via cadmium reduction and colorimetric reaction (Alpkem Corporation 1986a; Alpkem Corporation 1986b; Clackamas, OR, USA).

Tissue nutrient analyses. Plant tissue samples were assayed by Kansas State University's Soil Testing Lab (Manhattan, KS). Plant tissue was tested for total nitrogen (N), total carbon (C), P, K, Ca, Mg, SO_4^{2-} -S, Cu, Fe, Mn, and Zn. Exp. 1 additionally tested for nitrate (NO_3^-). In plant tissue, total N and C were analyzed via dry combustion (LECO TruSpec CN combustion analyzer, LECO Corp., St. Joseph, MI, USA). Nitrate was determined via KCl extraction, cadmium reduction, and analyzed by colorimetric reaction (Alpkem Corporation 1986a; Clackamas, OR, USA; Gelderman and Beegle 1998). Phosphorus and K were determined from a sulfuric peroxide digest (Lindner and Harley 1942; Thomas et al. 1967). Ca, Mg, SO_4 -S, Cu, Fe, Mn, and Zn were determined from a nitric-perchloric digest (Gieseking et al. 1935). All tissue nutrients except total N and C and NO_3 were analyzed with ICP-OES (Model 5800, Agilent Technologies, CA, USA).

Results and Discussion

Growth data. Cultivar. In Expt. 1, both SS and IS cultivars had similar width, fresh and dry weights (Table 1). In Expt. 2, SS was largest, followed by BM, and PH was smallest based on width and dry weight. SS and BM had similar fresh weight, but greater than PH (Table 1).

Growth differences across the four petunia cultivars were expected, as they represent a wide range of habit and vigor. These differences allowed us to evaluate the effect of overwatering across a wide genetic pool.

Water status. In Expt. 1, water status main effect indicated growth differences based on treatment: Over and Opt resulted in similar growth that was greater than for Under (Table 1, Fig. 1). These results are similar to those of Rebi et al. (2024) who found that greatest plant height, numbers of leaves and flowers, and shoot fresh and dry weights of grandiflora petunia occurred when plants were watered daily; growth was less when plants were watered at 3-day intervals, and reduced still when they were watered at 5-day intervals. In Expt. 2, Opt resulted in larger plants than Over based on width, fresh and dry weights. These results suggest that overwatering affects growth more significantly for less vigorous cultivars (PH, and to a lesser extent, BM) compared to the faster-growing and larger SS, especially if the root system may be damaged.

Fertilizer. In both experiments, both Gen and Pet fertilizers resulted in similar growth (Table 1). This result suggests that the primary purpose of Pet is cosmetic in abating or correcting the occurrence of young foliage chlorosis from overwatering (Fig. 2). For example, when plants have been overwatered and young leaf chlorosis occurs, a recommendation to remediate the problem, after reducing irrigation frequency, is application of an iron chelate (Veazie and Whipker 2022). Indeed, Jack's Classic Petunia FeED 20-6-22 is marketed as "...especially effective in hanging baskets and containers that are watered often," aligning additional quantity and chelated Fe sources with use when crops are overwatered (Petunia FeED 20-6-22).

In Expt. 1, no interactions were significant for width, fresh or dry weights; therefore, only main effect results are shown (Table 1). In Expt. 2, only the Cultivar x Water status interaction

was significant for width, fresh and dry weights ($P = <0.0001$ for all); therefore, no other interaction results are shown for these growth parameters. The Cultivar x Water status significance is explained by the performance of SS2 Over treatment being comparable to that of SS2 Opt and much better than the performance of the less vigorous BM and PH Over treatments (Table 1).

Root system evaluation. Root system evaluations occurred only in Expt. 2. SS2 developed roots on the surface of the substrate, whereas the less vigorous cultivars BM and PH did not (Table 1). Greenhouse-grown cowpeas (*Vigna unguiculata* L.) with increased formation and length of adventitious roots have been shown to possess enhanced tolerance to waterlogged conditions (Basavaraj et al. 2024); this is demonstrated with SS2 in Cultivar x Water status interaction because SS had more prolific surface/adventitious roots and subsequently heavier and wider plants compared to BM and PH. Similarly, SS2 had a greater root quality rating than either BM or PH (Table 1). Surface roots only occurred when plants were Over, not Opt-watered (Table 1). Surprisingly, Over did not result in poorer root system rating compared to Opt, which is explained by the only significant interaction: Cultivar x Water status. Root quality of the SS2 Over treatment was better than Opt treatments of both other cultivars. The SS cultivar had a more vigorous root system that developed more surface roots than other cultivars (Table 1); this suggests that SS had a means to overcome hypoxia in the root zone and contributed to its vigor (Steffens and Rasmussen 2016). No difference occurred in either root system parameter based on fertilizer (Gen or Pet) main effect. Similar to our results, when petunia root systems were evaluated over a range of irrigation frequencies, the heaviest root systems by fresh and dry weight were found in petunias that were irrigated every day compared to those irrigated at three-, five-, or seven-day intervals (Rebi et al. 2024).

Relative leaf greenness. In Expt. 1, leaf greenness (SPAD) was greater in IS than SS. Water status treatment main effect did not result in difference. However, leaves were greener in plants produced with Pet compared to Gen fertilizer (Table 1). All two-way interactions (but not three-way interaction) were significant for leaf greenness. The Cultivar x Water status interaction ($P = 0.015$) indicated that IS was always greener than SS except for the SS x Under treatment which was similar to IS SPAD levels (data not shown). This result aligns with the greener foliage of IS and suggests that less growth of SS when underwatered concentrated chlorophyll in smaller cells that matched IS foliar greenness. The Cultivar x fertilizer interaction ($P = 0.006$) showed that only SS x Gen treatments were less green than all other combinations (data not shown). Water status x fertilizer interaction ($P = 0.012$) indicates a difference between Over Pet treatments compared to Over Gen treatments (Figure 6); Over Pet is greater. In total, these SPAD results support the effectiveness of Pet fertilizer in overcoming symptoms of chlorosis associated with overwatering in the SS cultivar.

In Expt. 2, SS had greenest foliage that was similar to results in Expt. 1, followed by PH, then BM (Table 1). Interestingly, Over plants were greener than Opt and fertilizer main effect between Pet and Gen was not different in this study (Table 1). The only significant interaction was Cultivar x Fertilizer, which revealed that SS2 Pet treatment was greenest (50.2 SPAD units compared to 38.5 SPAD units for Gen), but Pet and Gen fertilizers resulted in similar foliage greenness within a cultivar for PH (37.9 and 42.6 SPAD units, respectively) and BM (30.1 and 34.7 SPAD units, respectively). When growth was not limited by irrigation frequency, Pet resulted in greener foliage.

Solution pH and EC data. The EC of substrate solution provides an overview of fertility level in soilless substrates. In Expt. 1, EC ranged from 1.9 to 2.3 dS m⁻¹ across treatments; in

Expt. 2, this range increased from 2.2 to 2.6 dS·m⁻¹ (Table 1). These ranges fall within an acceptable range for petunias of 2.0 to 3.5 dS·m⁻¹ from PourThru extraction (Hamrick 2003). While Fertilizer x Cultivar interaction was significant in Expt. 2 (P=0.0365), the Fertilizer main effect was dominant with Gen resulting in EC from 3.1 (Gen x BM) to 2.4 (Gen x PH) and Pet resulting in EC of 2.0 (Pet x PH) to 1.9 (Pet x SS2)

The pH of substrate solution provides an indication of solubility, and thus availability for plant uptake, of nutrients including Fe, Mn, and Mg. In both experiments, SS exhibited greater pH than other cultivars, 6.5 and 6.1 in Expts. 1 and 2, respectively. In Expt. 1, the Pet fertilizer resulted in greater substrate pH (6.6) than Gen, which does not support Pet contributing to lower pH to improve Fe and Mn solubility. In Expt. 2, pH main effect was not different between Pet and Gen fertilizers (5.8 for both; Table 1), which would maintain Fe and Mn solubility across forms. Pet does not reduce pH; in Expt. 1, pH is 0.4 units greater in Pet than Gen; in Expt. 2, no difference occurs based on fertilizer treatment. This indicates that Pet decreasing pH is not a mechanism of this fertilizer overcoming chlorosis. Pet fertilizer is not designed to reduce pH, as indicated by its potential acidity (420 pounds calcium carbonate equivalent per ton versus 407 CCE/ton for Gen). The two fertilizers have similar potential acidity and would behave similarly over the course of the production cycle.

Water status main effect showed that Over irrigation resulted in greater pH than Opt by 0.6 and 0.5 pH units in Expts. 1 and 2, respectively, indicating that Over results in decrease in protons in the substrate over time. Hypoxic conditions raise substrate pH primarily by altering its chemistry from an oxidative to a reductive state. After microbes have reduced all the O₂ during their cellular respiration, some will start reducing nitrate and in the process increase substrate pH (5.9B: Nitrate Reduction and Denitrification 2017). While Water status x Fertilizer interaction

was significant ($P=0.0285$), the main effect was dominant with Over resulting in pH from 6.0 (Over x Pet) to 6.2 (Over x Gen) and Opt resulting in pH of 5.7 (Opt x Pet) to 5.5 (Opt x Gen).

Tissue data. The visual symptomology associated with overwatering was different between cultivars. SS showed interveinal chlorosis of the youngest foliage or slightly chlorotic whole youngest leaf blades in Expt. 1, but very few symptoms in Expt. 2. Foliar symptoms associated with overwatering for BM and PH were speckled gray lower leaves which is potentially a symptom of Mn deficiency (Fig. 5; Alejandro et al. 2020). Because no symptoms of overwatering occurred with IS, tissue analyses was completed on SS leaves only in Expt. 1 (Table 2) While tissue was analyzed for all three cultivars in Expt. 2 (Appendix A), only results for SS2 are shown (Table 3) to allow focus on the symptoms of young foliage chlorosis when some petunia cultivars are overwatered.

All SS tissue levels fell within acceptable ranges for healthy petunia (Mills and Jones, 1996) except Ca, which was below the range in both experiments (Table 1 & 2). In Expt. 1, the Water status main effect indicated that Over plants had less P, K, Ca, SO₄-S, and Mn compared to Opt and Under. Mg, however, was lowest in Under plants. Again in Expt. 2, the Water status main effect indicated that Over plants had less P, Ca, and Mn compared to Opt, though SO₄-S and Fe were less in Opt. Notably, tissue Mn was lowest in the Over treatment in both experiments, but not Fe or Mg, which are frequently associated with interveinal chlorosis. As an immobile nutrient, interveinal chlorosis associated with Fe deficiency is typically manifested in the youngest leaves; as a mobile nutrient, interveinal chlorosis associated with Mg deficiency is typically manifested in the oldest leaves. Our results suggest that whether or not chlorosis of youngest foliage occurs during overwatering, Mn—not Fe or Mg—is more likely to be reduced in the tissue. One possible hypothesis for why lower, but not deficient, tissue Mn levels might be

leading to chlorosis of the youngest foliage in overwatered conditions is that overwatered conditions increase reactive oxygen species (ROS) in plant tissues which can subsequently lead to chlorosis (Habibi et al. 2023). Manganese is a cofactor for manganese superoxide dismutase which breaks down superoxide ions (type of ROS), so decreased levels of Mn might be limiting the amount ROS detoxification causing chlorosis to persist in overwatered conditions (Alejandro et al. 2020).

In Expt. 1, Fertilizer main effect showed little difference between tissue accumulation except that Gen was greater for P and $\text{SO}_4\text{-S}$ and less for Mg. In Expt. 2, Fertilizer main effect showed that Gen was greater or not different for all nutrients except $\text{SO}_4\text{-S}$, Mn, and Zn. Comparing Pet and Gen, respectively, at 150 ppm N solution Mg (ppm): 10 vs. 1.2. Tissue in Pet was greater for Mg.

Smith et al. (2004) found that chlorophyll content decreased when the ratio of tissue Fe to Mn was <0.71 for petunia, or Fe was $<112 \text{ ug.g}^{-1}$, or Mn was $>170 \text{ ug.g}^{-1}$. In Expt. 1 (SS only), when we saw visual symptomology of chlorotic foliage, Fe tissue levels in both Over and Opt treatments was less than 112 ug.g^{-1} ; in Expt. 2 (SS only), when we did not see chlorotic foliage, Fe tissue levels were greater than this threshold. We did not measure Mn levels $>170 \text{ ug.g}^{-1}$ as well as Fe:Mn <0.71 for water status treatments in both studies. SPAD in levels in Smith et al. (2004) for those optimal levels of Fe, Mn, and Fe:Mn were between approximately 40 and 50. The SPAD averages in Expt. 1 and 2 (SS) for fertilizer x water status range from 38.3 to 51.9. Pet supplied Fe and Mn at 0.74% Mn (Mn-EDTA) C111 was added to the macronutrient solution at 0.033, 0.067, or $0.134 \text{ g}\cdot\text{L}^{-1}$. Using Fe as an example, C111 supplied 0.5, 1.0 or $2.0 \text{ mg}\cdot\text{L}^{-1}$ Fe, respectively. Relative leaf greenness readings for both studies were similar being within the same range as each while ours observed chlorotic overwatered symptomology could depend on a

multitude of factors most prominently cultivar differences between the two studies could be playing a major role in the differences in experimental observations. Overall, comparing Smith et. al (2004)'s results to ours, it does not seem that Fe and Mn antagonism is occurring in our study based on SPAD readings and tissue nutrient analyses.

Solution nutrient data. Beyond pH and EC, complete analyses of the substrate solution from PourThru extraction was completed only in Expt. 2 at the end of the experiment (Table 4). Compared to Opt, Over plants had less or similar of every nutrient except $\text{NH}_4\text{-N}$. This may be directly related to the amount of leaching that occurred; with each irrigation, while more nutrients were delivered to the pot, they would be leached, limiting the increase of nutrient concentrations over time. Turning focus to the nutrients that substrate microbes would use as electron acceptors as oxygen is depleted in waterlogged conditions, NO_3 , Mn and Fe would be affected (Rabenhorst 2018). During this altered metabolism these chemical species are reduced making them unavailable (NO_3) or more soluble and more likely for them to be leached during irrigation events (Mn and Fe) (Zhang et al. 2025). We observe this trend, though Mn is not significantly greater in Over compared to Opt treatments.

Fertilizer main effect showed that Gen fertilizer had more $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, P, K, Mn, Zn, and Cu in solution than Opt, but less S, and Fe. Because the levels of Fe, Mn, and Mg are so much greater in Pet compared to Gen fertilizer, it is interesting that only Fe is only slightly greater in the extracted substrate solution.

Conclusions. The ability of a cultivar to generate surface roots, as in SS, allowed it to overcome overwatering stress and use additional available water to increase growth. Pet fertilizer is effective in overcoming chlorosis induced from overwatering-associated hypoxia, though it does not result in increased Fe or Mn in youngest leaf tissue—the location of visual

symptomology--to do so. Our results suggest effects of overwatering on substrate solution analyses that may be associated with changes in substrate microbial that occur under hypoxic conditions. More research is needed to quantify microbial activity in soilless substrates under a range of water status to determine if they may affect nutrient availability to iron inefficient crops, including petunia.

Table 1.1 Growth data and solution pH and EC data for Expt. 1 and Expt. 2, and root data for Expt. 2.

Table 1. Growth data and solution pH and EC data for Expt. 1 and Expt. 2, and root ratings data for Expt. 2. for petunia cultivars ['Indian Summer' (IS) and 'Strawberry Sky' (SS1) in Expt. 1 and 'Strawberry Sky' (SS2), 'Black Magic' (BM), and 'Phantom' (PH) in Expt. 2] by two fertilizers [General (Gen) and Petunia (Pet)] by three watering treatments [Overwatering (Over), Optimally watered (Opt), and Underwatered (Under)].

Experiment 1				Surface	Root	SPAD	pH	EC
				Roots	Quality			
<i>Cultivar (n=30)</i>	Width	Fresh Wt	Dry Wt	Rating	Rating			
	(cm)	(g)	(g)					
IS	48.8a	66.8	6.7	na	na	48.8a	6.2b	2.2a
SS1	43.8b	67.8	6.6	na	na	43.8b	6.5a	2.0b
Prob > F	<0.0001	ns	ns			<0.0001	<0.0001	0.0024
<i>Fertilizer (n=30)</i>								
Gen	34.0	69.4	6.8	na	na	44.4b	6.2b	1.9b
Pet	33.7	65.2	6.5	na	na	48.4a	6.6a	2.2a
Prob > F	ns	ns	ns			0.0009	<0.0001	<0.0001
<i>Water (n=20)</i>								
Over	36.5a	76.5a	7.8a	na	na	45.3	6.7a	2.3a
Opt	34.1ab	78.5a	6.9a	na	na	45.8	6.1b	1.9b
Under	30.4b	47.1b	5.2b	na	na	48.1	6.3b	2b
Prob > F	0.001	<0.0001	<0.0001			ns	<0.0001	<0.0001
Experiment 2								
<i>Cultivar (n=20)</i>								
SS2	48.6a	106.8a	9.9a	1.03a	3.2a	44.3a	6.1a	2.2b
BM	30.8b	99.4a	7.6b	0.02b	2.1b	32.8c	5.7b	2.6a
PH	24.1c	88.5b	6.6c	0b	1.7b	40.2b	5.7b	2.2b
Prob > F	<0.0001	<0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0153
<i>Fertilizer (n=30)</i>								
Gen	34.8	98.5	8.1	0.37	2.4	38.6	5.84	2.6a
Pet	34.2	95.2	8	0.33	2.3	39.6	5.85	2.0b
Prob > F	ns	ns	ns	ns	ns	ns	ns	<0.0001
<i>Water (n=30)</i>								
Over	32.7b	7.6b	7.6b	0.68a	2.3	41.1a	6.1a	2.1b
Opt	36.3a	8.5a	8.5a	0.05b	2.3	37.1b	5.6b	2.5a
Prob > F	0.0009	<0.0001	0.0009	<0.0001	ns	<0.0001	<0.0001	0.0003

Table 1.2 Tissue data analysis from Expt. 1, SS Only

Table 2. Tissue data analysis from Expt. 1, SS Only: two fertilizers [General (Gen) and Petunia (Pet)] by three watering treatments [Overwatering (Over), Optimally watered (Opt), and Underwatered (Under)].

Expt. 1	Total	P (%)	K (%)	Ca (%)	Mg	SO4-S	Fe	Mn	Zn	Cu
(SS1	N (%)				(%)	(%)	(ppm)	(ppm)	(ppm)	(ppm)
Only)										
<i>Water (n=10)</i>										
Over		0.47b	3.28b	0.4b	0.65a	0.44a	94.1	57.2b	57.1	20.8
Opt		0.63a	4.19a	0.93a	0.64a	0.37b	105.1	81.6a	42.1	11.2
Under		0.57a	4.00a	1.01a	0.34b	0.38b	87.7	94.3a	41.6	7.3
Prob > F	na	<.0001	0.0002	<0.0001	<.0001	0.0002	ns	<0.0001	ns	ns
<i>Fertilizer(n=15)</i>										
Gen		0.61a	3.87	0.75	0.51b	0.42a	98	80.5	50.4	18.5
Pet		0.50b	3.77	0.8	0.57a	0.38b	100.5	74.9	43.5	7.7
Prob > F	na	<.0001	ns	ns	0.0216	0.0033	ns	ns	ns	ns
<i>Irrigation*Fertilizer(n=5)</i>										
Over x Gen					0.29c	0.40b				
Over x Pet					0.38c	0.49a				
Opt x Gen					0.58b	0.37b				
Opt x Pet					0.71a	0.37b				
Under x Gen					0.68ab	0.37b				
Under x Pet					0.62ab	0.40b				
Prob > F	na	ns	ns	ns	0.0062	0.032	ns	ns	ns	ns
Range*		0.47-	3.13-	1.2-2.81	0.36-	0.33-	84-168	44-177	33-85	3-19
		0.93	6.65		1.37	0.8				

Table 1.3 Tissue data Analysis from Expt. 2, SS Only

Table 3. Tissue data analysis from Expt. 2, SS Only: two fertilizers [General (Gen) and Petunia (Pet)] by two watering treatments [Overwatering (Over) and Optimally watered (Opt)].

Expt. 2 (SS2 Only)	Total N (%)	P (%)	K (%)	Ca (%)	Mg (%)	SO4-S (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
<i>Water (n=15)</i>										
Over	5.95	0.42b	3.77	0.49b	0.74a	0.44a	130a	63b	49	8.9
Opt	6.11	0.50a	3.72	1.11a	0.34b	0.38b	119b	109a	43	9.1
Prob > F	ns	0.0093	ns	<0.0001	<.0001	0.0002	0.025	<0.0001	ns	ns
<i>Fertilizer (n=15)</i>										
Gen	6.36a	0.58a	3.96a	0.77	0.45b	0.43a	122	101a	51a	9.6
Pet	5.71b	0.34b	3.52b	0.83	0.63a	0.40b	127	71b	41b	8.4
Prob > F	0.0002	<.0001	0.0195	ns	<.0001	0.0223	ns	<0.0001	0.004	ns
<i>Water x Fert (n=5)</i>										
Over x Gen		0.49b			0.33c		122ab	70bc		
Over x Pet		0.34c			0.36c		138a	56c		
Opt x Gen		0.66a			0.58b		123ab	133a		
Opt x Pet		0.34c			0.90a		116b	85b		
Prob > F	ns	0.0093	ns	ns	<.0001	ns	0.0172	0.002	ns	ns

Table 1.4 Solution data analysis from Expt. 2

Table 4 Solution data analysis from Expt. 2 for petunia cultivars ‘Strawberry Sky’ (SS2), ‘Black Magic’ (BM), and ‘Phantom’ (PH) by two fertilizers [General (Gen) and Petunia (Pet)] by two watering treatments [Overwatering (Over) and Optimally watered (Opt)].

Treatments	pH	EC	Zn (ppm)	NH ₄ -N (ppm)	NO ₃ -N (ppm)	P (ppm)	Ca (ppm)	Mg (ppm)	K (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)	S (ppm)
<i>Variety</i>													
SS2	6.1a	2.21b	1.24a	59.45a	145.38b	43.35	93.23	66.47b	134.15	0.09a	1.74b	0.31b	97.87a
BM	5.7b	2.56a	1.26a	58.13a	189.40a	49.89	123.29	87.44a	134.10	0.09a	2.69a	0.47a	88.73ab
Ph	5.7b	2.20b	1.05b	49.29b	149.06b	38.70	102.02	68.66ab	120.79	0.07b	2.40a	0.37ab	77.44b
Prob > F	<.0001	0.0153	0.0038	0.0054	0.0004	ns	ns	0.0283	ns	<.0001	<.0001	0.0039	0.0102
<i>Water</i>													
Over	6.1a	2.11b	1.15	70.29a	149.32b	41.52	56.42b	43.05b	174.51a	0.08	1.39b	0.35	61.23b
Opt	5.6b	2.54a	1.22	40.95b	173.24a	46.43	155.94a	105.33a	84.86b	0.08	3.16a	0.42	114.81a
Prob > F	<.0001	0.0003	ns	<.0001	0.0131	ns	<.0001	<.0001	<.0001	ns	<.0001	ns	<.0001
<i>Fertilizer</i>													
Gen	5.8	2.65a	1.30a	73.49a	203.27a	74.66a	114.29	78.05	158.25a	0.09a	2.11b	0.48a	69.84b
Pet	5.9	1.99b	1.07b	37.76b	119.29b	13.29b	98.07	70.32	101.12b	0.07b	2.45a	0.28b	106.19a
Prob > F	ns	<.0001	<.0001	<.0001	<.0001	<.0001	ns	ns	<.0001	<.0001	0.0365	<.0001	<.0001
<i>Water*Fertilizer</i>													
Opt x Gen	5.5b	ns	1.41a	53.11b	226.63a	82.72a	176.13a	118.11a	100.36c	0.10a	3.16a	0.63a	89.69b
Opt x Pet	5.7b	ns	1.03b	28.80c	119.86c	10.15c	135.75b	92.56a	69.35d	0.07c	3.16a	0.21c	139.92a
Over x Pet	6.0a	ns	1.12b	46.73b	118.72c	16.44c	60.38c	48.09b	132.88b	0.08b	1.73b	0.35b	72.45b
Over x Gen	6.2a	ns	1.18b	93.86a	179.91b	66.61b	52.45c	38.00b	216.13a	0.08b	1.06c	0.34bc	50.00c
Prob > F	0.0285	ns	0.0029	<.0001	0.0178	0.0106	0.0243	0.0116	0.0013	<.0001	0.0355	<.0001	0.0111
<i>Variety*Fertilizer</i>													
Prob > F	ns	ns	ns	0.0479	ns	ns	ns	ns	ns	0.0159	0.0003	<.0001	ns
<i>Variety*Fertilizer</i>													
Prob > F	ns	0.0365	ns	0.0026	0.0199	0.021	ns	ns	ns	0.0040	0.0007	ns	ns
<i>Variety*Irrigation*Fertilizer</i>													

Prob > F

ns

ns

ns

ns

ns

ns

0.0391

ns

ns

ns

0.0128

0.0002

ns

'Strawberry Sky'

'Indian Summer'

Over

General

Petunia

General

Petunia

Optimal

General

Petunia

General

Petunia

Under

General

Petunia

General

Petunia

Figure 1.1 Overview of experimental treatments in Expt. 1

Figure 1. Overview of experimental treatments in Expt. 1 for petunia cultivars [‘Indian Summer’ and ‘Strawberry Sky’] by two fertilizers [General and Petunia] by three watering treatments [Overwatering (Over), Optimally watered (Opt), and Underwatered (Under)].



Figure 1.2 Comparison of foliar chlorosis symptomology associated with overwatering by fertilizer

Figure 2. Comparison of foliar chlorosis symptomology associated with overwatering by fertilizer [General and Petunia]



Figure 1.3 Overall root ratings (six-point Likert scale) of petunia in Expt. 2.

Figure 3. Overall root ratings (six-point Likert scale) of petunia in Expt. 2: 0: little to no fine roots, 1: small number of fine roots, 2: moderate number of fine roots, 3: robust root system of fine roots, 4: few large roots, 5: robust root system of large roots

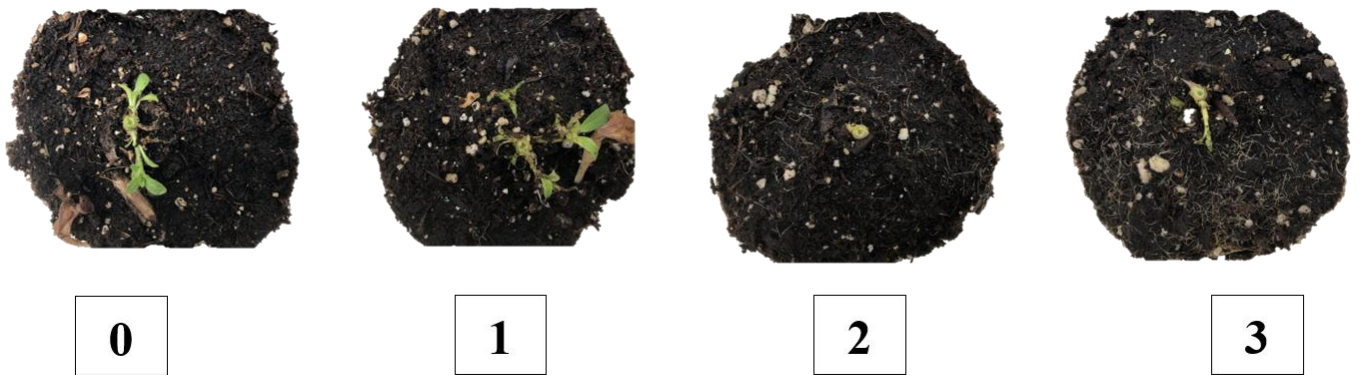


Figure 1.4 Surface root ratings (four-point Likert scale) of petunia in Expt.

Figure 4. Surface root ratings (four-point Likert scale) of petunia in Expt. 2: 0: no surface roots, 1: minimal surface roots, 2: moderate surface roots, 3: extensive surface roots

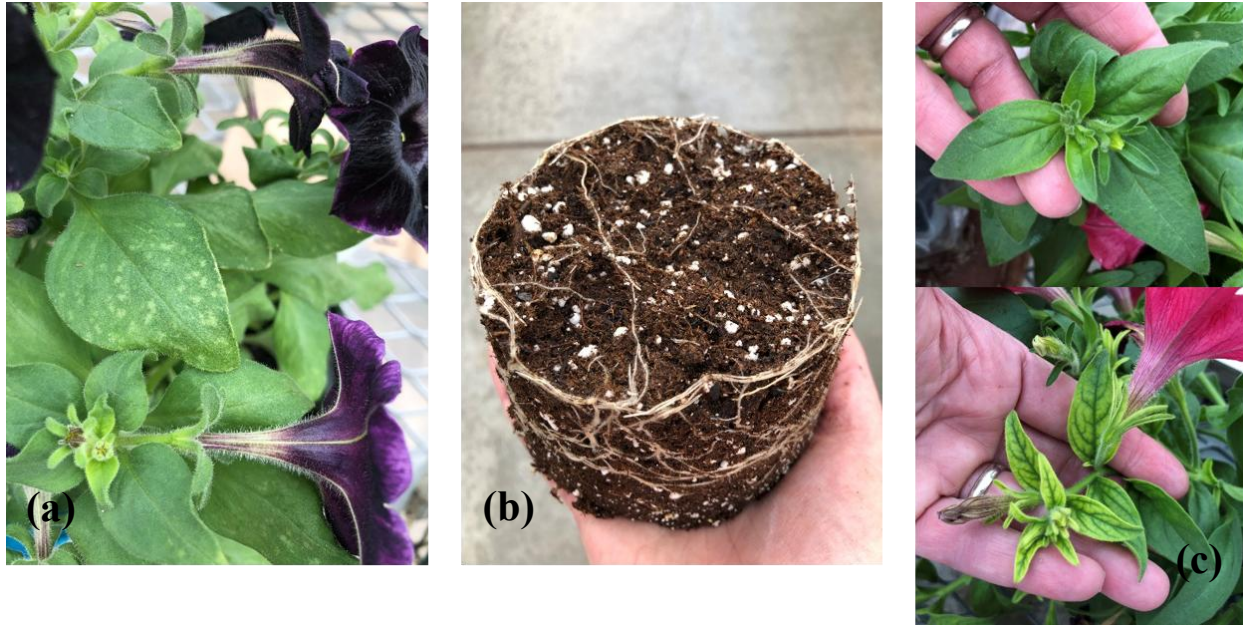


Figure 1.5 Foliar symptomology and root system development

Figure 5 Left (a): Lower leaf symptomology of Over PH (Expt. 2), Middle (b): Root system 12 days after potting at the time watering treatments began (Expt. 1), Right (c): Range of symptomology observed in tissue collected for analyses (Expt. 1)

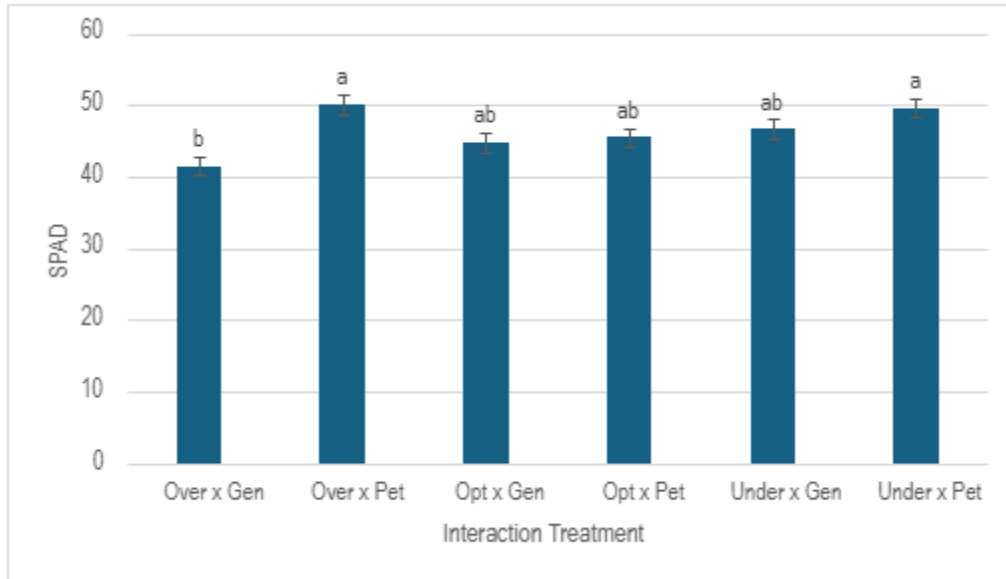


Figure 1.6 Water by Fertilizer interaction graph from Expt. 1

Figure 6: Relative leaf greenness (SPAD units) by interaction of water and fertilizer in Expt. 1

Chapter 2 - IRIS Film Study

Soil microbe-mediated Changes in Substrate Manganese, but Not Iron, Solubility May Cause Young Leaf Chlorosis in Overwatered Petunias

Keywords: Iron deficiency, IRIS films, Mn deficiency,

Abstract.

Waterlogged or hypoxic substrate conditions cause microbial mediated reduction of manganese and iron that changes their plant bioavailability and leaching potential. Iron-inefficient crops such as petunia are vulnerable to waterlogged conditions as the substrate availability of these micronutrients change. In Spring 2025 (Expt. 1), Petunia ‘Headliner Strawberry Sky’ (SS) plants were grown in a greenhouse under two different watering regimes: overwatered (Over; rewatered when weight of sentinel pots dropped to 90 to 95±5% of container capacity, CC) and optimally watered (Opt; 60±5% of CC) with four different fertilizer sources: Jack’s Professional General Purpose (JPGP, Gen), JPGP with additional Mn EDTA (Gen+Mn), JPGP with additional Fe EDTA (Gen+Fe), and Jack’s Classic Petunia Feed (Pet), all at 150 ppm N. In Spring 2026, two experiments were performed to assess the effect plant maturity plays in the overwatering stress. The first experiment (Expt. 2) had Young plant that were newly transplanted when experimental conditions started compared to the second experiment (Expt. 3) where plants used had time to establish before experimental conditions. Both were started with the three different treatment fertilizers: Gen, Gen+Mn, and Control (Con; no micros added except Mg and S) and an additional cultivar: ‘SureShot Dark Blue’ (DB). Both Fe and Mn Indicator of Reduction In Soils (IRIS) films were used to quantify the amount of Fe and Mn reduction in each experimental treatment. Plant weights, leaf greenness, nutrient levels from pour though extractions, and tissue nutrient content of youngest leaves were measured. Tissue Fe

levels were greater in Over than Opt in all three Expts., and tissue Mn was greater in Opt than Over in Expt. 1 and the same in Expts. 2 and 3. Across Expts. total manganese removal from IRIS films was greater in Over treatments between 10.9% and 61.4%. Total iron removal was greater in Over treatments as well; however, the percentages of removal across treatments were low between 0.4% and 1.6%. While SPAD differences occurred based on fertilizer formulation and water status, very little overwatered chlorotic foliage occurred, possibly indicating that more mature plants are less susceptible to developing symptomology in waterlogged conditions. Changes in leaf greenness in response to overwatering varied by plant maturity with young plants in Expt. 2 having greener leaves as the experiment progressed and more-well established plants in Expt. 3 having less green leaves.

Introduction

Overwatering has a variety of detrimental effects on the growth and development of crops in the spring greenhouse environment (e.g. Crum 2011). The practice leads to decreased air-filled porosity of the substrate that can result in hypoxia, or low oxygen conditions, that induce root stress by limiting oxygen for aerobic cellular respiration; this, in turn, reduces root growth and limits nutrient uptake (Xie et al. 2021). As an example, container grown roses irrigated to continuously maintain container capacity had no roots present in hypoxic areas of the substrate (Evans et al. 2009). Overwatering that causes hypoxia also provides a favorable environment for root pathogens such as *Pythium* and *Phytophthora* to infect already stressed roots (Chung and Lee 2020), which further inhibits roots' ability to absorb nutrients. Studies focusing on overwatering stress in herbaceous ornamental plants produced in a greenhouse environment are lacking.

Root hypoxia initiates abscisic acid synthesis in leaves that causes stomata to close and, subsequently, a reduction in root conductance. Limited root conductance decreases nutrient uptake (González-Guzmán et al. 2021; Sharma et al. 2021). This stomatal closing additionally results in an imbalance of carbon dioxide and light energy whereby excessive light energy leads to the creation of reactive oxygen species (ROS) and subsequent damage/inhibition of photosystem II (PSII) (Rankenberg et al. 2021). Tomatoes under stress-inducing, hypoxic conditions had decreased root dry weight and increased shoot dry weight, indicating a shift in dry-matter partitioning (Horchani et al. 2008).

Overwatering stress is ultimately manifested in shoot tissue with a symptom of chlorotic foliage. An early description of damage includes yellow margin of the leaf blade and/or interveinal chlorosis which ties the disorder to visual deficiency symptoms of the micronutrients iron (Fe) and manganese (Mn) (Poesch 1937). Iron plays essential roles in photosynthetic pathways by acting as a cofactor for formation of photosystem complexes. When Fe is deficient, photosynthetic capacity is decreased due to a reduction in the amount of chlorophyll, leading to leaf chlorosis (Spiller and Terry 1980; Terry 1980; Therby-Vale et al. 2022). Concentrations of chlorophyll a and b were less in Chinese cabbage grown under flooded conditions compared to optimally watered or droughty conditions (Šola et al. 2021). An upregulation of genes responsible for making chlorophyll-catabolizing enzymes could be one reason chlorophyll reduction/chlorosis occurs in waterlogged plants (Zhu et al. 2018). Some species are considered Fe-inefficient, i.e. crops that perform best when grown at lower pH to maintain solubility of micronutrients for plant uptake (Williams and Scoggins 2018). Deficiency of Mn can also manifest as chlorosis of young leaves (Schmidt et al. 2016). The ability of Mn to exist in many different redox states allows it to be an important component of several plant enzymes. Most

notably, Mn is part of the oxygen evolution complex (OEC) in photosystem II (PSII) that is needed to split water molecules and generate the H^+ electrochemical gradient that allows ATP synthase to function as well as (Schmidt and Husted 2019).

Anaerobic substrate conditions due to overwatering result in reduction of many chemical species, notably NO_3^- , Mn(IV)/Mn(III), and Fe(III), via microbial anaerobic cellular respiration (McBride 1994). These changes in redox state change plant bioavailability and leaching potential of essential plant nutrients. Saturated soil conditions cause heterotrophic microorganisms to quickly consume oxygen dissolved in the soil solution as these microbes reduce O_2 to H_2O using O_2 as a terminal electron acceptor during cellular respiration. After all O_2 is consumed, these microbes will switch to using nitrate as their terminal electron acceptor. Mn(III/IV) is reduced to Mn(II) after all nitrate is reduced, and Fe(III) is reduced to Fe(II) after all Mn(III/IV) is reduced (Rabenhorst et al. 2021); this phenomenon is termed the “redox ladder.” Iron(II) and Mn(II) are plant available as well as leachable/soluble chemical species (Alejandro et al. 2020; Rabenhorst et al. 2021; Bhat et al. 2024). The increased solubility associated with the change of their redox state caused by microbial reduction in waterlogged substrates could lead to increased nutrient flushing when crops are overirrigated in pots in the commercial greenhouse environment. Conversely, waterlogged wheat grown in acidic field soil has been shown to hyperaccumulate Fe and Mn to toxic levels (Khabaz-Saberi et al. 2006).

In continuous liquid feed (CLF) production systems, which are common in production of herbaceous greenhouse crops, nutrients are supplied to the rhizosphere with each irrigation event. The reasons why overwatered crops exhibit symptomology of nutrient deficiency require further study. Specific “petunia” fertilizer is already used in the greenhouse industry to address micronutrient problems in iron-inefficient crops such as petunia and calibrachoa, and this

formulation is commonly used to mitigate foliar chlorosis symptoms when they arise during production. Hayden et al. (2026) found that “petunia” fertilizer did not decrease substrate pH with use, which would support Fe solubility and, thus, uptake. They also found that ...However, causes of specific nutrient deficiency(ies) in overwatered crops is unknown.

The research gap at the center of this project is understanding what nutrient deficiencies occur and why in herbaceous ornamental crops production when overwatering occurs. The overall objective of this research was to determine if Mn and/or Fe are microbially reduced in hypoxic substrate conditions and if this phenomenon is correlated with foliar chlorosis in overwatered petunias. Based on substrate water status and fertilizer treatments, we 1) evaluated growth differences between optimally and overwatered plants, 2) measured macro- and micronutrient differences in the substrate, 3) determined effects of overwatering on nutrient status of youngest leaf tissue, 4) quantified rhizosphere Mn and Fe reduction in treatment substrates, and 5) evaluated effects of plant maturity on occurrence of the disorder.

Materials and Methods

Overview of experiments. Three experiments were conducted in a glass-glazed greenhouse of Kansas State University’s Throckmorton Plant Sciences Center in Manhattan, KS, USA. Methods of the experiments differed from each other in these several ways. In Expts. 1 and 3, plants were allowed to establish healthy root systems prior to implementing water status treatments that included overwatering, and Expt. 2 was conducted with freshly-potted rooted liners that were not yet established when treatments were started. Secondly, similar to the methods of Hayden et al. (2026), when overwatered status could not be maintained with once per day irrigation due to increased plant water use, pots were set in saucers to ensure substrate

saturation in Expt. 1. However, in Expts. 2 and 3, plants were allowed to drain freely and the overwatered treatment was irrigated twice per day.

The first experiment (Expt. 1) was conducted during Spring 2025 from 28 Mar to 6 May 2025. Petunia ‘Headliner Strawberry Sky’ (SS) was grown with two watering regimes of optimally watered (Opt) and overwatered (Over) crossed with four fertilizers [general purpose (Gen), general purpose plus Mn EDTA (Gen+Mn), general purpose plus Fe EDTA (Gen+Fe), and petunia feed (Pet)]. The experimental units were arranged in a completely random design (CRD). Two water regimes by four fertilizers by one cultivar at five replications with two pots per experimental unit totaled 80 pots of petunias in this experiment.

The second and third experiments (Expts. 2 and 3, respectively) were conducted during Spring 2026. Expt. 2 used young plants (Yng) and was conducted 9 Feb to 3 Mar 2026. Expt. 3 used established plants (Est) and was conducted from 4 Mar to 26 Mar 2026. Petunia ‘Headliner Strawberry Sky’ (SS) and ‘SureShot Dark Blue’ (DB) were grown with two watering regimes of optimally watered (Opt) and overwatered (Over) crossed with three fertilizers [general purpose (General), general purpose plus Mn EDTA (Gen+Mn), and a control fertilizer (Con)]. The experimental units were arranged in a CRD. Two water regimes by three fertilizers by two cultivars at four replications with two pots per experimental unit totaled 96 pots of petunias in each experiment. Supplemental lighting was used in Expts. 2 and 3.

For Exp. 1, rooted liners were potted into BM6 All-Purpose Mix (Berger, Saint-Modeste, QC, CA; Batch 15511031412400654071) in 5.5 in (1.92 L volume) black square deep perennial pots (HC Companies, Twinsburg, OH, USA). For Expts. 2 and 3 rooted liners were potted into BM6 All-Purpose Mix (Berger, Saint-Modeste, QC, CA; Batch 15511031392501154071) in 5 in (770 mL volume) azalea pots (HC Companies, Twinsburg, OH, USA). Pots were filled with

substrate, brushed off to level, and then lightly tapped three times to settle substrate. Then, additional substrate was added again to fill and brushed to level off. Bulk density was $0.07 \text{ g}\cdot\text{cm}^{-3}$, air-filled porosity was 27.0%, and total porosity was 83.2% based on five replications.

Cultivar treatments. For Expt. 1, rooted liners of SS were shipped from Tagawa Greenhouse Enterprises (TGI; Brighton, CO, USA) during week 8 and potted on 7 Mar 2025. Plants were grown for 21 days to establish a healthy root system prior to implementing the water status and fertilizer treatments. For Expt. 2 and 3, rooted liners of SS and DB were shipped from TGI during week 5 and potted on 6 Feb 2026. Young plants in Expt. 2 were given three days to establish before water and fertilizer treatments started. Established plants in Expt. 3 were given 26 days to establish before water and fertilizer treatments started.

Water status treatments. Two water status treatments were used in all three experiments: Opt and Over. For Opt pots, gravimetric moisture content was used throughout the experiments to determine when to rewater based on container capacity (CC; Jonas and Williams 2000; Yafuso and Fisher 2017). After fertilizer treatments were applied, pots were allowed to drain freely for about 30 min. Designated sentinel pots (one per treatment combination in all experiments) were then weighed. These sentinel pots were weighed daily throughout the experiments and if the average pot weight(s) fell below 60% of weight at CC, all pots of that water status treatment would be fertigated. In Expt. 1, Over water status treatments were similarly established by rewatering when sentinel pots averaged 95% of weight at CC). Once Over pots needed to be rewatered twice a day beginning 15 Apr (18 DAT), trays were set under pots to maintain Over treatment. In Expts. 2 and 3, plants in the Over treatment were rewatered every day in Expt. 2 (Yng) and twice a day in Expt. 3 (Est) to ensure conditions of overwatering.

Fertilizer treatments. Four fertilizer treatments were used in Expt. 1 and three were used in Expts. 2 and 3 (Table 1). The notable difference between these fertilizers for the purpose of this study is the amount and formulation of Mg, Fe, and Mn. Fertilizer treatments Gen+Mn and Gen+Fe were formulated to contain the same concentration of Mn and Fe, respectively, as Pet as to try to pinpoint whether it is additional Mn or Fe that that alleviates overwatering related chlorosis. Fertilizers injected using Dosatron model D25F1 at a dilution ratio of 1:100 (Dosatron International, LLC., Clearwater, FL, USA).

Expt. 1: Environmental data of temperature and light were recorded every ten minutes by an Apogee SM-600 sensor (Apogee Instruments, Logan, UT, USA) suspended from ceiling in the middle of the greenhouse. Greenhouse day and night temperatures, 77 and 72°F, respectively, were set by Argus (Argus Control Systems Limited, Langley, BC, CA). The average recorded temperatures included: a daily temperature of 24.5°C, a nightly temperature of 22.1°C, a high of 30.2°C, and a low of 19.3°C. The light values include an average day value of 358.55 $\mu\text{mol m}^{-2} \text{s}^{-1}$, a high value of 1095.41 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and a low value of 0.00 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Whitewash was applied on 18 Mar 25*.

Expt. 2 and 3: Environmental data of light was recorded every 30 minutes by a HOBO Pendant MX Temp/Light Data Logger (HOBO Data Loggers, Bourne, MA) placed at pot-level on experiment bench. Greenhouse day and night temperatures, 73 and 67 °F, respectively, were set by Wadsworth STEP Up controller (Wadsworth Control Systems Inc, Arveda, CO). The light values include an average day value of 358.55 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Thimijan and Heins 1983).

Data collection and analyses. For Expt. 1, plant yield data excluding SPAD was collected by replication on 6 to 7 May 2025 (39 to 40 DAT). Root ratings were assigned for each of two pots in an experimental unit based on a Likert-type scale where 5 possessed the most

vigorous root mass and best color (Fig. 1). Leaf greenness was determined by averaging five readings per experimental unit taken on youngest leaves using SPAD-502 meter (Konica Minolta, Ramsey, NJ, USA) on 5 May (38 DAT). These youngest leaves were harvested for tissue analyses on 6 May to 7 May (39 to 40 DAT) by removing enough youngest leaf blade tissue from both pots in an experiment unit until 10 g fresh weight was recovered for analyses. Fresh weight of whole above-ground plant shoots was measured and then placed in a 70°C forced-air oven. After several days, dry weights were measured. Dried tissue was ground into powder for tissue analyses using a mortar and pestle that was wiped clean between samples to avoid contamination. PourThru solution sampling was conducted on 11 Apr, 25 Apr, and 6 May (14, 28, and 39 DAT, respectively) (Cavins and Williams 2018). Plants were fertigated as normal, allowed to drain for 30 min, then 33 mL distilled water was added to pots and allowed to drain into saucer under each pot. Samples were then stored in a refrigerator until analyses occurred.

For Expt. 2 (Yng), plant yield data excluding SPAD was collected on 3 Mar 2026 (22 DAT). For Expt. 3 (Est), plant yield data was collected 25 Mar (21 DAT). Plant width was determined by averaging two perpendicular measurements of plant diameter for both pots of an experimental unit. Leaf greenness was determined by averaging three readings per experimental unit taken on youngest leaves using SPAD-502 meter (Konica Minolta, Ramsey, NJ, USA) on 20 Feb (11 DAT) and 2 Mar (21 DAT) in Expt. 2 (Yng) and 13 Mar (9 DAT) and 24 Mar (20 DAT) in Expt. 3 (Est). These youngest leaves were harvested for tissue analyses on 3 Mar (22 DAT) in Expt. 2 and 25 Mar (21 DAT) in Expt. 3 by removing enough youngest leaf blade tissue from both pots in an experimental unit until 5 g was recovered. Dried tissue was ground into powder for tissue analyses using a mortar and pestle as described for Expt. 1. Fresh and dry weights of

whole above-ground plant shoots were determined as described for Expt. 1. Pour through solution sampling was conducted on 2 Mar (21 DAT) in Expt. 2 and 24 Mar (20 DAT) in Expt. 3 as described for Expt. 1.

Tissue nutrient analyses. For all experiments, plant tissue samples were assayed by Kansas State University's Soil Testing Lab (Manhattan, KS). Plant tissue was tested for total nitrogen (N) and carbon (C), P, K, Ca, Mg, SO₄-S, Cu, Fe, Mn, and Zn. Expt. 1 additionally tested for nitrate (NO₃-N). In plant tissue, total N and C were analyzed via dry combustion (LECO TruSpec CN combustion analyzer, LECO Corp., St. Joseph, MI, USA). Nitrate was determined via KCl extraction, cadmium reduction, and analyzed by colorimetric reaction (Alpkem Corporation 1986a; Clackamas, OR, USA; Gelderman and Beegle 1998). Phosphorus and K were determined from a sulfuric peroxide digest (Lindner and Harley 1942; Thomas et al. 1967). Ca, Mg, SO₄-S, Cu, Fe, Mn, and Zn were determined from a nitric-perchloric digest (Giesecking et al. 1935). All tissue nutrients except total N, C and NO₃-N were analyzed with ICP-OES (Model 5800, Agilent Technologies, CA, USA).

Solution nutrient analyses. For all experiments, pH (Bluelab Combo Meter, Bluelab Corp., Tauranga, NZ) and EC (Accumet Excel XL20, Fisher Scientific, Waltham, MA) were measured on each pour through sample. Probes were rinsed and dried between samples to avoid contamination. Plant water samples were assayed by Kansas State University's Soil Testing Lab (Manhattan, KS). Leachate was tested for Zn, NO₃-N, NH₄-N, P, Ca, Mg, K, Cu, Fe, Mn, and S. Expt. 2 additionally tested for Na. Elements in leachate samples (Zn, P, Ca, Mg, Na, K, Cu, Fe, Mn, and S) were determined by ICP-OES (Model 5800, Agilent Technologies, CA, USA). NH₄-N was determined via indophenol colorimetric reaction and NO₃⁻-N determined via cadmium

reduction and colorimetric reaction (Alpkem Corporation 1986a; Alpkem Corporation 1986b; Clackamas, OR, USA).

IRIS film analyses. In all experiments, Indicator of Reduction In Soils (IRIS) PVC films (Advanced IRIS Oxides Inc., LLC, Riverdale, MD) were used to measure electrochemical reduction of Fe and Mn in substrate solution via microorganism metabolism. In Expt. 1, a 12.7 cm Mn film was installed in one pot of each e.u. and a 12.7 cm Fe film was installed in the other pot of each e.u. at the beginning of the experiment. Expts. 2 and 3 had 6.4 cm Mn films installed in one pot of each e.u. and Fe films installed in the other pot of only two e.u.s for each treatment. Films were removed from pots and cleaned on 8 May 2025 in Expt. 1, 4 Mar 2026 in Expt. 2, and 3 Mar 2026 in Expt. 3 (Fig. 4). Films were then scanned (Epson WorkForce ES-50, Epson America, Inc., Los Alamitos, CA, USA) and results uploaded into image analysis software (Fig. 5; ImageJ, Madison, WI, USA) which was used to quantify the percentage of Mn or Fe oxide removal on the respective Mn or Fe films (code in Appendix B). Expt. 1 films were analyzed for removal percentage by total area as well as by 2.54 cm sections that reflected layers of substrate depth, with 1 (at top of the pot) descending to 5 (at bottom of pot). Expts. 2 and 3 films were also analyzed by for removal by total by sections 1 (top of pot) and 2 (bottom of pot).

Statistical analyses. JMP Pro ver. 16 software (SAS Institute, Cary, NC) was used for all statistical analyses. For Expt. 1, two-way ANOVA was performed, and for Expts. 2 and 3, three-way ANOVA was performed. When a particular F test was significant, the means were separated with Tukey HSD test at a probability level of $\alpha=0.05$. For root rating only, which was ordinal data, contingency analysis of Water and Fertilizer main effects only were performed to determine Pearson's Contingency Coefficient.

Results and Discussion

Expt. 1. Growth data. Fresh and dry weights, and SPAD were different between the two water status treatments (Table 2). Over irrigation resulted in heavier plants for fresh and dry weight as well as greener foliage. Increased growth in Over irrigation indicated that root systems were not damaged by the water treatment, despite continually sitting in fertilizer solution beginning 18 DAT. Similar to our results, larger petunias based on all growth parameters, including fresh and dry weight, were observed by Rebi et al. (2024) when petunias were watered daily as opposed to less frequently. SPAD readings also differed by fertilizer formulation, with Gen+Fe fertilizer resulting in greenest plants, followed by Gen, with Gen+Mn and Pet least green. Minimal chlorosis was observed in this experiment (Fig. 3) with experimental treatments yielding similar looking plants at the end of the experiment (Fig. 2). No interactions were significant for growth data (Table 2).

Solution data. For both water treatments, pH was between 5.4 and 5.9 over the course of the experiment (Fig. 6a), which was within the recommended range of 5.4 to 6.0 for iron-inefficient crops that maintains Fe solubility (Williams and Scoggins 2018). Solution EC was near 2 dS·m⁻¹ for both water treatments on DAT 14, then increased to between 2.5 and 3.5 dS·m⁻¹ for Opt and Over treatments, respectively, by DAT 39 (Fig. 6b). These EC ranges are similar to the 3.0 to 3.5 dS·m⁻¹ range that resulted in largest ‘Dreams White’ petunia (Alem et al. 2015), indicating that fertilization in our study was adequate to maximize growth but not excessive so as to induce osmotic stress. Ammonium differed by water status at 28 DAT (Fig. 6c), by fertilizer DAT 14, 28, and 39 (Fig. 7c), and by interaction at DAT 14. Nitrate differed by water status at DAT 28 and 39 (Fig. 7d), by fertilizer at DAT 14, 28, and 39 (Fig. 7d), and by interaction at DAT 28. Iron differed by water status by water status (Fig. 6e) and by fertilizer (Fig. 7e) as well as had significant interactions for all three sampling dates. Mn differed by water status (Fig. 6f)

and by fertilizer (Fig. 7f) as well as had significant interactions for all three sampling dates. Significantly greater Mn in Over solution versus Opt at all three sample dates. As the experiment progressed, Mn accumulated in substrate solutions of Over treatments because plants were sitting in nutrients solution beginning DAT 18, but nutrients leached from Opt treatments. Pet fertilizer having reduced concentrations of nutrients in leachate analysis compared to Gen, Gen+Mn, and Gen+Fe despite having the same or greater concentrations of micronutrients in fertilizer solution could be due to matrix effects during leachate nutrient analysis caused by chelated metals (Ganga Hettiarachchi, personal communication). It is worth noting that both Gen+Mn and Gen+Fe fertilizer stock solutions were mixed and stored in opaque, black buckets to inhibit photodegradation associated with chelated metals (Albano and Miller 2001).

Tissue data. No differences in total N for main effects nor interactions (Table 3). There were differences in nutrient content based on water status for total C, P, Ca, Mg, SO₄-S, Fe, Mn, and NO₃, but not K, Cu, or Zn. Opt notably had greater levels of Mn and NO₃ as well as P, Ca, and Mg. Over had notably greater levels of Fe as well as SO₄-S and total C. There were differences among fertilizer formulations for P, K, Mg, Cu, Fe, Mn, and Zn. Gen, Gen+Mn, and Gen+Fe were highest notably in Mn and Fe, as well as P, K, and Zn. Pet had greater Mg. Pet, Gen+Fe, and Gen+Mn had greater Cu. There were interactions between water status and fertilizer for Fe and Zn.

Opt likely had greater NO₃ because of the greater availability of O₂ in the substrate relative to Over. Nitrification is an aerobic process so there would be decreases in the conversion of ammonia to nitrate in waterlogged conditions (Norton and Ouyang 2019). The decreased availability of O₂ in waterlogged conditions would enable denitrification, an anerobic process, to occur which would be converting nitrate into other nitrogen species (Devol 2008). Reduced Mn

in Over may be the result of increased Mn reduction in the waterlogged substrate causing an increased Mn solubility/leaching potential (Rabenhorst et al. 2021). A hypothesis that connects to the increased amount of Fe in Over tissue relates to the methodology of having Over treatments sitting in saucers during the latter part of Expt. 1; this method allowed for a longer period of increased nutrient uptake, including for micronutrients that were applied at each irrigation.

IRIS film data. Iron IRIS films had less removal compared to Mn films. Over had more removal than Opt for the total area of the film (0.4% vs. 0.2%, respectively) (Table 4). While there was statistical significance, these results are not biologically significant. This trend was also observed for sections 1 and 2 of the film (sections closest to top of pot). Low removal percentages across main effects and interactions suggest that Fe reduction is not occurring in significant manner in overwatered substrate. Removal that is occurring is likely due to a combination of scratches on IRIS films during installation process and root mediated reduction of Fe (Ning et al. 2023). A much greater amount of Mn removal was occurring (Table 5). Over treatments had more Mn removed across all five sections of the film, as well as the total, compared to Opt. By fertilizer, the lowest amount of Total Mn removal was in the Gen+Mn treatments. The interaction of water status x fertilizer had reduced removal across all Opt followed by Over x Pet and Over x Gen-Mn. Based on depth of container, Mn removal increased towards the bottom of the pot, peaking at 83% removal in the bottom two inches of the pots. Because the plants were sitting in solution after 18 DAT, the waterlogged conditions ensured Mn reduction in these zones.

Based on the evidence from Expt. 1, significant reduction of Mn is clearly happening in waterlogged conditions compared to optimally watered ones. Increased Mn removal for Over x

Fe and -Gen compared to Over x Pet and -Mn shown in water status x fertilizer interaction suggests that additional Mn in the latter fertilizer treatments lessens the reduction of Mn in overwatered conditions. Manganese in these fertilizers is delivered via Mn EDTA which contains Mn(II) as it is the plant available form. Manganese(IV/III) are the forms that facultative microbes reduce to Mn(II) in waterlogged conditions. One possible hypothesis as to why this is that the conversion of Mn(IV/III) to Mn(II) shifts with additional Mn(II) as it changes this chemical equilibrium (Vepraskas and Craft 2016).

Expt. 2: *Growth data.* Cultivars differed by SPAD1 (11 DAT), SPAD2 (22 DAT), Width, fresh and dry weights. DB was greater for all except SPAD1 (Table 6). Young foliage in Over treatment was less green than Opt at 11 DAT, but not different from Opt at harvest, 22 DAT. Fresh weight was greater in Over compared to Opt plants at harvest This suggests that petunias can be susceptible to the disorder early in the production cycle, but can grow out of it. The fertilizer main effect shows that Gen and Gen+Mn were similar and had greater SPAD1, SPAD2, and fresh and dry weights compared to the Con treatment.

Solution analyses. Solution pH at harvest was 5.6 to 5.7, and EC ranged from 1.8 to 2.0 dS·m⁻¹. These results suggest that Fe and Mn were soluble, and thus available for uptake, and that plants were fertilized at an appropriate level. Cultivars differed in NH₄-N, P, and K, with SS always greater than DB (Table 8). Based on water status, Over has notably greater NH₄-N and Mn as well as Cu and K. We speculate that Mn was greater in the Over treatments because the fertilizer was more frequently applied—daily at each irrigation—compared to the Opt treatments. Opt had notably greater NO₃-N as well as Zn, P, Ca, Mg, Na, and S. Fertilizer main effect showed that Gen+Mn had greater NH₄-N, NO₃-N, Fe, and Mn as well as Zn and Cu. Gen+Mn

and Gen had greater P, Ca, Mg, K, and S. There were interactions between water status x fertilizer for Zn, NH₄-N, NO₃-N, P, Ca, Mg, Na, Fe, and S.

Tissue analysis. Cultivars differed in tissue content of total N, P, K, Ca, Mg, SO₄-S, Cu, Fe, Mn, and Zn (Table 7). Based on water status main effect, Over plants notably had greater Fe as well as total N, P, and K. Opt plants had greater Ca and Mg. Specifically, Fe was greater in Over plants while Mn was similar in Over and Opt; in Expt. 1, we found a similar result with Fe but Mn was reduced in Over compared to Opt. Based on fertilizer main effect, Gen and Gen+Mn notably had greater Fe as well as total C. Con had greater K, Ca, Mg, SO₄-S, Mn, and Zn. The water status x fertilizer interaction was significant for Cu and Zn.

Substantially greater Mn and Zn in Con could be because of reduced growth due to minimal overall micronutrients that allowed for Mn and Zn to be more concentrated in youngest tissue compared to that of the other two fertilizers where sufficient micronutrients supported additional growth, diluting Mn and Zn in their youngest tissues. Manganese and Zn could have also been hyperaccumulating in Con's youngest tissues as a response to Fe deficiency; genes upregulated in response to Fe deficiency can also transport Mn and Zn (Robe et al. 2020).

IRIS film data. At Section 2 (bottom half of pot), DB had greater Mn removal compared to SS (Table 9). Based on water status main effect, Over resulted in greater Mn removal in Section 2 and for the total area of the film. There was an interaction between water status x fertilizer in Section 2. There were no significant main effects nor interactions for Fe removal. Compared to Expt. 1 when Over pots sat in saucers to ensure waterlogged conditions, Fe removal was slightly greater.

Expt. 3. Growth data. At end of the experiment, cultivars differed in SPAD2, width, fresh and dry weights (Table 10). Based on water status main effect, Over resulted in greater width,

fresh and dry weights, whereas Opt had greater SPAD2 on 20 DAT, but not SPAD1 at 9 DAT. This trend is opposite compared to in Expt. 2, where younger plants overcame the effect of overwatering and young leaves were similarly green between Over and Opt treatments by harvest. However, the trend is similar to results of Expt. 1 where plants were already established when overwatering began, and young foliage was lighter green at the end of the study. The fertilizer main effect shows that Gen and Gen+Mn fertilizers resulted in similar leaf greenness to each other, and had greater SPAD1 and SPAD2 readings compared to the Con treatment (Table 10).

Solution analyses. In Expt. 3, pH was about a 0.2 to 0.5 units lower (5.0 to 5.4) compared to Expt. 2; in these ranges, multivalent cationic micronutrients remain soluble. EC levels also indicate adequate, but not excessive, fertility in this experiment, as in Expt. 2. However, EC was greater in Opt treatments, rising to 2.3 compared to 1.8 in Over treatments. Cultivars differed by $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, P, Na, K, Fe, Mn, and S (Table 12). Based on water status main effect, Over had greater $\text{NH}_4\text{-N}$, Cu, and Mn, whereas Opt had greater $\text{NO}_3\text{-N}$, P, Ca, Mg, Na, and S. The same trend occurred as in Expt. 2, with Fe similar between Over and Opt but Mn considerably greater in Over petunia tissue. Based on fertilizer main effect, Gen+Mn had greater EC, Zn, $\text{NO}_3\text{-N}$, Ca, Mg, Na, Fe, Mn, and S; and Gen+Mn and Gen had greater P and Cu. There were interactions between water status x fertilizer for $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, Ca, Mg, Cu, Mn, and S.

Tissue analysis. Cultivars differed by tissue content of total N, P, K, Ca, Mg, $\text{SO}_4\text{-S}$, Cu, Fe, Mn, and Zn (Table 11). By water status, Over had greater total N, total C, P, and Fe whereas Opt had greater K, Ca, Mg, $\text{SO}_4\text{-S}$, and Zn. As in Expt. 2, Fe was greater in the tissue of Over and Mn was not different between Over and Opt treatments. Based on fertilizer main effect, Gen+Mn and Gen were similar and had greater total C and Fe; Gen+Mn and Con had greater Ca

and Mn; Con had greater P, Mg, Cu, and Zn. There were interactions between water status x fertilizer for total C, Ca, Mg, SO₄-S, Cu, Fe, Mn, and Zn.

IRIS film data. Greater Fe removal for both Section 2 and total area was observed in Over versus Opt (Table 13). Based on fertilizer, Gen had greater removal than Con for section 2 and total area with Gen in between. Iron removal percentages were greater in Expt. 3 compared to Expt. 2. Greater Fe removal could be due to the increased irrigation in Expt. 3; removal from Fe IRIS films in Expt. 3 visually appeared to be closely associated with roots. Over had greater Mn removal from the total area of the film as well as both Sections 1 and 2 (Table 13). There were no other significant main effect nor interaction differences for Mn removal.

Plants in both Expt. 2 and 3 did not result in differences between their Over and Opt treatments for tissue Mn content. Although this could suggest that Mn deficiency is not to blame for overwatered symptomology, young tissue chlorosis was minimal in the Over plants in both of these experiments, as reflected in the SPAD data. However, we did observe interesting differences in SPAD readings between the two experiments that may reflect a plant's ability to overcome (Expt. 2) or succumb to (Expt. 3) the disorder. Multi-factors contribute to this disorder, and a successful strategy to overcome overwatering is by using a high porosity soilless substrate. The peat and perlite substrate used in our experiments could have abated the Over treatment and allowed petunias to overcome the conditions that typically result in the disorder. Another contributing factor may be low light conditions when overwatering occurs because there reduced light meaning reduced evapotranspiration and subsequently the rhizosphere stays wetter because roots are not taking up as much water.

Tissue analysis discussion. In all three experiments, greater tissue Fe in Over versus Opt treatments provides strong evidence that Fe deficiency is likely not to blame for overwatered symptomology.

One of the key differences between the experimental design of Expt. 1 vs Expt. 2 and 3 is the fact that the Over pots in Expt. 1 had their overwatered treatment maintained via sitting in the respective fertilizer solution in trays beginning 18 DAT whereas in Expt. 2 and 3 the overwatered treatment was maintained by increased frequency of irrigation events. Expt. 1 conditions were likely more consistently saturated throughout the experiment which would explain increased Mn removal from IRIS films in that experiment compared to Expts. 2 and 3. The petunias in Expts. 2 and 3 had more dry down between irrigation events, especially as plants grew larger and evapotranspiration increased. These conditions led to increased O₂ diffusion limiting reduction of Mn in the rhizosphere. The saucers in Expt. 1 were also likely influencing the concentrations of leachate nutrients. The fertilizer solution sitting in the trays was evaporating which increased the concentration of fertilizer nutrients in the substrate solution (evident by greater EC in Over vs Opt) which subsequently may have increased absorption of those nutrients by those Over plants, as in Expt. 1 (Fig. 6b). In Expt. 2 and 3, where fertilizer solution drained away between fertigation events, the greater substrate solution EC was in the Opt versus Over plants which was likely because of salts building up in Opt pots through wet and dry cycles (greater EC in Opt in both Expt. 2 and 3). Over pots in these experiments had a more constant stream of fertilizer solution running through them, so there was less evaporation/salt build up. A fundamental limitation of the interpretation of this study is reduced EC of Over treatments. Wetting and drying cycles of substrate under CLF in Opt causes soluble salts

to build up over time whereas Over has less drying/evaporation occurring. Soluble salts therefore are reduced in this treatment which potentially decreases those salts' uptake by petunia.

IRIS film discussion. Percentage Fe removal from the IRIS films was greater in Expts. 2 and 3 (Tables 9 and 13, respectively) compared to Expt. 1 (Table 4), which is explained by the closer association of roots with the Fe removal in Fe IRIS films in Expts. 2 and 3. Removal in Expt. 1 was more minimal and seemed to be less associated with roots. Percentage Mn removal from the IRIS films was reduced in Expts. 2 & 3 (Tables 9 and 13, respectively) compared to Expt. 1 (Table 5), which is explained by the use of saucers under pots in the Over treatment in Expt. 1 to ensure substrate saturation, but not in Expts. 2 and 3.

Conclusion

The level of establishment of petunia, young versus more well-established plants, appears to play a role in changes to leaf greenness in overwatered conditions based on the results of this study. Overwatered young petunia had less green leaves halfway through the experiment and then at the end had approximately the same leaf greenness. More well-established plants had the opposite response. Mature plants might have a more difficult time adjusting to overwatering compared to younger plants facing this stress earlier on in their life cycle. Chlorosis of the youngest foliage when petunia are overwatered is consistent with the visual symptomology of iron deficiency; however, across all three experiments, tissue iron levels were actually greater in overwatered treatments compared to optimally watered. Iron deficiency does not seem to be to blame for this disorder. Reduced tissue Mn in Expt. 1 in overwatered treatments potentially points to Mn deficiency at least being part of this disorder. This reduction in Mn in over- versus optimally watered plants not being observed in Expts. 2 and 3 could be due to the increased rate of fertilization replenishing nutrient levels; although, there was also increased nutrient flushing

in the overwatered treatments in these experiments. Often overlooked microbial interactions in substrate are occurring and potentially changing nutrient availability. Separate from the potential overwatered nutrient deficiencies, the evidence from these experiment does show that Mn is being reduced in overwatered conditions in the greenhouse whereas reduction of iron was not occurring in a substantial manner. It is not currently understood why the reduction of nitrate is seemingly passed over in the stepwise anerobic reduction. Additionally, is unknown whether low light levels impact the development of overwatered symptomology. Both these questions merit further investigation.

Table 2.1 Fertilizer treatment nutrient comparison for Expts. 1, 2, and 3

Table 1: Fertilizer treatment nutrient comparison for Expts. 1, 2, and 3				
(@ 150 ppm N)	Fe (ppm)	Mn (ppm)	Mg (ppm)	Fertilizer Source
Expt. 1				
Gen	0.375	0.1875	1.2	Jack's Professional 20-10-20 General Purpose
Gen+Mn	0.375	0.375	1.2	" " + Mn EDTA 1.07 g Mn EDTA 8L ⁻¹
Gen+Fe	1.5	0.1875	1.2	" " + Fe EDTA (5.92 g Fe EDTA 8L ⁻¹)
Pet	1.5	0.375	10	Jack's Professional 20-3-19 Petunia FeED Plus Mg
Expts. 2 & 3				
Con	0*	0*	1.2	Reagent grade KH ₂ PO ₄ , KNO ₃ , NH ₄ NO ₃ , and MgSO ₄ : 20-10-20
Gen	0.375	0.1875	1.2	Same as Expt. 1
Gen+Mn	0.375	0.375	1.2	Same as Expt. 1

*Baseline micronutrients added to substrate --> See Berger report, Jack's Professional (JR Peters Inc., Allentown, PA, USA), Mn EDTA (Combi-Blocks, Inc., San Diego, CA), Fe (Sigma-Aldrich, St. Louis, MO), Reagent grade salts: Fisher Scientific Company, Fair Lawn, NJ

Table 2.2 Fresh and dry weights, leaf greenness (SPAD), and root ratings from petunia ‘Strawberry Sky’ plants in Expt. 1

Table 2: Fresh and dry weights, leaf greenness (SPAD), and root ratings for petunia cultivar ‘Strawberry Sky’ by four fertilizers [General (Gen), General + Mn EDTA (Gen+Mn), General + Fe EDTA (Gen+Fe), and Petunia (Pet)] by two watering treatments [Overwatering (Over) and Optimally watered (Opt)]. Least-squared mean values with different letters are significantly different based on Tukey’s HSD at $\alpha = 0.05$				
Treatment	FWt (g)	DWt (g)	SPAD	ROOTS¹
<i>Water (n=20)</i>				
Over	255a	28.782a	43.5a	1.7
Opt	200b	21.263b	41.3b	3.1
Prob > F (Pearson*)	<.0001	<.0001	0.0008	0.0015*
<i>Fertilizer (n=10)</i>				
Gen	219a	24.406a	42.6ab	2.4
Gen+Mn	223a	24.946a	41.3b	2.5
Gen+Fe	243a	26.150a	44.6a	2.3
Pet	226a	24.587a	40.9b	2.5
Prob > F	ns	ns	0.0004	ns*
<i>Water*Fertilizer (n=5)</i>				
Over x Gen				
Over x Gen+Mn				
Over x Gen+Fe				
Over x Pet				
Opt x Gen				
Opt x Gen+Mn				
Opt x Gen+Fe				
Opt x Pet				
Prob > F	ns	ns	ns	na
¹ Root quality ratings scale where 0=small number of fine roots and 5=robust system of large roots. *For root rating only, which was ordinal data, contingency analysis of Water and Fertilizer main effects only were performed to determine Pearson’s Contingency Coefficient				

Table 2.3 Tissue data analysis from Expt. 1

Table 3: Tissue data analysis from Expt. 1 for petunia cultivar ‘Strawberry Sky’ by four fertilizers [General (Gen), General + Mn EDTA (Gen+Mn), General + Fe EDTA (Gen+Fe), and Petunia (Pet)] by two watering treatments [Overwatering (Over) and Optimally watered (Opt)]. Least-squared mean values with different letters are significantly different based on Tukey’s HSD at $\alpha = 0.05$												
Treatment	Total N (%)	Total C (%)	P (%)	K (%)	Ca (%)	Mg (%)	SO ₄ -S (%)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)	NO ₃ (ppm)
<i>Water (n=20)</i>												
Over	5.71	43.79a	0.411b	4.50	0.934b	0.510b	0.407a	6.6	184.3a	105.5b	39.6	19655b
Opt	5.93	42.14b	0.522a	4.56	1.503a	0.781a	0.388b	7.1	159.7b	131.1a	42.0	33347a
Prob > F	ns	<.0001	<.0001	ns	<.0001	<.0001	0.0189	ns	0.0305	0.0002	ns	<.0001
<i>Fertilizer (n=10)</i>												
Gen	5.84	42.66	0.522a	4.94a	1.19	0.573b	0.386	6.4b	192.9a	131.6a	44.0a	27699
Gen+Mn	5.97	43.08	0.504a	4.94a	1.17	0.569b	0.400	6.8ab	166.8ab	133.2a	43.3a	27909
Gen+Fe	5.92	43.01	0.519a	4.41a	1.26	0.644b	0.400	6.6ab	201.3a	121.5a	42.1a	28165
Pet	5.55	43.10	0.321b	3.83b	1.26	0.796a	0.408	7.5a	127.1b	87.0b	33.8b	22232
Prob > F	ns	ns	<.0001	<.0001	ns	0.0002	ns	0.0499	0.0001	<.0001	0.0002	ns
<i>Water*Fertilizer (n=5)</i>												
Over x Gen	ns	ns	ns	ns	ns	ns	ns	ns	211.6ab	ns	40.4ab	ns
Over x Gen+Mn	ns	ns	ns	ns	ns	ns	ns	ns	150.8bc	ns	39.9ab	ns
Over x Gen+Fe	ns	ns	ns	ns	ns	ns	ns	ns	235.8a	ns	39.4ab	ns
Over x Pet	ns	ns	ns	ns	ns	ns	ns	ns	139.0c	ns	33.2b	ns
Opt x Gen	ns	ns	ns	ns	ns	ns	ns	ns	174.2abc	ns	47.5a	ns
Opt x Gen+Mn	ns	ns	ns	ns	ns	ns	ns	ns	150.8bc	ns	46.7a	ns
Opt x Gen+Fe	ns	ns	ns	ns	ns	ns	ns	ns	166.7abc	ns	44.9a	ns
Opt x Pet	ns	ns	ns	ns	ns	ns	ns	ns	115.14c	ns	34.3b	ns
Prob > F	ns	ns	ns	ns	ns	ns	ns	ns	0.0197	ns	0.0281	ns

Table 2.4 Fe IRIS film data analysis from Expt. 1

Table 4: Fe IRIS film data analysis from Expt. 1 for petunia cultivar ‘Strawberry Sky’ by four fertilizers [General (Gen), General + Mn EDTA (Gen+Mn), General + Fe EDTA (Gen+Fe), and Petunia (Pet)] by two watering treatments [Overwatering (Over) and Optimally watered (Opt)]. Least-squared mean values with different letters are significantly different based on Tukey’s HSD at $\alpha = 0.05$						
Treatment	(% of Fe removal from IRIS film)					
	Section 1 (top of pot)	Section 2	Section 3	Section 4	Section 5 (bottom of pot)	Total
<i>Water (n=20)</i>						
Over	0.6a	0.4a	0.3	0.3	0.5	0.4a
Opt	0.2b	0.1b	0.2	0.3	0.5	0.2b
Prob > F	0.0026	0.0004	ns	ns	ns	0.0011
<i>Fertilizer (n=10)</i>						
Gen	0.5	0.3	0.2a	0.2a	0.5	0.3
Gen+Mn	0.4	0.1	0.2a	0.2a	0.4	0.2
Gen+Fe	0.4	0.4	0.5a	0.5a	0.7	0.4
Pet	0.3	0.1	0.2a	0.2a	0.4	0.2
Prob > F	ns	ns	0.0406	0.0359	ns	ns
<i>Water*Fertilizer (n=5)</i>						
Over x Gen	1.0a	ns	ns	ns	0.1a	ns
Over x Gen+Mn	0.5ab	ns	ns	ns	0.3a	ns
Over x Gen+Fe	0.7ab	ns	ns	ns	1.0a	ns
Over x Pet	0.5ab	ns	ns	ns	0.4a	ns
Opt x Gen	0.1ab	ns	ns	ns	0.9a	ns
Opt x Gen+Mn	0.3ab	ns	ns	ns	0.5a	ns
Opt x Gen+Fe	0.0b	ns	ns	ns	0.4a	ns
Opt x Pet	0.0b	ns	ns	ns	0.4a	ns
Prob > F	0.0333	ns	ns	ns	0.0202	ns

Table 2.5 Mn IRIS film data analysis from Expt. 1

Table 5: Mn IRIS film data analysis from Expt. 1 for petunia cultivar ‘Strawberry Sky’ by four fertilizers [General (Gen), General + Mn EDTA (Gen+Mn), General + Fe EDTA (Gen+Fe), and Petunia (Pet)] by two watering treatments [Overwatering (Over) and Optimally watered (Opt)]. Least-squared mean values with different letters are significantly different based on Tukey’s HSD at $\alpha = 0.05$						
Treatment	(% of Mn removal from IRIS film)					
	Section 1 (top of pot)	Section 2	Section 3	Section 4	Section 5 (bottom of pot)	Total
<i>Water (n=20)</i>						
Over	37a	57a	76a	83a	83a	61a
Opt	6b	10b	18b	22b	33b	13b
Prob > F	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
<i>Fertilizer (n=10)</i>						
Gen	26.a	43a	53a	56	61	43a
Gen+Mn	19ab	27b	41b	47	53	32c
Gen+Fe	28a	36ab	50ab	56	63	41ab
Pet	14b	29b	45ab	51	54	33bc
Prob > F	0.0027	0.0017	0.0122	ns	ns	0.0008
<i>Water*Fertilizer (n=5)</i>						
Over x Gen	46a	74a	87a	ns	ns	72a
Over x Gen+Mn	34ab	43c	65b	ns	ns	51b
Over x Gen+Fe	49a	64ab	83a	ns	ns	70a
Over x Pet	21bc	48bc	71ab	ns	ns	53b
Opt x Gen	7c	10d	20c	ns	ns	15c
Opt x Gen+Mn	5c	11d	17c	ns	ns	13c
Opt x Gen+Fe	7c	9d	17c	ns	ns	13c
Opt x Pet	7c	10d	18c	ns	ns	14c
Prob > F	0.0022	0.0031	0.0277	ns	ns	0.002

Table 2.6 Yield data analysis from Expt. 2

Table 6: Yield data analysis from Expt. 2 for petunia cultivars ['Dark Blue' (DB) and 'Strawberry Sky' (SS) by three fertilizers [General (Gen), General + Mn EDTA (Gen+Mn), and Control (Con)] by two watering treatments [Overwatering (Over) and Optimally watered (Opt)]. Least-squared mean values with different letters are significantly different based on Tukey's HSD at $\alpha = 0.05$					
Treatment	SPAD1 (11 DAT)	SPAD2 (21 DAT)	WDTH (cm) (22 DAT)	FWt (g) (22 DAT)	DWt (g) (22 DAT)
<i>Cultivar (n=24)</i>					
DB	32.6b	39.5a	26a	31a	2.29a
SS	37.5a	37.1b	22b	26b	1.89b
Prob > F	<.0001	0.0017	<.0001	0.0243	0.0204
<i>Water (n=24)</i>					
Over	33.4b	38.4	25	31a	2.13
Opt	36.7a	38.2	23	26b	2.05
Prob > F	0.0001	ns	ns	0.0100	ns
<i>Fertilizer (n=16)</i>					
Gen	36.6a	40.2a	25a	31a	2.31a
Gen+Mn	37.2a	39.3a	25a	31a	2.22ab
Con	31.3b	35.4b	22a	24b	1.75b
Prob > F	<.0001	<.0001	0.0308	0.0113	0.0177
<i>Variety*Water</i>					
Prob > F	ns	ns	ns	ns	ns
<i>Variety*Fertilizer</i>					
Prob > F	ns	ns	ns	ns	ns
<i>Water*Fertilizer</i>					
Prob > F	ns	ns	ns	ns	ns
<i>Variety*Water*Fertilizer</i>					
Prob > F	0.0012	ns	ns	ns	ns

Table 2.7 Tissue data analysis from Expt. 2

Table 7: Tissue data analysis from Expt. 2 for petunia cultivars [‘Dark Blue’ (DB) and ‘Strawberry Sky’ (SS) by three fertilizers [General (Gen), General + Mn EDTA (Gen+Mn), and Control (Con)] by two watering treatments [Overwatering (Over) and Optimally watered (Opt)]. Least-squared mean values with different letters are significantly different based on Tukey’s HSD at $\alpha = 0.05$											
Treatment	Total N (%)	Total C (%)	P (%)	K (%)	Ca (%)	Mg (%)	SO ₄ -S (%)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
<i>Cultivar (n=24)</i>											
DB	7.71a	40.97	0.920a	5.33b	0.895a	0.500a	0.388b	6.0a	96.60b	101.6b	47.6b
SS	7.13b	41.23	0.815b	5.82a	0.720b	0.439b	0.415a	4.0b	108.62a	120.6a	59.8a
Prob > F	<.0001	ns	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.011	<.0001	<.0001
<i>Water (n=24)</i>											
Over	7.64a	40.99	0.945a	5.67a	0.750b	0.457b	0.402	4.8	110.28a	108.4	52.2
Opt	7.20b	41.20	0.790b	5.48b	0.866a	0.482a	0.401	5.2	94.94b	112.8	55.2
Prob > F	<.0001	ns	<.0001	0.0072	<.0001	0.0089	ns	ns	0.0016	ns	ns
<i>Fertilizer (n=16)</i>											
Gen	7.41	41.30a	0.850	5.50b	0.795b	0.462b	0.396b	4.4	109.90a	91.8b	44.6b
Gen+Mn	7.42	41.31a	0.856	5.46b	0.774b	0.453b	0.394b	5.3	116.36a	94.1b	45.5b
Con	7.42	40.68b	0.896	5.76a	0.854a	0.494a	0.415a	5.3	81.57b	147.5a	71.0a
Prob > F	ns	0.0004	ns	0.0009	0.0008	0.0017	0.0088	ns	<.0001	<.0001	<.0001
<i>Water*Fertilizer (n=8)</i>											
Over x Gen								4.5ab			41.8b
Over x Gen+Mn								5.5ab			47.5b
Over x Con								4.5ab			67.3a
Opt x Gen								4.3b			47.4b
Opt x Gen+Mn								5.0ab			43.5b
Opt x Con								6.2a			74.8a
Prob > F	ns	ns	ns	ns	ns	ns	ns	0.0288	ns	ns	0.0304
<i>Variety*Water</i>											
Prob > F	0.0280	ns	ns	ns	ns	0.0469	ns	0.0021	ns	0.0359	0.0321
<i>Variety*Fertilizer</i>											
Prob > F	ns	ns	ns	ns	ns	ns	0.0135	ns	ns	ns	<.0001
<i>Variety*Water*Fertilizer</i>											
Prob > F	ns	ns	ns	0.0346	0.0332	ns	0.0101	ns	ns	ns	ns

Table 2.8 Solution data analysis from Expt. 2

Table 8: Solution data analysis from Expt. 2 for petunia cultivars [‘Dark Blue’ (DB) and ‘Strawberry Sky’ (SS) by three fertilizers [General (Gen), General + Mn EDTA (Gen+Mn), and Control (Con)] by two watering treatments [Overwatering (Over) and Optimally watered (Opt)]. Least-squared mean values with different letters are significantly different based on Tukey’s HSD at $\alpha = 0.05$														
Treatment	pH	EC	Zn (ppm)	NH ₄ -N (ppm)	NO ₃ -N (ppm)	P (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)	K (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)	S (ppm)
<i>Cultivar (n=24)</i>														
DB	5.7	1.907	0.36	30.91b	130.96	41.57b	81.50	54.62	66.35	131.11b	0.02	0.33	0.15	38.50
SS	5.6	1.906	0.38	33.79a	133.43	43.53a	81.56	55.91	66.11	141.73a	0.02	0.34	0.17	37.57
Prob > F	ns	ns	ns	0.0426	ns	0.0010	ns	ns	ns	0.0018	ns	ns	ns	ns
<i>Water (n=24)</i>														
Over	5.6b	1.829b	0.35b	33.89a	126.52b	41.12b	75.66b	50.45b	63.17b	139.75a	0.019a**	0.34	0.21a	34.50b
Opt	5.7a	1.986a	0.39a	30.81b	137.87a	43.99a	87.04a	60.09a	69.30a	133.09b	0.015b**	0.33	0.11b	41.56a
Prob > F	0.0008	<.0001	0.0023	0.0309	<.0001	<.0001	<.0001	<.0001	<.0001	0.0412	0.0322	ns	<.0001	<.0001
<i>Fertilizer (n=16)</i>														
Gen	5.6ab	2.025b	0.39b	32.14b	141.15b	47.64a	88.04a	60.22a	68.87a	151.92a	0.02b	0.47b	0.17b	39.44a
Gen+Mn	5.556b*	2.119a	0.55a	39.39a	153.72a	49.12a	90.44a	60.61a	68.15a	156.03a	0.03a	0.52a	0.21a	38.87a
Con	5.7a	1.576c	0.17c	25.52c	101.71c	30.89b	66.11b	44.97b	61.67b	101.30b	0.0004c**	0.02c	0.10c	35.78b
Prob > F	0.0467	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
<i>Water*Fertilizer (n=8)</i>														
Over x Gen		1.969b	0.37c	37.90ab	138.20b	46.64b	81.22b	53.27b	65.04b			0.51a		35.48bc
Over x Gen+Mn		1.940b	0.49b	43.91a	135.13b	46.06b	74.22b	49.55b	63.38b			0.51a		34.58c
Over x Con		1.575c	0.17d	19.87d	106.22c	30.65c	71.54bc	48.53bc	61.06b			0.01c		33.43c
Opt x Gen		2.081b	0.41bc	26.39cd	144.10b	48.64b	94.86a	67.18a	72.70a			0.43b		43.40a
Opt x Gen+Mn		2.299a	0.61a	34.88b	172.32a	52.18a	106.67a	71.68a	72.92a			0.54a		43.16a
Opt x Con		1.578c	0.16d	31.17bc	97.20c	31.14c	60.68c	41.42c	62.27b			0.03c		38.13b
Prob > F	ns	<.0001	0.0063	<.0001	<.0001	0.0005	<.0001	<.0001	0.0033	ns	ns	<.0001	ns	0.0287
<i>Variety*Water</i>														
Prob > F	ns	ns	ns	ns	ns	<.0001	ns	ns	<.0001	0.0334	ns	ns	ns	0.0004
<i>Variety*Fertilizer</i>														
Prob > F	0.0078	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.0445
<i>Variety*Water*Fertilizer</i>														
Prob > F	ns	ns	ns	ns	ns	na	ns	ns	0.0218	ns	ns	ns	ns	0.006

Table 2.9 Mn and Fe IRIS film data analysis from Expt. 2

Table 9: Mn and Fe IRIS film data analysis from Expt. 2 for petunia cultivars [‘Dark Blue’ (DB) and ‘Strawberry Sky’ (SS) by three fertilizers [General (Gen), General + Mn EDTA (Gen+Mn), and Control (Con)] by two watering treatments [Overwatering (Over) and Optimally watered (Opt)]. Least-squared mean values with different letters are significantly different based on Tukey’s HSD at $\alpha = 0.05$						
Treatment	(% of Mn or Fe removal from IRIS film)					
	Mn 1	Mn 2	Mn Total	Fe 1	Fe 2	Fe Total
<i>Cultivar (Mn: n=24, Fe: n=12)</i>						
DB	3	16a	9	0.1	0.8	0.4
SS	5	10b	8	0.2	0.4	0.3
Prob > F	ns	0.0142	ns	ns	ns	ns
<i>Water (Mn: n=24, Fe: n=12)</i>						
Over	5	17a	12a	0.2	0.9	0.5
Opt	9	9b	6b	0.1	0.3	0.2
Prob > F	ns	0.0008	0.005	ns	ns	ns
<i>Fertilizer (Mn: n=16, Fe: n=8)</i>						
Gen	2	13	7	0.1	0.6	0.4
Gen+Mn	4	14	9	0.1	0.8	0.4
Con	5	13	9	0.3	0.3	0.3
Prob > F	ns	ns	ns	ns	ns	ns
<i>Water*Fertilizer (Mn: n=8, Fe: n=4)</i>						
Over x Gen		19a				
Over x Gen+Mn		21a				
Over x Con		12ab				
Opt x Gen		7b				
Opt x Gen+Mn		7b				
Opt x Con		13ab				
Prob > F	ns	0.0209	ns	ns	ns	ns
<i>Variety*Water</i>						
Prob > F	0.0389	ns	ns	ns	ns	ns
<i>Variety*Fertilizer</i>						
Prob > F	ns	ns	ns	ns	ns	ns

<i>Variety*Water*Fertilizer</i>						
Prob > F	ns	ns	ns	ns	ns	ns

Table 2.10 Yield data analysis from Expt. 3.

Table 10: Yield data analysis from Expt. 3 for petunia cultivars [‘Dark Blue’ (DB) and ‘Strawberry Sky’ (SS) by three fertilizers [General (Gen), General + Mn EDTA (Gen+Mn), and Control (Con)] by two watering treatments [Overwatering (Over) and Optimally watered (Opt)]. Least-squared mean values with different letters are significantly different based on Tukey’s HSD at $\alpha = 0.05$					
Treatment	SPAD1 (9 DAT)	SPAD2 (20 DAT)	WDTH (cm) (21 DAT)	FWt (g) (21 DAT)	DWt (g) (21 DAT)
<i>Cultivar (n=24)</i>					
DB	41.0	41.5a	43a	123a	11.46a
SS	41.7	36.8b	34b	88b	7.77b
Prob > F	ns	<.0001	<.0001	<.0001	<.0001
<i>Water (n=24)</i>					
Over	41.7	37.2b	40a	119a	10.29a
Opt	41.0	41.1a	37b	93b	8.95b
Prob > F	ns	<.0001	0.0003	<.0001	0.0015
<i>Fertilizer (n=16)</i>					
Gen	42.2a	40.9a	39	104	9.57a
Gen+Mn	43.1a	40.7a	40	111	10.22a
Con	38.7b	35.8b	38	103	9.07a
Prob > F	<.0001	<.0001	ns	ns	ns
<i>Variety*Water</i>					
Prob > F	ns	ns	ns	ns	ns
<i>Variety*Fertilizer</i>					
Prob > F	ns	ns	ns	ns	ns
<i>Water*Fertilizer</i>					
Prob > F	ns	ns	ns	ns	ns
<i>Variety*Water*Fertilizer</i>					
Prob > F	ns	ns	ns	ns	ns

Table 2.11 Tissue data analysis from Expt. 3

Table 11: Tissue data analysis from Expt. 3 for petunia cultivars [‘Dark Blue’ (DB) and ‘Strawberry Sky’ (SS) by three fertilizers [General (Gen), General + Mn EDTA (Gen+Mn), and Control (Con)] by two watering treatments [Overwatering (Over) and Optimally watered (Opt)]. Least-squared mean values with different letters are significantly different based on Tukey’s HSD at $\alpha = 0.05$											
Treatment	Total N (%)	Total C (%)	P (%)	K (%)	Ca (%)	Mg (%)	SO ₄ -S (%)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
<i>Cultivar (n=24)</i>											
DB	6.8a	41.87	0.636a	5.02b	0.831a	0.461a	0.393a	4.1a	119.57b	124.1b	35.0b
SS	6.42b	41.96	0.606b	5.37a	0.549b	0.388b	0.365b	2.6b	154.50a	165.7a	50.5a
Prob > F	<.0001	ns	0.0189	0.0015	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
<i>Water (n=24)</i>											
Over	6.78a	42.28a	0.681a	5.03b	0.503b	0.407b	0.367b	3.4	151.76a	142.4	40.8b
Opt	6.44b	41.55b	0.561b	5.36a	0.876a	0.442a	0.391a	3.4	122.32b	147.4	44.7a
Prob > F	<.0001	<.0001	<.0001	0.0024	<.0001	0.0007	<.0001	ns	<.0001	ns	0.0064
<i>Fertilizer (n=16)</i>											
Gen	6.53	42.21a	0.596b	5.33	0.657b	0.396b	0.373	3.0b	165.99a	131.41b	33.4b
Gen+Mn	6.61	42.23a	0.596b	5.15	0.687ab	0.412b	0.382	3.0b	167.66a	152.8a	37.2b
Con	6.69	41.31b	0.671a	5.10	0.726a	0.466a	0.382	4.1a	77.46b	150.4a	57.7a
Prob > F	ns	<.0001	<.0001	ns	0.0039	<.0001	ns	0.0039	<.0001	0.0008	<.0001
<i>Water*Fertilizer (n=8)</i>											
Over x Gen		42.32a			0.474c	0.380b	0.366bc	2.7b	189.47a	137.1c	33.2c
Over x Gen+Mn		42.55a			0.524c	0.412b	0.376bc	3.6ab	185.12a	162.8ab	38.9c
Over x Con		41.98a			0.513c	0.429b	0.360c	3.9ab	80.68c	127.2c	50.4b
Opt x Gen		42.10a			0.840b	0.411b	0.381bc	3.3ab	142.51b	125.8c	33.6c
Opt x Gen+Mn		41.91a			0.850b	0.412b	0.388ab	2.4b	150.20b	142.8bc	35.5c
Opt x Con		40.64b			0.939a	0.504a	0.404a	4.4a	74.25c	173.7a	65.0a
Prob > F	ns	0.0099	ns	ns	0.0406	0.0091	0.0117	0.0359	0.0005	<.0001	<.0001
<i>Variety*Water</i>											
Prob > F	ns	ns	ns	ns	<.0001	<.0001	ns	ns	ns	ns	ns
<i>Variety*Fertilizer</i>											

Prob > F	ns	ns	ns	ns	ns	ns	ns	ns	<.0001	ns	<.0001
<i>Variety*Water*Fertilizer</i>											
Prob > F	ns	ns	ns	ns	ns	ns	ns	0.0064	ns	ns	0.0059

Table 2.12 Solution data analysis from Expt. 3

Table 12: Solution data analysis from Expt. 3 for petunia cultivars [‘Dark Blue’ (DB) and ‘Strawberry Sky’ (SS) by three fertilizers [General (Gen), General + Mn EDTA (Gen+Mn), and Control (Con)] by two watering treatments [Overwatering (Over) and Optimally watered (Opt)]. Least-squared mean values with different letters are significantly different based on Tukey’s HSD at $\alpha = 0.05$														
Treatment	pH	EC	Zn (ppm)	NH ₄ -N (ppm)	NO ₃ -N (ppm)	P (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)	K (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)	S (ppm)
<i>Cultivar (n=24)</i>														
DB	5.3a	2.048	0.37	39.48b	142.87b	58.73a	108.42	58.03	76.71a	107.32b	0.02	0.69a	0.32b	51.25a
SS	4.9b	2.027	0.41	42.60a	152.87a	51.59b	101.02	71.47	62.20b	166.61a	0.02	0.61b	0.46a	40.74b
Prob > F	<.0001	ns	ns	0.0265	0.0251	<.0001	ns	ns	<.0001	0.0155	ns	0.0147	<.0001	<.0001
<i>Water (n=24)</i>														
Over	4.9b	1.825b	0.40	47.25a	131.20b	49.01b	80.75b	39.01b	59.70b	110.98	0.02a	0.64	0.50a	36.37b
Opt	5.4a	2.250a	0.39	34.83b	164.54a	61.31a	128.69a	71.47a	79.21a	112.96	0.01b	0.66	0.28b	55.62a
Prob > F	<.0001	<.0001	ns	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	ns	0.0495	ns	<.0001	<.0001
<i>Fertilizer (n=16)</i>														
Gen	5.0b	2.000b	0.43b	40.97ab	142.71b	59.63a	91.61b	49.35b	65.47b	140.22a	0.02a	0.87b	0.37b	42.05b
Gen+Mn	5.1b	2.350a	0.55a	44.56a	178.80a	60.19a	138.58a	73.06a	82.26a	91.92c	0.03a	1.06a	0.59a	52.54a
Con	5.3a	1.762c	0.20c	37.59b	122.10c	45.66b	83.97b	43.31b	60.64b	103.75b	0.01b	0.02c	0.20c	43.40b
Prob > F	0.0029	<.0001	<.0001	0.0007	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
<i>Water*Fertilizer (n=8)</i>														
Over x Gen				42.83b	129.46cd		75.87cd	38.29cd			0.025ab		0.50b	35.73d
Over x Gen+Mn				55.99a	151.42bc		102.26bc	48.30bcd			0.033a		0.75a	40.99cd
Over x Con				42.93b	122.71d		64.11d	30.44d			0.0004c**		0.24c	32.93d
Opt x Gen				39.11bc	155.95b		107.36b	60.41b			0.024ab		0.25c	48.63bc
Opt x Gen+Mn				33.14c	206.18a		174.89a	97.82a			0.018b		0.43b	64.09a
Opt x Con				32.25c	131.49cd		103.84b	56.18bc			0.003c		0.17c	54.42b
Prob > F	ns	ns	ns	<.0001	0.0038	ns	0.0071	0.0054	ns	ns	0.0099	ns	0.0002	0.0343
<i>Variety*Water</i>														
Prob > F	ns	ns	ns	ns	0.0036	ns	0.0278	ns	ns	ns	ns	ns	0.0002	ns
<i>Variety*Fertilizer</i>														
Prob > F	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Variety*Water*Fertilizer</i>														
Prob > F	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.0046

Table 2.13 Mn and Fe IRIS film data analysis from Expt. 3

Table 13: Mn and Fe IRIS film data analysis from Expt. 3 for petunia cultivars ['Dark Blue' (DB) and 'Strawberry Sky' (SS) by three fertilizers [General (Gen), General + Mn EDTA (Gen+Mn), and Control (Con)] by two watering treatments [Overwatering (Over) and Optimally watered (Opt)]. Least-squared mean values with different letters are significantly different based on Tukey's HSD at $\alpha = 0.05$						
Treatment	(% of Mn or Fe removal from IRIS film)					
	Mn 1	Mn 2	Mn Total	Fe 1	Fe 2	Fe Total
<i>Cultivar (Mn: n=24, Fe: n=12)</i>						
DB	7	31	19	0.2	1.8	1.0
SS	5	26	15	0.2	1.7	0.9
Prob > F	ns	ns	ns	ns	ns	ns
<i>Water (Mn: n=24, Fe: n=12)</i>						
Over	9a	42a	25.a	0.3	2.9a	1.6a
Opt	3b	15b	9b	0.1	0.6b	0.3b
Prob > F	0.0012	<.0001	<.0001	ns	0.0008	0.0009
<i>Fertilizer (Mn: n=16, Fe: n=8)</i>						
Gen	7	25	16	0.2	2.2ab	1.2ab
Gen+Mn	5	29	17	0.3	2.4a	1.3a
Con	5	31	18	0.1	0.7b	0.4b
Prob > F	ns	ns	ns	ns	0.0371	0.0326
<i>Variety*Water</i>						
Prob > F	ns	0.0057	0.0399	ns	ns	ns
<i>Variety*Fertilizer</i>						
Prob > F	ns	ns	ns	ns	ns	ns
<i>Water*Fertilizer</i>						
Prob > F	ns	ns	ns	ns	0.0447	0.0500
<i>Variety*Water*Fertilizer</i>						
Prob > F	ns	ns	ns	ns	ns	ns

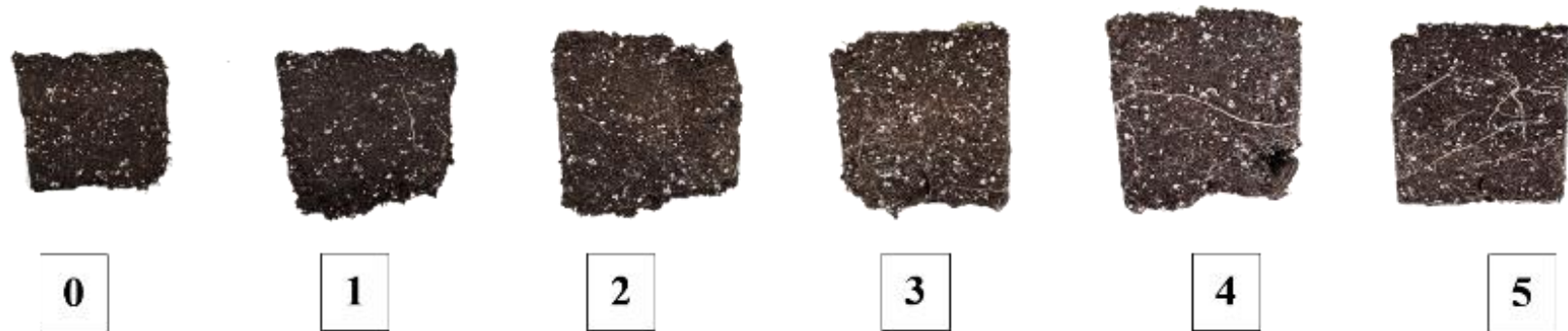


Figure 2.1 Root ratings (six-point Likert scale) of petunia in Expt. 1

Figure 1. Root ratings (six-point Likert scale) of petunia in Expt. 1: 0: little to no fine roots, 1: small number of fine roots, 2: moderate number of fine roots, 3: robust root system of fine roots, 4: few large roots, 5: robust root system of large roots

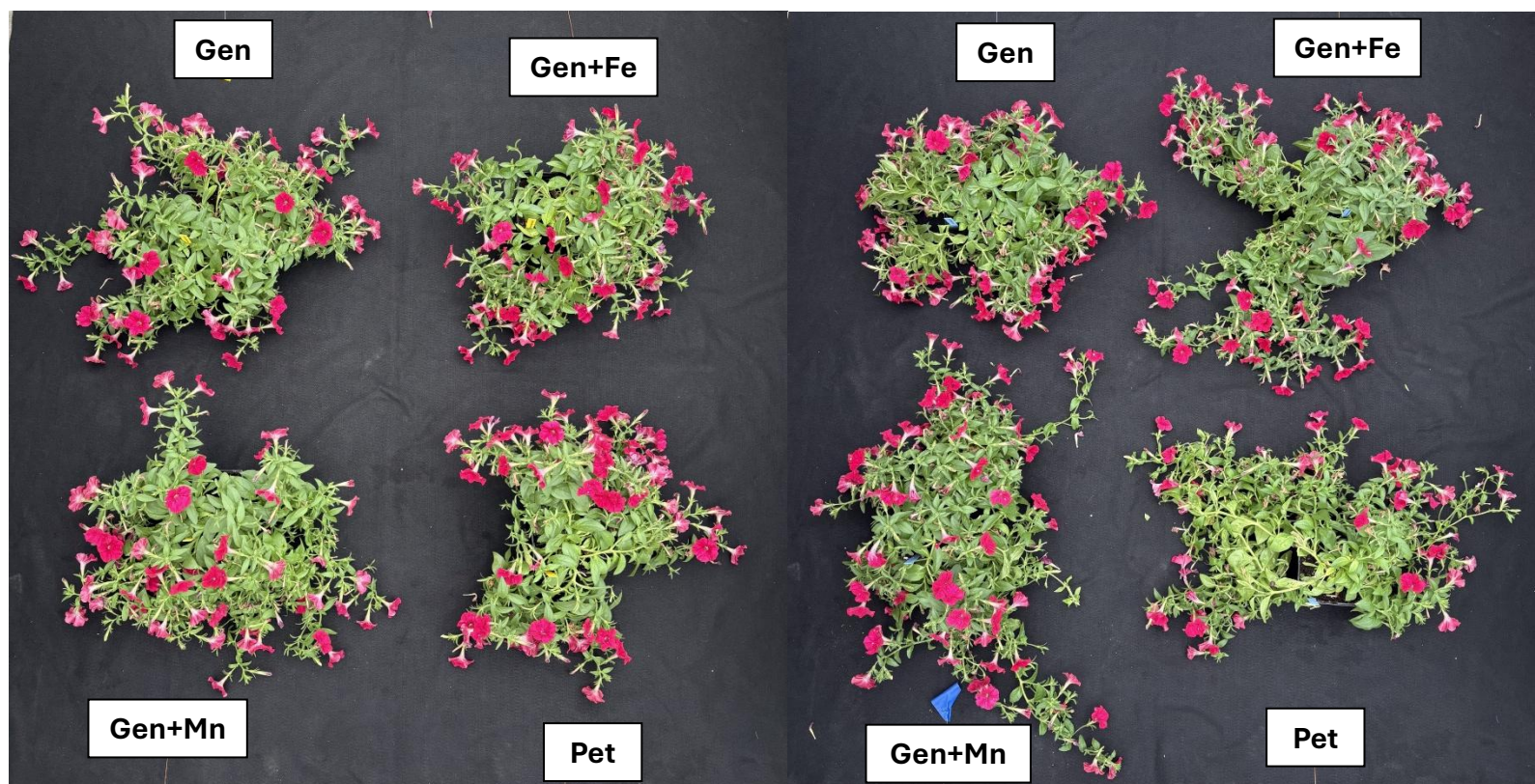


Figure 2.2 Expt. 1 end of experiment overview images

Figure 2: Expt. 1 end of experiment overview images Left: Opt, Right: Over



Figure 2.3 Range of visual symptomology in Expt. 1

Figure 3 Range of visual symptomology of youngest foliage in Expt. 1

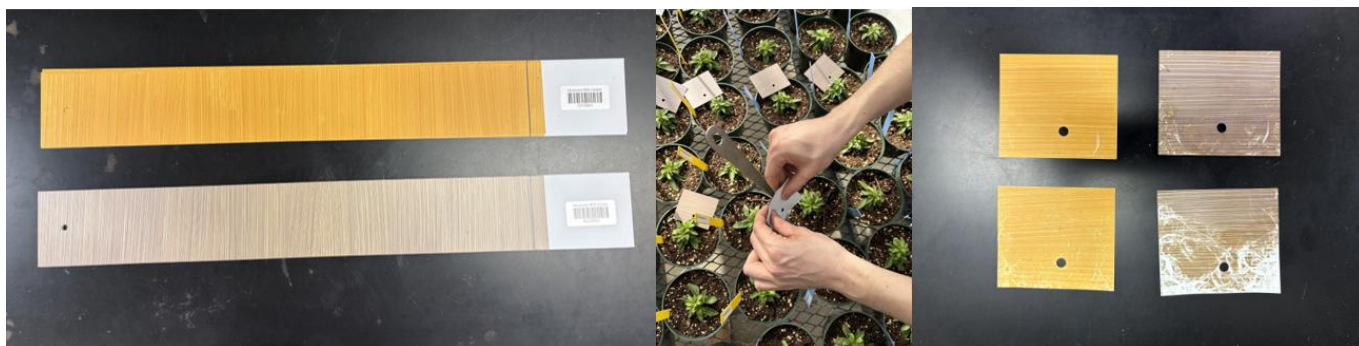


Figure 2.4 Overview of IRIS Film installation and retrieval

Figure 4: Left; full-length IRIS Films (top: Fe, bottom: Mn) before being cut into pot-sized strips, Center; Installation of Mn IRIS Films during Young Plant Experiment (2026), Right; Fe and Mn IRIS after being removed from pots and cleaned in Established Plant Experiment (2026) before image analysis

IRIS (Indicator of Reduction in Soils) Film Image Analysis: Fiji

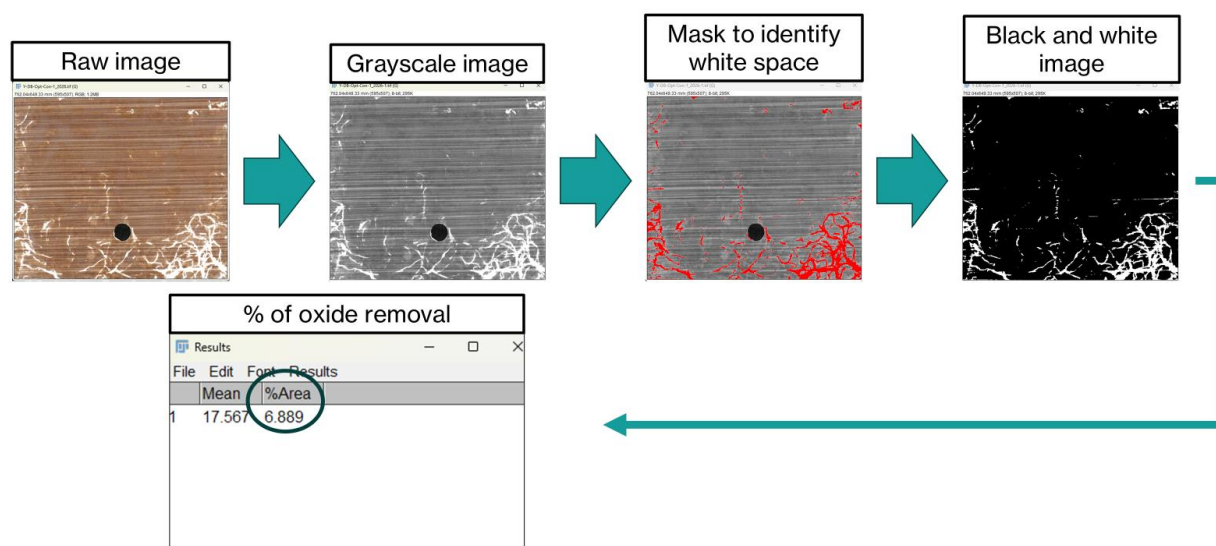


Figure 2.5 IRIS Film Analysis Overview

Figure 5: IRIS Film Analysis Overview: Processing raw IRIS film image into intermediate images to be able to quantify percentage of oxide removal via image analysis

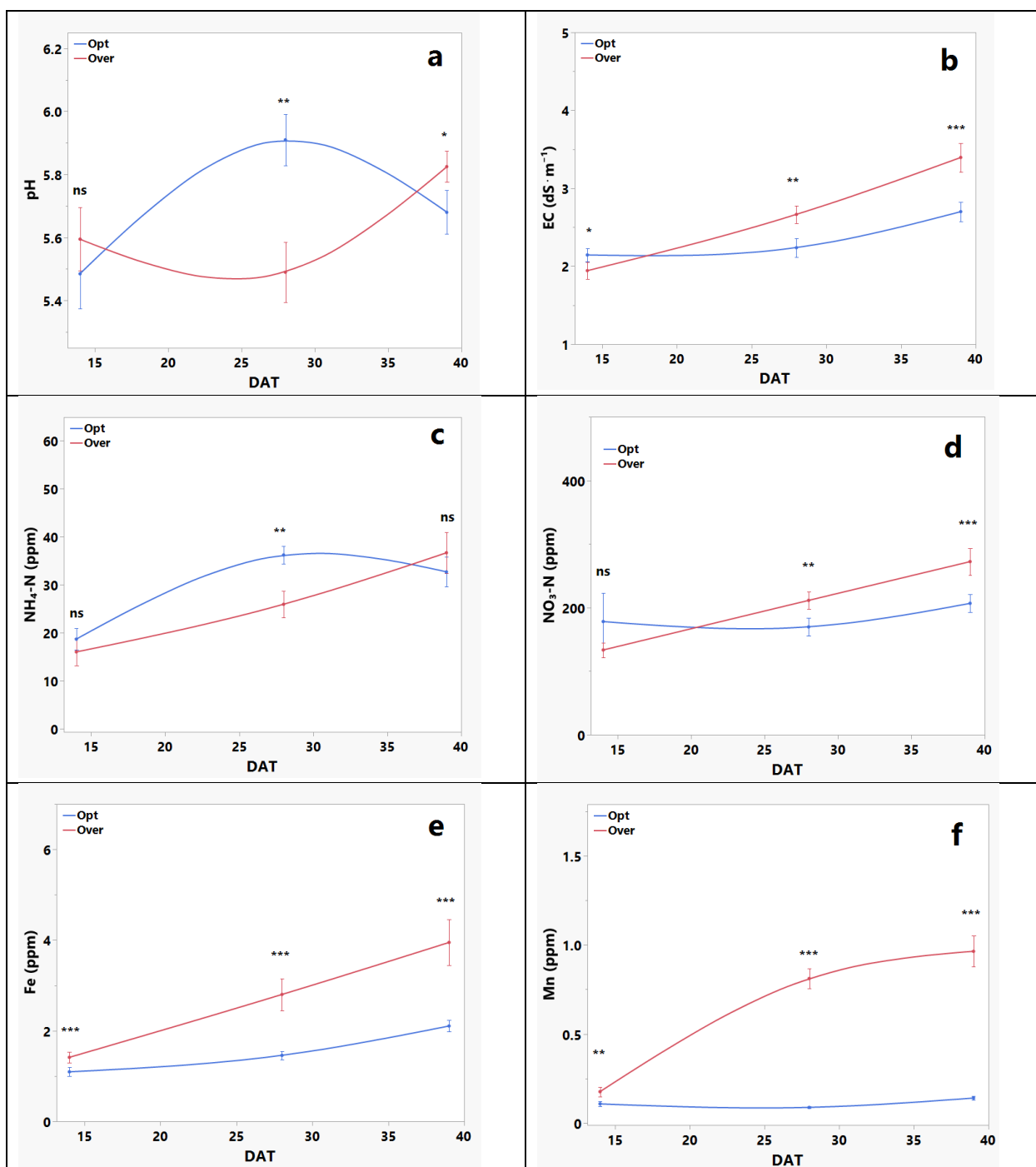


Figure 2.6 Solution graphs by Water from Expt. 1 for pH (a), EC (b), Ammonium (c), Nitrate (d), Mn (e), and Fe (f)

Figure 6. Solution graphs by Water from Expt. 1 for pH (a), EC (b), Ammonium (c), Nitrate (d), Mn (e), and Fe (f)

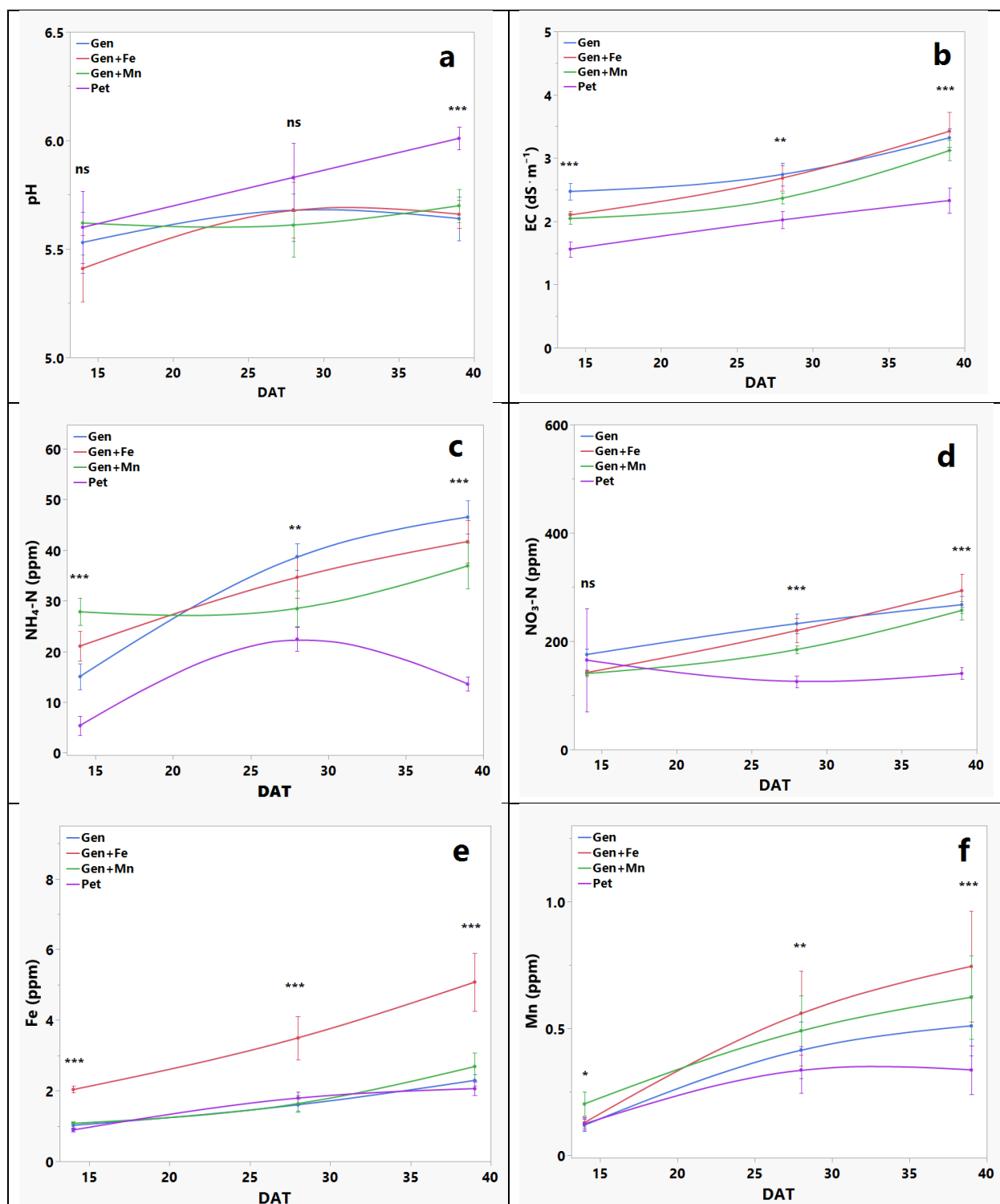


Figure 2.7 Solution graphs by Fertilizer from Expt. 1 for pH (a), EC (b), Ammonium (c), Nitrate (d), Mn (e), and Fe (f)

Figure 7. Solution graphs by Fertilizer from Expt. 1 for pH (a), EC (b), Ammonium (c), Nitrate (d), Mn (e), and Fe (f)

References

Chapter 1

- 5.9B: Nitrate Reduction and Denitrification. 2017. Biol. Libr.
[https://bio.libretexts.org/Bookshelves/Microbiology/Microbiology_\(Boundless\)/05%3A_Microbial_Metabolism/5.09%3A_Anaerobic_Respiration/5.9B%3A_Nitrate_Reduction_and_Denitrification](https://bio.libretexts.org/Bookshelves/Microbiology/Microbiology_(Boundless)/05%3A_Microbial_Metabolism/5.09%3A_Anaerobic_Respiration/5.9B%3A_Nitrate_Reduction_and_Denitrification). [accessed 25 June 2026].
- Albano JP, Miller WB. 2001. Photodegradation of FeDTPA in Nutrient Solutions. I. Effects of Irradiance, Wavelength, and Temperature. *HortScience*. 36(2):313–316.
<https://doi.org/10.21273/HORTSCI.36.2.313>.
- Alejandro S, Höller S, Meier B, Peiter E. 2020. Manganese in Plants: From Acquisition to Subcellular Allocation. *Front Plant Sci*. 11. <https://doi.org/10.3389/fpls.2020.00300>.
- Alem P, Thomas PA, Van Iersel MW. 2015. Substrate Water Content and Fertilizer Rate Affect Growth and Flowering of Potted Petunia. *HortScience*. 50(4):582–589.
<https://doi.org/10.21273/HORTSCI.50.4.582>.
- Alpkem Corporation. 1986a. RFA Methodology no. A303-S021. Ammonia Nitrogen.
- Alpkem Corporation. 1986b. RFA Methodology no. A303-S170. Nitrate+Nitrite.
- Basavaraj PS, Jangid KK, Babar R, Gangana Gowdra VM, Gangurde A, Shinde S, Tripathi K, Patil D, Boraiah KM, Rane J, Harisha CB, Halli H, Sammi Reddy K, Prabhakar M. 2024. Adventitious root formation confers waterlogging tolerance in cowpea (*Vigna unguiculata* (L.) Walp.). *Front Sustain Food Syst*. 8.
<https://doi.org/10.3389/fsufs.2024.1373183>.
- Bhat MA, Mishra AK, Shah SN, Bhat MA, Jan S, Rahman S, Baek K-H, Jan AT. 2024. Soil and Mineral Nutrients in Plant Health: A Prospective Study of Iron and Phosphorus in the Growth and Development of Plants. *Curr Issues Mol Biol*. 46(6):5194–5222.
<https://doi.org/10.3390/cimb46060312>.
- Cavins T, Williams KA. 2018. Root: Testing and Interpretation, p 119–135. *Water, Root Media, and Nutrient Management for Greenhouse Crops*. University of California, Agriculture and Natural Resources.
- Chung H, Lee Y-H. 2020. Hypoxia: A Double-Edged Sword During Fungal Pathogenesis? *Front Microbiol*. 11. <https://doi.org/10.3389/fmicb.2020.01920>.
- Crum D. 2011. Teach Your Staff To Avoid Overwatering. *Greenh. Grow*.
<https://www.greenhousegrower.com/production/plant-culture/teach-your-staff-to-avoid-overwatering/>. [accessed 17 Nov 2025].

- Devol AH. 2008. Denitrification Including Anammox, p 263–301. Nitrogen in the Marine Environment. Elsevier. <https://doi.org/10.1016/B978-0-12-372522-6.00006-2>.
- Evans RY, Hansen J, Dodge LL. 2009. Growth of rose roots and shoots is highly sensitive to anaerobic or hypoxic regions of container substrates. *Sci Hortic*. 119(3):286–291. <https://doi.org/10.1016/j.scienta.2008.07.033>.
- Gelderman RH, Beegle D. 1998. p 18–19. Recommended Chemical Soil Test Procedures for the North Central Region. Missouri Agricultural Experiment Station, Columbia, MO.
- Getter K. 2014. Have you thought about your greenhouse watering strategy lately? MSU Ext. https://www.canr.msu.edu/news/have_you_thought_about_your_greenhouse_watering_strategy_lately. [accessed 8 Oct 2025].
- Gibson JL, Pitchay DS, Williams-Rhodes AL, Whipker BE, Nelson PV, Dole JM. Nutrient Deficiencies in Bedding Plants: A Pictorial Guide for Identification and Correction.
- Gieseking JE, Snider HJ, Getz CA. 1935. Destruction of Organic Matter in Plant Material by the Use of Nitric and Perchloric Acids. *Ind Eng Chem Anal Ed*. 7(3):185–186. <https://doi.org/10.1021/ac50095a021>.
- González-Guzmán M, Gómez-Cadenas A, Arbona V. 2021. Absciscic Acid as an Emerging Modulator of the Responses of Plants to Low Oxygen Conditions. *Front Plant Sci*. 12. <https://doi.org/10.3389/fpls.2021.661789>.
- Habibi F, Liu T, Shahid MA, Schaffer B, Sarkhosh A. 2023. Physiological, biochemical, and molecular responses of fruit trees to root zone hypoxia. *Environ Exp Bot*. 206:105179. <https://doi.org/10.1016/j.envexpbot.2022.105179>.
- Hamrick D. 2003. Plant Nutrition, p 31. Ball Redbook. Ball Publishing, Batavia. Illinois.
- Horchani F, Aloui A, Brouquisse R, Aschi-Smiti S. 2008. Physiological Responses of Tomato Plants (*Solanum lycopersicum*) as Affected by Root Hypoxia. *J Agron Crop Sci*. 194(4):297–303. <https://doi.org/10.1111/j.1439-037X.2008.00313.x>.
- Jiménez J de la C, Mustroph A, Pedersen O, Weits DA, Schmidt-Schippers R. 2024. Flooding stress and responses to hypoxia in plants. *Funct Plant Biol*. 51(4). <https://doi.org/10.1071/FP24061>.
- Jonas VM, Williams KA. 2000. 644 Optimum Irrigation and Fertilization Regimes for Ivy Geranium (*Pelargonium peltatum*). :508D – 508. <https://doi.org/10.21273/HORTSCI.35.3.508D>.
- Khabaz-Saberi H, Setter TL, Waters I. 2006. Waterlogging Induces High to Toxic Concentrations of Iron, Aluminum, and Manganese in Wheat Varieties on Acidic Soil. *J Plant Nutr*. 29(5):899–911. <https://doi.org/10.1080/01904160600649161>.

- Lindner RC, Harley CP. 1942. A Rapid Method for the Determination of Nitrogen in Plant Tissue. *Science*. <https://doi.org/10.1126/science.96.2503.565>.
- McBride MB. 1994. *Environmental chemistry of soils*. New York : Oxford University Press.
- Ning X, Lin M, Huang G, Mao J, Gao Z, Wang X. 2023. Research progress on iron absorption, transport, and molecular regulation strategy in plants. *Front Plant Sci*. 14:1190768. <https://doi.org/10.3389/fpls.2023.1190768>.
- Norton J, Ouyang Y. 2019. Controls and Adaptive Management of Nitrification in Agricultural Soils. *Front Microbiol*. 10:1931. <https://doi.org/10.3389/fmicb.2019.01931>.
- Ozores-Hampton M. 2013. Effective Strategies to Correct Iron Deficiency in Florida Vegetable Crops. *HortTechnology*. 23(5):548–552. <https://doi.org/10.21273/HORTTECH.23.5.548>.
- Petunia FeED 20-6-22. JR Peters. <https://www.jrpeters.com/online-store/Petunia-FeED-20-6-22-p184835392>. [accessed 17 June 2026].
- Poesch GH. 1937. *Greenhouse potted plants*. Ohio Agricultural Experiment Station, Wooster Ohio.
- Rabenhorst MC. 2018. A System for Making and Deploying Oxide-Coated Plastic Films for Environmental Assessment of Soils. *Soil Sci Soc Am J*. 82(5):1301–1307. <https://doi.org/10.2136/sssaj2018.05.0178>.
- Rabenhorst MC, Drohan PJ, Galbraith JM, Moorberg C, Spokas L, Stolt MH, Thompson JA, Turk J, Vasilas BL, Vaughan KL. 2021. Manganese-coated IRIS to document reducing soil conditions. *Soil Sci Soc Am J*. 85(6):2201–2209. <https://doi.org/10.1002/saj2.20301>.
- Rankenberg T, Geldhof B, van Veen H, Holsteens K, Van de Poel B, Sasidharan R. 2021. Age-Dependent Abiotic Stress Resilience in Plants. *Trends Plant Sci*. 26(7):692–705. <https://doi.org/10.1016/j.tplants.2020.12.016>.
- Rebi A, Ejaz I, Khatana MA, Alvi ABA, Irfan M, Wang G, Gang YY, Wang L, Meng Y, Ghazanfar S, Zhou J. 2024. Effect of irrigation levels on the physiological responses of petunia cultivars for selection. *Ecol Front*. 44(1):206–216. <https://doi.org/10.1016/j.chnaes.2023.12.001>.
- Robe K, Gao F, Bonillo P, Tissot N, Gaymard F, Fourcroy P, Izquierdo E, Dubos C. 2020. Sulphur availability modulates *Arabidopsis thaliana* responses to iron deficiency. *PLOS ONE*. 15(8):e0237998. <https://doi.org/10.1371/journal.pone.0237998>.
- Runkle E, Fisher P. 2014. Greening Up Calibrachoa & Petunia. *Greenh. Prod. News*. 42.
- Schmidt SB, Husted S. 2019. The Biochemical Properties of Manganese in Plants. *Plants*. 8(10):381. <https://doi.org/10.3390/plants8100381>.

- Sharma S, Sharma J, Soni V, Kalaji HM, Elsheery NI. 2021. Waterlogging tolerance: A review on regulative morpho-physiological homeostasis of crop plants. *J Water Land Dev.* :16–28. <https://doi.org/10.24425/jwld.2021.137092>.
- Smith BR, Fisher PaulR, Argo WR. 2004. Nutrient Uptake in Container-grown Impatiens and Petunia in Response to Root Substrate pH and Applied Micronutrient Concentration. *HortScience.* 39(6):1426–1431. <https://doi.org/10.21273/HORTSCI.39.6.1426>.
- Šola I, Stić P, Rusak G. 2021. Effect of flooding and drought on the content of phenolics, sugars, photosynthetic pigments and vitamin C, and antioxidant potential of young Chinese cabbage. *Eur Food Res Technol.* 247(8):1913–1920. <https://doi.org/10.1007/s00217-021-03759-1>.
- Spiller S, Terry N. 1980. Limiting Factors in Photosynthesis: II. IRON STRESS DIMINISHES PHOTOCHEMICAL CAPACITY BY REDUCING THE NUMBER OF PHOTOSYNTHETIC UNITS 1 2. *Plant Physiol.* 65(1):121–125. <https://doi.org/10.1104/pp.65.1.121>.
- Steffens B, Rasmussen A. 2016. The Physiology of Adventitious Roots. *Plant Physiol.* 170(2):603–617. <https://doi.org/10.1104/pp.15.01360>.
- Terry N. 1980. Limiting Factors in Photosynthesis: I. USE OF IRON STRESS TO CONTROL PHOTOCHEMICAL CAPACITY IN VIVO 1. *Plant Physiol.* 65(1):114–120. <https://doi.org/10.1104/pp.65.1.114>.
- Therby-Vale R, Lacombe B, Rhee SY, Nussaume L, Rouached H. 2022. Mineral nutrient signaling controls photosynthesis: focus on iron deficiency-induced chlorosis. *Trends Plant Sci.* 27(5):502–509. <https://doi.org/10.1016/j.tplants.2021.11.005>.
- Thimijan RW, Heins RD. 1983. Photometric, Radiometric, and Quantum Light Units of Measure: A Review of Procedures for Interconversion. *HortScience.* 18(6):818–822. <https://doi.org/10.21273/HORTSCI.18.6.818>.
- Thomas RL, Sheard RW, Moyer JR. 1967. Comparison of Conventional and Automated Procedures for Nitrogen, Phosphorus, and Potassium Analysis of Plant Material Using a Single Digestion1. <https://doi.org/10.2134/agronj1967.00021962005900030010x>.
- Veazie P, Whipker BE. 2022. The Irony of Overwatering Your Plants. *Greenh. Grow.* www.e-grow.org.11.36 e-GRO Alert Iron Watering.pptx. [accessed 16 June 2026].
- Vepraskas MJ, Craft CB. 2016. Wetland soils: genesis, hydrology, landscapes, and classification, Second edition. Taylor & Francis, Boca Raton, FL.
- Williams KA, Scoggins H. 2018. Formulating Fertilization Programs, p 148–149. *Water, Root Media, and Nutrient Management for Greenhouse Crops.* University of California, Agriculture and Natural Resources.

- Xie R, Zheng L, Jiao Y, Huang X. 2021. Understanding physiological and molecular mechanisms of citrus rootstock seedlings in response to root zone hypoxia by RNA-Seq. *Environ Exp Bot*. 192:104647. <https://doi.org/10.1016/j.envexpbot.2021.104647>.
- Yafuso EJ, Fisher PR. 2017. Oxygenation of Irrigation Water during Propagation and Container Production of Bedding Plants. *HortScience*. 52(11):1608–1614. <https://doi.org/10.21273/HORTSCI12181-17>.
- Zhang Y, Chen X, Geng S, Zhang X. 2025. A review of soil waterlogging impacts, mechanisms, and adaptive strategies. *Front Plant Sci*. 16. <https://doi.org/10.3389/fpls.2025.1545912>.
- Zhu X, Li X, Jiu S, Zhang K, Wang C, Fang J. 2018. Analysis of the regulation networks in grapevine reveals response to waterlogging stress and candidate gene-marker selection for damage severity. *R Soc Open Sci*. 5(6):172253. <https://doi.org/10.1098/rsos.172253>.

Chapter 2

- 5.9B: Nitrate Reduction and Denitrification. 2017. Biol. Libr. [https://bio.libretexts.org/Bookshelves/Microbiology/Microbiology_\(Boundless\)/05%3A_Microbial_Metabolism/5.09%3A_Anaerobic_Respiration/5.9B%3A_Nitrate_Reduction_and_Denitrification](https://bio.libretexts.org/Bookshelves/Microbiology/Microbiology_(Boundless)/05%3A_Microbial_Metabolism/5.09%3A_Anaerobic_Respiration/5.9B%3A_Nitrate_Reduction_and_Denitrification). [accessed 25 June 2026].
- Albano JP, Miller WB. 2001. Photodegradation of FeDTPA in Nutrient Solutions. I. Effects of Irradiance, Wavelength, and Temperature. *HortScience*. 36(2):313–316. <https://doi.org/10.21273/HORTSCI.36.2.313>.
- Alejandro S, Höller S, Meier B, Peiter E. 2020. Manganese in Plants: From Acquisition to Subcellular Allocation. *Front Plant Sci*. 11. <https://doi.org/10.3389/fpls.2020.00300>.
- Alem P, Thomas PA, Van Iersel MW. 2015. Substrate Water Content and Fertilizer Rate Affect Growth and Flowering of Potted Petunia. *HortScience*. 50(4):582–589. <https://doi.org/10.21273/HORTSCI.50.4.582>.
- Alpkem Corporation. 1986a. RFA Methodology no. A303-S021. Ammonia Nitrogen.
- Alpkem Corporation. 1986b. RFA Methodology no. A303-S170. Nitrate+Nitrite.
- Basavaraj PS, Jangid KK, Babar R, Gangana Gowdra VM, Gangurde A, Shinde S, Tripathi K, Patil D, Boraiah KM, Rane J, Harisha CB, Halli H, Sammi Reddy K, Prabhakar M. 2024. Adventitious root formation confers waterlogging tolerance in cowpea (*Vigna unguiculata* (L.) Walp.). *Front Sustain Food Syst*. 8. <https://doi.org/10.3389/fsufs.2024.1373183>.
- Bhat MA, Mishra AK, Shah SN, Bhat MA, Jan S, Rahman S, Baek K-H, Jan AT. 2024. Soil and Mineral Nutrients in Plant Health: A Prospective Study of Iron and Phosphorus in the Growth and Development of Plants. *Curr Issues Mol Biol*. 46(6):5194–5222. <https://doi.org/10.3390/cimb46060312>.

- Cavins T, Williams KA. 2018. Root: Testing and Interpretation, p 119–135. Water, Root Media, and Nutrient Management for Greenhouse Crops. University of California, Agriculture and Natural Resources.
- Chung H, Lee Y-H. 2020. Hypoxia: A Double-Edged Sword During Fungal Pathogenesis? *Front Microbiol.* 11. <https://doi.org/10.3389/fmicb.2020.01920>.
- Crum D. 2011. Teach Your Staff To Avoid Overwatering. *Greenh. Grow.* <https://www.greenhousegrower.com/production/plant-culture/teach-your-staff-to-avoid-overwatering/>. [accessed 17 Nov 2025].
- Devol AH. 2008. Denitrification Including Anammox, p 263–301. Nitrogen in the Marine Environment. Elsevier. <https://doi.org/10.1016/B978-0-12-372522-6.00006-2>.
- Evans RY, Hansen J, Dodge LL. 2009. Growth of rose roots and shoots is highly sensitive to anaerobic or hypoxic regions of container substrates. *Sci Hortic.* 119(3):286–291. <https://doi.org/10.1016/j.scienta.2008.07.033>.
- Gelderman RH, Beegle D. 1998. p 18–19. Recommended Chemical Soil Test Procedures for the North Central Region. Missouri Agricultural Experiment Station, Columbia, MO.
- Getter K. 2014. Have you thought about your greenhouse watering strategy lately? *MSU Ext.* https://www.canr.msu.edu/news/have_you_thought_about_your_greenhouse_watering_strategy_lately. [accessed 8 Oct 2025].
- Gibson JL, Pitchay DS, Williams-Rhodes AL, Whipker BE, Nelson PV, Dole JM. Nutrient Deficiencies in Bedding Plants: A Pictorial Guide for Identification and Correction.
- Gieseking JE, Snider HJ, Getz CA. 1935. Destruction of Organic Matter in Plant Material by the Use of Nitric and Perchloric Acids. *Ind Eng Chem Anal Ed.* 7(3):185–186. <https://doi.org/10.1021/ac50095a021>.
- González-Guzmán M, Gómez-Cadenas A, Arbona V. 2021. Absciscic Acid as an Emerging Modulator of the Responses of Plants to Low Oxygen Conditions. *Front Plant Sci.* 12. <https://doi.org/10.3389/fpls.2021.661789>.
- Habibi F, Liu T, Shahid MA, Schaffer B, Sarkhosh A. 2023. Physiological, biochemical, and molecular responses of fruit trees to root zone hypoxia. *Environ Exp Bot.* 206:105179. <https://doi.org/10.1016/j.envexpbot.2022.105179>.
- Hamrick D. 2003. Plant Nutrition, p 31. Ball Redbook. Ball Publishing, Batavia. Illinois.
- Horchani F, Aloui A, Brouquisse R, Aschi-Smiti S. 2008. Physiological Responses of Tomato Plants (*Solanum lycopersicum*) as Affected by Root Hypoxia. *J Agron Crop Sci.* 194(4):297–303. <https://doi.org/10.1111/j.1439-037X.2008.00313.x>.

- Jiménez J de la C, Mustroph A, Pedersen O, Weits DA, Schmidt-Schippers R. 2024. Flooding stress and responses to hypoxia in plants. *Funct Plant Biol.* 51(4). <https://doi.org/10.1071/FP24061>.
- Jonas VM, Williams KA. 2000. 644 Optimum Irrigation and Fertilization Regimes for Ivy Geranium (*Pelargonium peltatum*). :508D – 508. <https://doi.org/10.21273/HORTSCI.35.3.508D>.
- Khabaz-Saberi H, Setter TL, Waters I. 2006. Waterlogging Induces High to Toxic Concentrations of Iron, Aluminum, and Manganese in Wheat Varieties on Acidic Soil. *J Plant Nutr.* 29(5):899–911. <https://doi.org/10.1080/01904160600649161>.
- Lindner RC, Harley CP. 1942. A Rapid Method for the Determination of Nitrogen in Plant Tissue. *Science.* <https://doi.org/10.1126/science.96.2503.565>.
- McBride MB. 1994. Environmental chemistry of soils. New York : Oxford University Press.
- Ning X, Lin M, Huang G, Mao J, Gao Z, Wang X. 2023. Research progress on iron absorption, transport, and molecular regulation strategy in plants. *Front Plant Sci.* 14:1190768. <https://doi.org/10.3389/fpls.2023.1190768>.
- Norton J, Ouyang Y. 2019. Controls and Adaptive Management of Nitrification in Agricultural Soils. *Front Microbiol.* 10:1931. <https://doi.org/10.3389/fmicb.2019.01931>.
- Ozores-Hampton M. 2013. Effective Strategies to Correct Iron Deficiency in Florida Vegetable Crops. *HortTechnology.* 23(5):548–552. <https://doi.org/10.21273/HORTTECH.23.5.548>.
- Petunia FeED 20-6-22. JR Peters. <https://www.jrpeters.com/online-store/Petunia-FeED-20-6-22-p184835392>. [accessed 17 June 2026].
- Poesch GH. 1937. Greenhouse potted plants. Ohio Agricultural Experiment Station, Wooster Ohio.
- Rabenhorst MC. 2018. A System for Making and Deploying Oxide-Coated Plastic Films for Environmental Assessment of Soils. *Soil Sci Soc Am J.* 82(5):1301–1307. <https://doi.org/10.2136/sssaj2018.05.0178>.
- Rabenhorst MC, Drohan PJ, Galbraith JM, Moorberg C, Spokas L, Stolt MH, Thompson JA, Turk J, Vasilas BL, Vaughan KL. 2021. Manganese-coated IRIS to document reducing soil conditions. *Soil Sci Soc Am J.* 85(6):2201–2209. <https://doi.org/10.1002/saj2.20301>.
- Rankenberg T, Geldhof B, van Veen H, Holsteens K, Van de Poel B, Sasidharan R. 2021. Age-Dependent Abiotic Stress Resilience in Plants. *Trends Plant Sci.* 26(7):692–705. <https://doi.org/10.1016/j.tplants.2020.12.016>.
- Rebi A, Ejaz I, Khatana MA, Alvi ABA, Irfan M, Wang G, Gang YY, Wang L, Meng Y, Ghazanfar S, Zhou J. 2024. Effect of irrigation levels on the physiological responses of

- petunia cultivars for selection. *Ecol Front.* 44(1):206–216.
<https://doi.org/10.1016/j.chnaes.2023.12.001>.
- Robe K, Gao F, Bonillo P, Tissot N, Gaymard F, Fourcroy P, Izquierdo E, Dubos C. 2020. Sulphur availability modulates *Arabidopsis thaliana* responses to iron deficiency. *PLOS ONE.* 15(8):e0237998. <https://doi.org/10.1371/journal.pone.0237998>.
- Runkle E, Fisher P. 2014. Greening Up Calibrachoa & Petunia. *Greenh. Prod. News.* 42.
- Schmidt SB, Husted S. 2019. The Biochemical Properties of Manganese in Plants. *Plants.* 8(10):381. <https://doi.org/10.3390/plants8100381>.
- Sharma S, Sharma J, Soni V, Kalaji HM, Elsheery NI. 2021. Waterlogging tolerance: A review on regulative morpho-physiological homeostasis of crop plants. *J Water Land Dev.* :16–28. <https://doi.org/10.24425/jwld.2021.137092>.
- Smith BR, Fisher PaulR, Argo WR. 2004. Nutrient Uptake in Container-grown Impatiens and Petunia in Response to Root Substrate pH and Applied Micronutrient Concentration. *HortScience.* 39(6):1426–1431. <https://doi.org/10.21273/HORTSCI.39.6.1426>.
- Šola I, Stić P, Rusak G. 2021. Effect of flooding and drought on the content of phenolics, sugars, photosynthetic pigments and vitamin C, and antioxidant potential of young Chinese cabbage. *Eur Food Res Technol.* 247(8):1913–1920. <https://doi.org/10.1007/s00217-021-03759-1>.
- Spiller S, Terry N. 1980. Limiting Factors in Photosynthesis: II. IRON STRESS DIMINISHES PHOTOCHEMICAL CAPACITY BY REDUCING THE NUMBER OF PHOTOSYNTHETIC UNITS 1 2. *Plant Physiol.* 65(1):121–125.
<https://doi.org/10.1104/pp.65.1.121>.
- Steffens B, Rasmussen A. 2016. The Physiology of Adventitious Roots. *Plant Physiol.* 170(2):603–617. <https://doi.org/10.1104/pp.15.01360>.
- Terry N. 1980. Limiting Factors in Photosynthesis: I. USE OF IRON STRESS TO CONTROL PHOTOCHEMICAL CAPACITY IN VIVO 1. *Plant Physiol.* 65(1):114–120.
<https://doi.org/10.1104/pp.65.1.114>.
- Therby-Vale R, Lacombe B, Rhee SY, Nussaume L, Rouached H. 2022. Mineral nutrient signaling controls photosynthesis: focus on iron deficiency-induced chlorosis. *Trends Plant Sci.* 27(5):502–509. <https://doi.org/10.1016/j.tplants.2021.11.005>.
- Thimijan RW, Heins RD. 1983. Photometric, Radiometric, and Quantum Light Units of Measure: A Review of Procedures for Interconversion. *HortScience.* 18(6):818–822.
<https://doi.org/10.21273/HORTSCI.18.6.818>.
- Thomas RL, Sheard RW, Moyer JR. 1967. Comparison of Conventional and Automated Procedures for Nitrogen, Phosphorus, and Potassium Analysis of Plant Material Using a Single Digestion1. <https://doi.org/10.2134/agronj1967.00021962005900030010x>.

- Veazie P, Whipker BE. 2022. The Irony of Overwatering Your Plants. Greenh. Grow. www.e-grow.org.11.36 e-GRO Alert Iron Watering.pptx. [accessed 16 June 2026].
- Vepraskas MJ, Craft CB. 2016. Wetland soils: genesis, hydrology, landscapes, and classification, Second edition. Taylor & Francis, Boca Raton, FL.
- Williams KA, Scoggins H. 2018. Formulating Fertilization Programs, p 148–149. Water, Root Media, and Nutrient Management for Greenhouse Crops. University of California, Agriculture and Natural Resources.
- Xie R, Zheng L, Jiao Y, Huang X. 2021. Understanding physiological and molecular mechanisms of citrus rootstock seedlings in response to root zone hypoxia by RNA-Seq. *Environ Exp Bot.* 192:104647. <https://doi.org/10.1016/j.envexpbot.2021.104647>.
- Yafuso EJ, Fisher PR. 2017. Oxygenation of Irrigation Water during Propagation and Container Production of Bedding Plants. *HortScience.* 52(11):1608–1614. <https://doi.org/10.21273/HORTSCI12181-17>.
- Zhang Y, Chen X, Geng S, Zhang X. 2025. A review of soil waterlogging impacts, mechanisms, and adaptive strategies. *Front Plant Sci.* 16. <https://doi.org/10.3389/fpls.2025.1545912>.
- Zhu X, Li X, Jiu S, Zhang K, Wang C, Fang J. 2018. Analysis of the regulation networks in grapevine reveals response to waterlogging stress and candidate gene-marker selection for damage severity. *R Soc Open Sci.* 5(6):172253. <https://doi.org/10.1098/rsos.172253>.

Appendix A - Petunia Cultivar Study

Appendix Figure A.1 Tissue nutrient analysis for SS, BM, and PH in Expt. 1

Treatment	Total N (%)	Total C (%)	P (%)	K (%)	Ca (%)	Mg (%)	SO4-S (%)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
Cultivar*Irrigation	<.0001	<.0001	0.0459	<.0001	<.0001	<.0001	0.0033	ns	ns	<.0001	0.0006
Cultivar*Fertilizer	ns	ns	0.0135	ns	0.0355	ns	ns	ns	0.0197	ns	ns
Cultivar*Irrigation*Fertilizer	ns	ns	0.0351	ns	ns	ns	ns	ns	ns	ns	ns
Cultivar											
SS2	6.03b	43.20a	ns	3.74b	0.800c	0.544c	0.412b	9.0a	124.7a	86.0b	45.8a
BM	6.40a	40.48b	ns	4.06b	1.765a	0.595b	0.405b	7.3b	102.4b	114.9a	34.6b
Ph	5.74b	40.91b	ns	4.39a	1.296b	0.651a	0.480a	6.0c	112.7ab	90.0b	48.4a
Prob > F	<.0001	<.0001	ns	<.0001	<.0001	<.0001	<.0001	<.0001	0.0009	<.0001	<.0001
Irrigation											
Over	5.58b	43.04a	0.431b	3.86b	0.877b	0.417b	0.457a	ns	119.3a	73.5b	50.7a
Opt	6.54a	40.02b	0.492a	4.27a	1.697a	0.777a	0.408b	ns	107.2b	120.4a	35.2b
Prob > F	<.0001	<.0001	0.0003	0.0006	<.0001	<.0001	0.0002	ns	0.0098	<.0001	<.0001
Fertilizer											
General	6.35a	ns	0.550a	4.28a	ns	0.530b	ns	7.8a	ns	114.7a	47.9a
P-feed	5.76b	ns	0.374b	3.85b	ns	0.663a	ns	7.0b	ns	79.2b	38.0b
Prob > F	<.0001	ns	<.0001	0.0003	ns	<.0001	ns	0.047	ns	<.0001	<.0001
Irrigation*Fertilizer											
Opt,Gen	ns	ns	0.596a	ns	ns	0.657b	ns	ns	ns	149.0a	ns
Opt,Pet	ns	ns	0.388c	ns	ns	0.897a	ns	ns	ns	91.8b	ns
Over,Pet	ns	ns	0.359c	ns	ns	0.430c	ns	ns	ns	66.63c	ns
Over,Gen	ns	ns	0.504b	ns	ns	0.404c	ns	ns	ns	80.45b	ns
Prob > F	ns	ns	0.0488	ns	ns	<.0001	ns	ns	ns	<.0001	ns

Appendix B - IRIS Film Study

Appendix Figure B.1. Macros for IRIS Film Analyses

Mn IRIS Removal Macro Expt. 1

```
run("Set Scale...", "distance=0.7808 known=1 unit=mm global");
run("8-bit");
run("Duplicate...", " ");
makeRectangle(0, 0, 595, 198);
run("Crop");
setThreshold(193, 255, "raw");
run("Convert to Mask");
run("Measure");
close;
run("Duplicate...", " ");
makeRectangle(0, 198, 595, 396);
run("Crop");
setThreshold(193, 255, "raw");
run("Convert to Mask");
run("Measure");
close;
run("Duplicate...", " ");
makeRectangle(0, 396, 595, 594);
run("Crop");
setThreshold(193, 255, "raw");
run("Convert to Mask");
run("Measure");
close;
run("Duplicate...", " ");
makeRectangle(0, 594, 595, 792);
run("Crop");
setThreshold(193, 255, "raw");
```

```

run("Convert to Mask");
run("Measure");
close;
run("Duplicate...", " ");
makeRectangle(0, 792, 595, 990);
run("Crop");
setThreshold(193, 255, "raw");
run("Convert to Mask");
run("Measure");
close;
setThreshold(193, 255, "raw");
run("Convert to Mask");
run("Measure");
close;

```

Fe IRIS Removal Macro 2025 Expt. 1

```

run("Set Scale...", "distance=0.7808 known=1 unit=mm global");
run("8-bit");
run("Duplicate...", " ");
makeRectangle(0, 0, 595, 198);
run("Crop");
setThreshold(205, 255, "raw");
run("Convert to Mask");
run("Measure");
close;
run("Duplicate...", " ");
makeRectangle(0, 198, 595, 396);
run("Crop");
setThreshold(205, 255, "raw");
run("Convert to Mask");
run("Measure");
close;

```

```

run("Duplicate...", " ");
makeRectangle(0, 396, 595, 594);
run("Crop");
setThreshold(205, 255, "raw");
run("Convert to Mask");
run("Measure");
close;
run("Duplicate...", " ");
makeRectangle(0, 594, 595, 792);
run("Crop");
setThreshold(205, 255, "raw");
run("Convert to Mask");
run("Measure");
close;
run("Duplicate...", " ");
makeRectangle(0, 792, 595, 990);
run("Crop");
setThreshold(205, 255, "raw");
run("Convert to Mask");
run("Measure");
close;
setThreshold(205, 255, "raw");
run("Convert to Mask");
run("Measure");
close;

```

Mn IRIS Removal Macro 2026 Expts. 2 & 3

```

run("8-bit");
run("Duplicate...", " ");
makeRectangle(0, 0, 595, 250.5);
run("Crop");
setThreshold(193, 255, "raw");

```

```

run("Convert to Mask");
run("Measure");
close;
run("Duplicate...", " ");
makeRectangle(0, 250.5, 595, 501);
run("Crop");
setThreshold(193, 255, "raw");
run("Convert to Mask");
run("Measure");
close;
setThreshold(193, 255, "raw");
run("Convert to Mask");
run("Measure");
close;

```

Fe IRIS Removal Macro 2026 Expts. 2 & 3

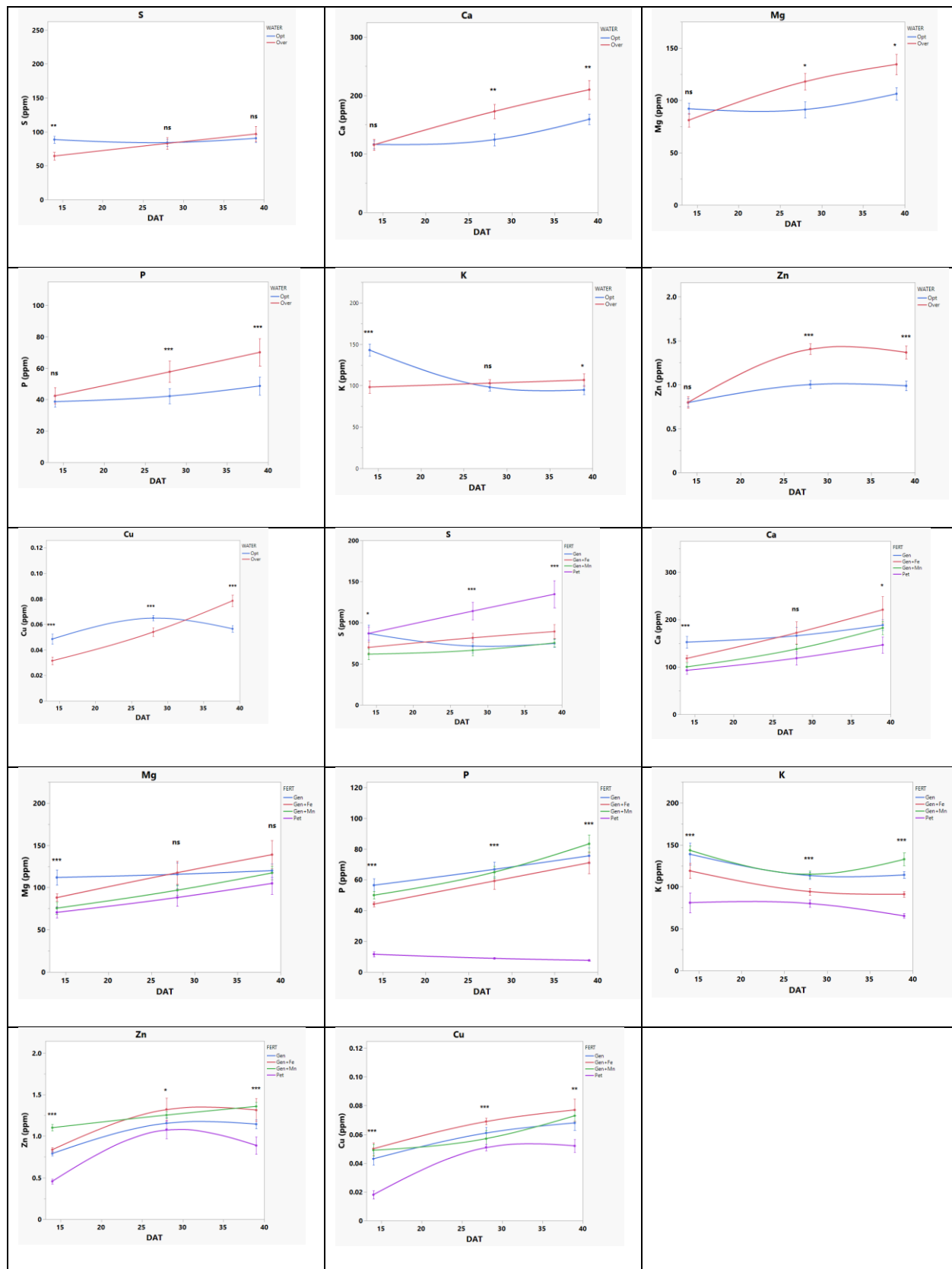
```

run("8-bit");
run("Duplicate...", " ");
makeRectangle(0, 0, 595, 250.5);
run("Crop");
setThreshold(205, 255, "raw");
run("Convert to Mask");
run("Measure");
close;
run("Duplicate...", " ");
makeRectangle(0, 250.5, 595, 501);
run("Crop");
setThreshold(205, 255, "raw");
run("Convert to Mask");
run("Measure");
close;
setThreshold(205, 255, "raw");

```

```
run("Convert to Mask");  
run("Measure");  
close;
```

Appendix Figure B.2 Additional solution graphs for Water and by Fert for Expt. 1



Appendix Figure B.3 Berger BM6 Substrate Nutrient Analysis Report 2026

Attached

Appendix Figure B.4 Control Fertilizer Salts Sources and Amounts

KH_2PO_4 – Fisher Scientific Company, Fair Lawn, NJ

- 230 g/16L at 100X

KNO_3 - Fisher Scientific Company, Fair Lawn, NJ

- 366g/16L at 100X

NH_4NO_3 – Fisher Scientific Company, Fair Lawn, NJ

- 581g/16L at 100X

MgSO_4 – Fisher Scientific Company, Fair Lawn, NJ

- 9g/16L at 100X