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Growth and Pigment Content of Container-grown Impatiens and Petunia in Relation to Root Substrate pH and Applied Micronutrient Concentration

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Abstract. **The objective was to quantify the effect of substrate pH and micronutrient concentration on growth and pigment content for two floricultural crop species, *Petunia ×hybrida* 'Priscilla' and *Impatiens wallerana* 'Rosebud Purple Magic'. A 70% peat : 30% perlite medium was amended with dolomitic hydrated lime to achieve five substrate pH's ranging from pH 4.4 to 7.0. Plants were grown in 10-cm-diameter pots in a greenhouse for 4 weeks, and irrigated with a fertilizer containing (in mg·L^{-1}) 210N–31P–235K–200Ca–49Mg. Micronutrients were applied using an EDTA (ethylenedinitrilotetraacetic acid) chelated micronutrient blend (C111), at 1×, 2×, and 4× concentrations (in mg·L^{-1}) of 0.50Fe–0.25Mn–0.025Zn–0.04Cu–0.075B–0.01Mo. Petunia shoot dry mass and stem caliper decreased as substrate pH increased, whereas leaf length and width remained unchanged. The highest level of C111 resulted in higher dry mass and smaller leaf area compared with other C111 levels. Overall, substrate pH and C111 had little effect on plant size or mass for impatiens. For both species, increasing substrate pH above 5.3 resulted in a decline in chlorophyll, carotenoids, and the SPAD chlorophyll index (measured with a Minolta-502 SPAD meter) compared with the lowest three pH levels. Chlorosis was observed at pH 7 after 2 weeks of growth. Increasing C111 concentration had no effect on pigment content below pH 5.3, but increased pigment content at higher pH levels. The SPAD index was highly correlated with chlorophyll content. This research emphasizes that an acceptable range in substrate pH can vary depending on fertilizer practices, with higher micronutrient concentration compensating for lower solubility at high substrate pH.**

A common recommended pH range for most floriculture crops grown in a media without soil is 5.6 to 6.2 (Bailey and Nelson, 1998). Chlorosis and necrosis are likely to occur if plants are grown outside of the acceptable pH range because pH affects micronutrient solubility. Necrosis caused by Fe and Mn toxicity is a common problem when Fe-efficient plants such as *Tagetes erecta* L. and *Pelargonium×hortorum* are grown in media with a low pH (high Fe and Mn solubility) (Albano and Miller, 1998; Hulme and Ferry, 1999). In contrast, if the pH of the growing medium is excessively high, plants can display micronutrient deficiency symptoms due to limited micronutrient solubility (Fisher et al., 2003; Marschner, 1995; Nelson, 1994). Iron deficiency is most prevalent in Fe-inefficient species including *Petunia ×hybrida* Hort. Vilm.-Andr. or *Calibrachoa ×hybrida* Cerv. (Fisher et al., 2003; Nelson, 1994), but can be induced in other plants if Fe supply is sufficiently limited.

When foliar content of Fe is below critical levels, changes in pigment concentration and composition can occur, resulting in a reduction in chlorophyll content in young leaves and a disruption of the photosynthetic apparatus (Abadia, 1992; Abadia et al., 1991; Marschner, 1995; Terry and Abadia, 1986). Iron deficiency tends to have a greater effect on chlorophylls than it does on carotenoids (Abadia, 1992). The loss of chlorophyll resulting from Fe deficiency, and the corresponding decrease in light-harvesting capability eventually results in a loss of plant vigor, reduced growth rates, and can delay flower development (Marschner, 1995; Pushnik and Miller, 1989).

Chlorosis induced by Fe deficiency at high pH can be alleviated by increasing the applied concentration of micronutrients (Bailey and Nelson, 1998; Fisher et al., 2003). In addition, substrate pH can be decreased to a level where micronutrient solubility is greater using acidification of irrigation water, acid drenches, or use of ammonium-based fertilizer (Bailey, 1996; Bishko et al., 2003).

Micronutrient concentration can vary widely in commercial floriculture management. Our review of commercially available blended water-soluble greenhouse fertilizers [Champion Fertilizers, (SQM North America, Atlanta, Ga.), Greencare Fertilizers (GreenCare Fertilizers, Inc, Kankakee, Ill.), Masterblend Fertilizers (Masterblend International, Chicago, Ill.), Peter's Fertilizers and Excel Fertilizer (Scotts Co., Marysville, Ohio), Plantex Fertilizers (Plant Products Co., LTD., Brampton, Ont., Canada), Technigro Fertilizers, (Sun Gro Horticulture, Seba Beach, Alb., Canada)] used in North America found that median micronutrient concentrations were 0.5 mg·L^{-1} Fe (range: 0.1 to 6 mg·L^{-1}), 0.2 mg·L^{-1} Mn (range: <0.01 to 1), 0.05 mg·L^{-1} Cu (range: <0.01 to 0.5), 0.1 mg·L^{-1} Zn (range: <0.01 to 0.55 mg·L^{-1}), 0.05 mg·L^{-1} B (range: <0.01 to 1.0), and 0.03 mg·L^{-1} Mo (range: <0.001 to 0.5 mg·L^{-1}) for every 100 mg·L^{-1} N.

The recommended N application rate for floricultural crops can range from 50 to >400 mg·L^{-1}, depending on the species, stage of development, medium, and leaching rate (Biernbaum et al., 1992, Nelson, 1994). Because it is common for growers to use blended fertilizer that contain several of the essential nutrients in addition to N, the concentration of applied N will affect the concentration of other nutrients applied to a crop. For example, Fe concentration in the median blended water-soluble fertilizer would be 0.25, 0.5, 1.0, and 2.0 mg·L^{-1} Fe, respectively, at N concentrations of 50, 100, 200, and 400 mg·L^{-1} N.

We hypothesized that the critical pH for the appearance of micronutrient deficiencies and their subsequent effect on plant growth depends on the concentration of micronutrients applied to the crop. The species were selected because hybrid petunia (*Petunia ×hybrida* Hort. Vilm.-Andr.) has been found to be prone to Fe deficiency symptoms at high substrate pH (Argo and Fisher, 2002; Nelson, 1994), whereas Argo and Biernbaum (1996) found that growth of impatiens (*Impatiens wallerana* Hook. F.) was insensitive to a wide range in substrate pH levels.

We further aimed to evaluate the Minolta SPAD chlorophyll index meter (a portable, hand-held device that indirectly measures chlorophyll content of a leaf in a nondestructive manner) as a tool for quantifying the plant health response of floricultural species to substrate pH. SPAD values are determined by measuring the ratio of light transmitted through the leaf at a red wavelength (650 nm) and an infrared wavelength (940 nm). Peryea and Kammereck (1997) found that the SPAD meter could quantify the severity of leaf chlorosis in pear trees associated with Fe deficiency. Marschner (1995) noted that a decrease in chlorophyll concentration may occur in response to Fe deficiency before changes in leaf expansion or biomass can be detected.

Materials and methods

Design. Two species, *Petunia ×hybrida* 'Priscilla' and *Impatiens wallerana* 'Rosebud Purple Magic', were grown in a 70% peat : 30% perlite medium at five preplant lime incorporation rates, and irrigated using a complete water-soluble fertilizer that contained one of three different micronutrient concentrations

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in a factorial design (five lime rates × three micronutrient concentrations). The experiment was a randomized, complete-block design conducted on four benches in the same greenhouse compartment, with each bench representing a block. Combinations of micronutrient concentration and lime rate were randomized within each block, and each combination contained 10 replicate pots per block. Data were analyzed using analysis of variance (ANOVA) and Tukey's HSD mean comparison test in PROC GLM of SAS (SAS Institute, Cary, N.C.). In addition, we performed quadratic regression analysis to correlate SPAD index and chlorophyll content for both species.

Growing conditions. Rooted cuttings were planted in 450-cm^3 containers (10-cm standard round pots) spaced 13.5 cm apart on center. The experiment took place on four 1.2 × 4.6-m hard plastic benches in a 6.1 × 9.1-m glasshouse for 4 weeks. Greenhouse air and soil temperature was measured by a datalogger (model CR10X; Campbell Scientific Inc., Logan, Utah) using type-T thermocouples (Omega Engineering, Stamford, Conn.). The air sensor was aspirated and shielded, and mean daily air temperature (± standard deviation) was 21.0 °C ± 0.4. Soil temperature was 20.2 °C ± 1.0. When ambient PAR light fell below 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ between 06:30 and 17:30, we provided supplemental lighting with high-pressure sodium lamps at 46 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Growing medium. Both species were grown in a 70% (by volume) Canadian sphagnum peat (Fisons professional black bale peat, Sun Gro Horticulture, Bellevue, Wash.) and 30% super coarse perlite (Whittemore Co., Lawrence, Mass.). At mixing, 0.9 $kg \cdot m^{-3}$ of a 6N–4P–7.5K–10Ca–1Mg–0.3Fe–0.04Mn–0.02Zn–0.036Cu–0.049B–0.0097Mo preplant nutrient charge fertilizer (GreenCare Fertilizers, Inc., Chicago, Ill.) was added. Preplant nutrients were derived from KNO_3, $Ca(NO_3)_2$, triple superphosphate (19.8P), $CaSO_4$, $MgSO_4$, Fe_2O_3, MnO_2, CuO, H_3BO_3, and $(NH_4)_6Mo_7O_{24}$. Type N microfine dolomitic hydrated lime [63.2% $Ca(OH)_2$, 33.8% MgO, 0.8% SiO_2, 0.4% H_2O, 0.2% Al_2O_3, 0.1% Fe_2O_3, 0.03% S; 92% of which passed through a 45-µm screen (National Lime and Stone, Findlay, Ohio)] was incorporated at 1.1, 1.5, 1.9, 2.4, or 3.1 $kg \cdot m^{-3}$ to give initial substrate pH levels of (se ± 0.01) 4.5, 4.8, 5.3, 6.1 and 7.2, and with initial electroconductivity (EC) of (se ± 0.01) 1.1, 1.2, 1.2, 1.3, and 1.3 $dS \cdot m^{-1}$, respectively. A deionized water solution containing 1.4 $mL \cdot L^{-1}$ of a wetting agent (Psi Matric; Aquatrols, Cherry Hill, N.J.) was added to all media at 0.14 $L \cdot m^{-3}$, and the medium was allowed to equilibrate for 5 d before planting.

Fertilization. All plants were fertilized with the same macronutrient solution consisting of (in $mg \cdot L^{-1}$) 210N–31P–235K–200Ca–49Mg from 5 mm $Ca(NO_3)_2$, 5 mm KNO_3, 2 mm $MgSO_4$, and 1 mm KH_2PO_4, dissolved in deionized water. The three micronutrient levels were supplied by adding different amounts of Compound 111 (abbreviated to C111 in this text, Scotts Co., Marysville, Ohio) EDTA-chelated micronutrient complex [0.74% Mg ($MgSO_4$), 0.232% B (H_3BO_3), 0.1136% Cu (Cu-EDTA), 1.5% Fe (Fe-EDTA), 0.74% Mn (Mn-EDTA), 0.0242% Mo (Na_2MoO_4), 0.075% Zn (Zn-EDTA)]. C111 was added to the macronutrient solution at either 0.033, 0.067, or 0.134 $g \cdot L^{-1}$. Using Fe as an example, C111 at the three concentrations supplied 0.5, 1.0, or 2.0 $mg \cdot L^{-1}$ Fe, respectively.

Tensiometers (Mini "LT", Irrometer, Riverside, Calif.) were randomly placed in three pots per species. We top-watered all plants of an individual species with respective treatment solutions when medium dryness exceeded –5 kPa. Each pot received ≈110 mL of solution at each irrigation, with a mean leaching rate of <8%.

Data collection. To measure pH and EC, a saturated paste was prepared from the media using deionized water as the extractant (Warnke, 1995). Substrate pH was measured directly in the slurry, and electroconductivity was measured on the filtered extract 0, 7, 14, and 21 d after planting from two destructively sampled replicates per block. Final pH and EC were collected 28 d after planting on all replications. Substrate pH was measured using a pH probe (model 6165 Sure-Flow Solid State pHuture Probe; Orion Technologies, Beverly, Mass.) with a pH meter (model 620; Orion), and EC was measured with an electroconductivity meter (model 130; Orion).

Plant growth data. The mean length and width for leaves 4, 5, and 6 were measured on one randomly selected stem per plant, where leaf number four was defined as the fourth leaf from the apex that was >1.0 cm, and averaged to provide a mean leaf length and width per plant. Stem caliper was measured at the media surface on one randomly selected stem per plant using digital calipers. Shoot tissue was harvested from each pot and was rinsed in a 0.5% nonionic, phosphate-free detergent (Aquet, Bel-Art, Pequannoc, N.J.) distilled water solution. The shoot was dried in a 50 °C oven for 7 d and dry mass was measured. Final shoot dry mass were subtracted from the mass at day 0 (transplant, 0.2 g for both species) to calculate change in mass. All mass data analyzed in this article represent change in mass from day 0 to 28.

Chlorophyll and carotenoids. Five discs were randomly selected from each plant using a 0.64-cm-diameter 1-hole punch, then frozen until analysis. For analysis, discs were ground in a 13 × 100-mm pyrex tissue grinder (Corning, Corning, N.Y.) using a pinch of sand and 2 mL 95% ethanol. The extract was diluted with an additional 3 mL of 95% ethanol, then 2 mL of final solution was centrifuged (Beckman Microfuge 11) for 120 s at 220 $r \cdot s^{-1}$ and 11,600 g_n. Solution was measured for absorbance at 470, 649, and 664 nm using a spectrophotometer (Spectronic 21D; Milton Roy, Ivyland, Pa.). Total chlorophyll and carotenoids were determined according to the method of Lichtenthaler (1987), based on the mean dry mass of five discs.

SPAD. The Minolta SPAD meter was used to measure 10 random leaves per plant and the mean value per plant was recorded. Quadratic regression analysis between SPAD and the spectrophotometric-based measurements of chlorophyll was performed separately for each species, combining the mean values of four replicate plants per block, with three blocks per treatment combination. An F test was conducted to check whether SPAD and chlorophyll correlation curves differed between the two species, using the method described by Zar (1999).

SPAD was measured on the fourth most newly expanded leaf on four replications per

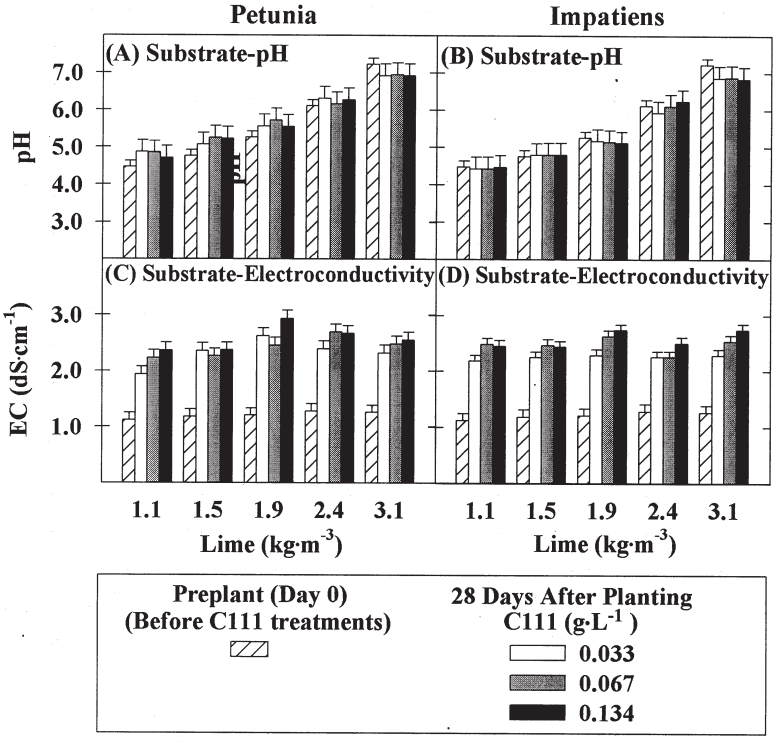

Fig. 1. Substrate pH and electroconductivity (EC) for petunia (**A** and **C**) and impatiens (**B** and **D**) before planting and 28 d after fertilization with either 0.033, 0.067, or 0.134 $g \cdot L^{-1}$ C111 EDTA-micronutrient complex. Measurements represent the least-square means for each C111 treatment (four replications) ±95% confidence intervals.

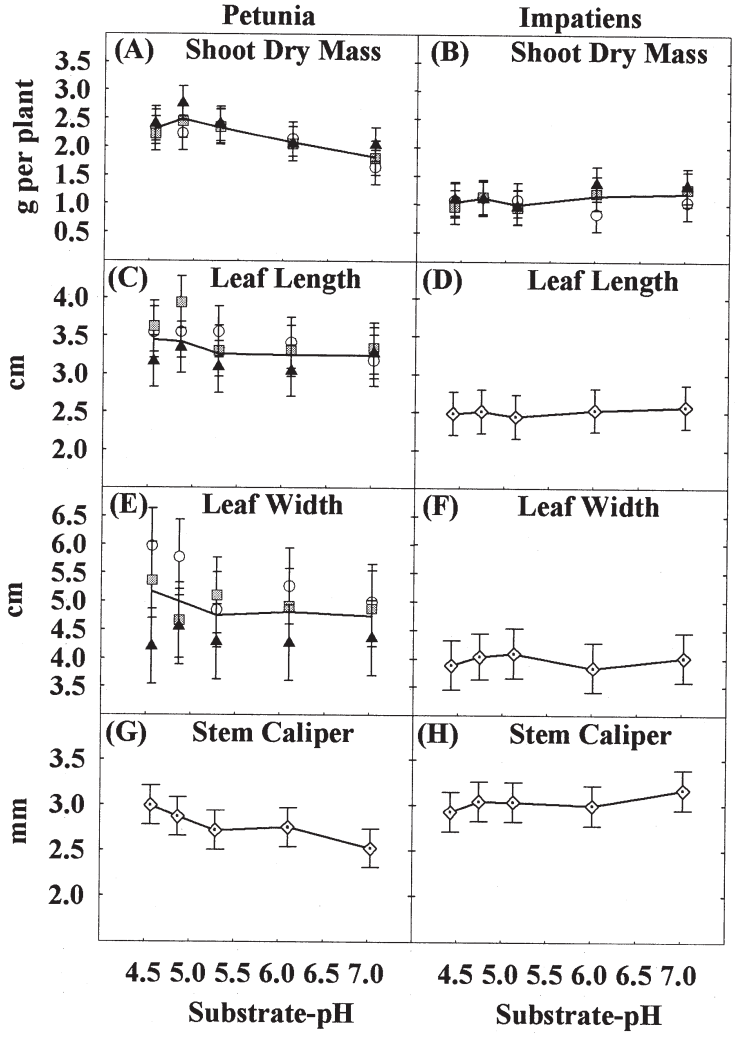

Fig. 2. The effect of substrate pH and micronutrient fertilizer (C111) concentration on petunia (**A**) shoot dry mass, (**C**) leaf length, (**E**) leaf width, and (**G**) stem caliper and impatiens (**B**) shoot dry mass, (**D**) leaf length, (**F**) leaf width, and (H) stem caliper 28 d after planting. Solid lines represent least-square means for each pH. Symbols represent least-square means for each C111 treatment (four replications) ± 95% confidence intervals (❍ 0.033 g·L^{-1}, ■ 0.067 g·L^{-1}, and ▲ 0.134 g·L^{-1}). In cases where there was no effect of C111, the symbol (✧) represents the least-square mean for each substrate pH ± 95% confidence intervals.

treatment, 0, 1, 2, 3, and 4 weeks after transplant, in order to determine the measurement date when substrate pH treatments first affected chlorophyll content. ANOVA was performed on data from each measurement date separately for each species, and chlorophyll was not measured by spectrophotometer for these leaves.

Results and Discussion

pH and EC. Within a plant species, each lime incorporation rate resulted in a different substrate pH from that of other lime rates (Fig. 1A and B). Over the 28 d of the experiment, substrate pH was affected by plant species, lime rate, and their interaction ($p < 0.001$), but was not affected by C111. Substrate pH changed over time ($p < 0.001$), by up to 0.5 pH units between days 0 and 28 (Fig. 1A and B). Overall, petunia had a slightly higher substrate pH (by up to 0.2 units) than impatiens (which was significant when data were pooled from both species and tested with ANOVA). For petunia, the least-square mean pH's of lime treatments over time (se ± 0.02) were 4.6, 4.9, 5.3, 6.1, and 7.0 pH units, whereas for impatiens the mean pH values were 4.4, 4.7, 5.1, 6.0, and 7.0. The term substrate pH discussed here refers to these least-square means over time.

The least-square mean EC before planting was 1.2 dS·m^{-1}, averaged between lime rates, and increased over time (Fig. 1C and D). Substrate EC did not differ between plant species. The least-square mean EC at the end of the experiment for the 0.033, 0.067, and 0.134 g·L^{-1} C111 treatments was 2.29, 2.45, and 2.57 dS·m^{-1} respectively, which is within the acceptable range in EC for container media using a saturated medium extract method (Warncke, 1995). The lowest EC occurred at the lowest lime concentration, but final EC did not differ by more than 0.3 dS·m^{-1} between lime rates.

Plant growth. Petunias grown at pH levels above 5.3 had a lower dry mass than those grown at the lowest three pH levels (Fig. 2A). Increasing the applied C111 concentration resulted in increased shoot dry mass at the pH 4.8 and 7.0 levels but did not affect dry mass at other pH levels (Fig. 2A). In petunia, increasing C111 resulted in a decrease in leaf length and width (Fig. 2). Increasing pH resulted in a decrease in petunia stem caliper.

Plant growth measurements were less affected by substrate pH and C111 for impatiens compared with petunia (Fig. 2). There was an interactive effect of substrate pH and C111 on shoot dry mass for impatiens (Fig. 2B). The higher C111 levels increased dry mass at pH 6.0 compared with that of plants grown at low C111. Leaf width and length and stem caliper were not affected by any treatment in impatiens.

Pigment and SPAD measurements. With all treatments where the mean SPAD chlorophyll index was below 40 for petunia and 50 for impatiens, as presented in Fig. 3A and 3B, there was a visually obvious decrease in green color (i.e., onset of chlorosis) to the extent that market value of plants would be impacted. Visual chlorosis symptoms worsened as SPAD decreased.

For both species, substrate pH affected SPAD, chlorophyll, and carotenoid levels. C111 affected these three pigment measurements in all cases except carotenoids in impatiens. There was a significant C111 and substrate pH interaction for SPAD in both species. This interaction occurred because at the two highest pH levels, increasing C111 increased SPAD (i.e., decreased chlorosis), whereas there was no effect of C111 on SPAD at the lower pH levels (Fig. 3).

For petunias grown with the 0.033 g·L^{-1} C111 concentration, increasing substrate pH above 5.3 resulted in a decline in SPAD, chlorophyll, and carotenoids compared with measurements from plants grown at the lowest three pH levels (Fig. 3A, C, and E). Compared with pigment measurements from plants grown at pH 5.3, plants grown at pH 7.0 had SPAD and chlorophyll 34% and 45% lower, respectively. Increasing the applied concentration of C111 from the 0.033 g·L^{-1} rate to higher rates raised the pH from 5.3 to 6.1 before SPAD and chlorophyll content declined (Fig. 3A and C).

With impatiens grown with the 0.033 g·L^{-1} C111 concentration, pigment levels decreased as substrate pH increased above pH 5.1 (Fig. 3B, D, and F). For impatiens grown at pH 7.0, SPAD, chlorophyll, and carotenoids were 18%, 28%, and 24% lower, respectively, than for plants grown at pH 5.1. Increasing the applied C111 concentration from the 0.033 g·L^{-1}rate to the higher rates raised the pH level to 6.0 before SPAD and chlorophyll content declined (Fig. 3B and D). However, carotenoid levels in impatiens were unaffected by pH.

With both petunias and impatiens, SPAD measurements were highly correlated with chlorophyll content (Fig. 4). However, the correlation curve for petunia was different from that determined for impatiens ($p < 0.001$).

With both petunias and impatiens, chlorosis developed after 2 weeks when grown at the highest pH (Fig. 5). With petunias, the chlorosis became progressively more severe between week 2 and 4. With impatiens, the chlorosis decreased in severity between week 2 and 4.

Iron-deficiency reportedly does not reduce all photosynthetic pigments to the same extent (Marschner, 1995). Chlorophyll a and b tend to decrease more than certain carotenoids under Fe-deficient conditions, yet certain xanthophylls are not reduced in the same

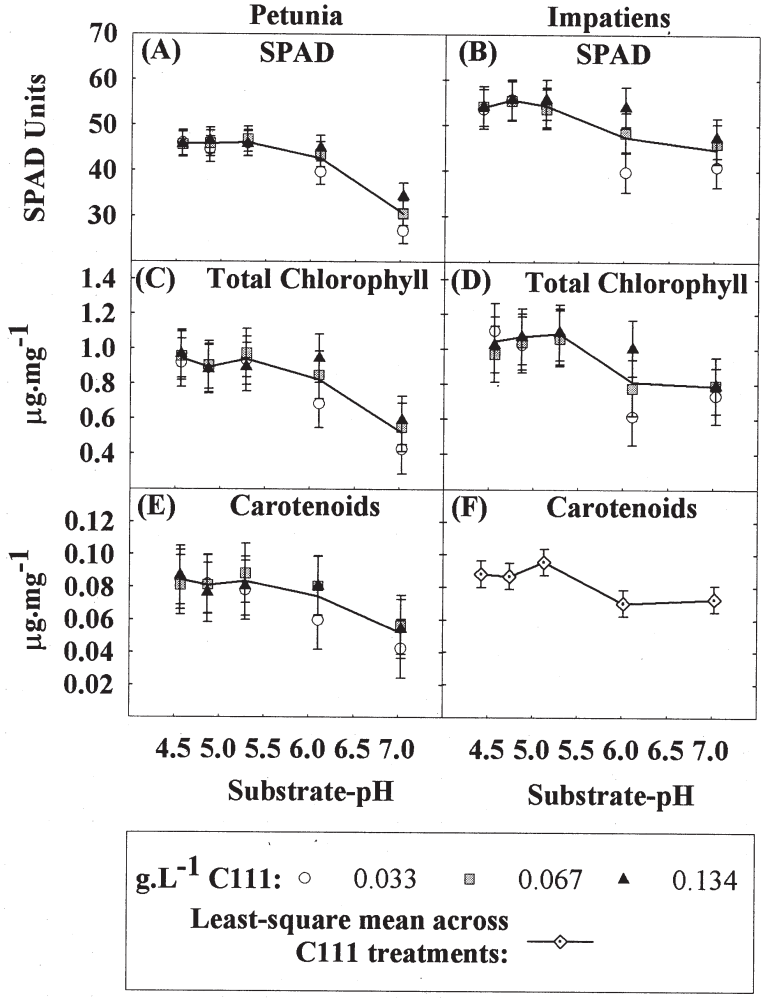

Fig. 3. The effect of substrate pH and fertilizer micronutrient concentration (C111) on petunia (**A**) SPAD, (**C**) chlorophyll, and (**E**) carotenoids, and impatiens (**B**) SPAD, (**D**) chlorophyll, and (**F**) carotenoids, 28 d after planting. Solid lines represent least-square means for each pH. Symbols represent least-square means for each C111 treatment (four replications) ±95% confidence intervals (❍ 0.033 g·L^{-1}, ■ 0.067 g·L^{-1}, and ▲ 0.134 g·L^{-1}). In cases where there was no effect of C111, the symbol (✧) represents the least-square mean for each substrate pH ± 95% confidence intervals.

manner, and may actually increase under conditions of Fe-deficient chlorosis (Abadia, 1992; Quilez et al., 1992; Terry and Abadia, 1986). In our research, total chlorophyll and carotenoids had similar trends with pH (Fig. 3), although chlorophyll showed a greater decline at pH 7.0 in both species compared with carotenoid level.

Our results support previous research showing little effect of substrate pH on dry mass accumulation for impatiens (Argo and Biernbaum, 1996). In our experiment, leaf area in impatiens did not increase at higher substrate pH levels to compensate for lower chlorophyll content. Presumably, however, the light-harvesting capacity was not limiting to whole-plant photosynthesis rate during this short-term experiment (28 d). Longer-term experiments would be expected to magnify effects of fertilizer treatments on tissue nutrients and pigment concentration (for example, as preplant nutrient charge is exhausted over time), and also would emphasize the resulting effects on growth. However, Argo and Biernbaum (1996) found that impatiens grown over a 17-week period at pH levels ranging from 4.5 to 8.5 showed little difference in shoot dry mass.

Conclusions

We define an acceptable substrate pH for a floricultural crop as a pH range that results in a concentration of available nutrients in the media solution that is sufficient for normal plant metabolism, without any detrimental effects on plant growth or appearance. In this experiment, pH responses were measured in terms of pigment content and plant growth. When SPAD index declined below about 40 for petunia and 50 for impatiens, chlorosis was visually obvious to the extent that it would affect market value. Based on pigment analysis, the acceptable pH range for petunia and impatiens was between 4.5 and 6.1 at the two highest C111 concentrations (which provided 1 or 2 mg·L^{-1} Fe), or the pH range 4.5 to 5.3 at the lowest C111 concentration (0.5 mg·L^{-1} Fe). Petunia dry mass decreased at pH 6.1 regardless of C111 level, with the smallest plants grown at pH 7.0 and the low C111 level. In contrast, plant growth measurements for impatiens showed little growth response in the range 4.4 to 7.0. Because floricultural crops are grown for both vigor and appearance, a pH level of 7.0 was too high for impatiens based on observed chlorosis, although it was not excessive for plant growth during this trial.

Recommended pH ranges for soilless media vary between floricultural crop species depending on Fe efficiency and the resulting susceptibility to Fe and Mn toxicity at low pH or Fe deficiency at high pH (Argo and Fisher, 2002; Bailey, 1996; Bethke, 1993; Nelson, 1994). Most plants (including impatiens) can be grown with a pH range from 5.6 to 6.2. In contrast, Fe-efficient plants (including *Pelargonium hortorum* L.H. Bail) are generally recommended to be grown at pH 6.0 to 6.6 to limit micronutrient solubility, and an Fe-inefficient group (including petunia) at pH 5.4 to 6.2 to increase micronutrient solubility.

Overall, our results were consistent with recommended pH ranges for impatiens and petunia (Argo and Fisher, 2002; Nelson, 1994). Based on this one experiment, we would not recommend growing impatiens and petunia at a substrate pH below pH 5.4, even though growth was not negatively affected, because nutrient imbalances may occur over the long term at very low substrate pH, differences in Fe efficiency between cultivars are likely (Albano and Miller, 1998; Marschner, 1995), and this experiment emphasizes that an acceptable pH varies with differing nutritional conditions. Furthermore,

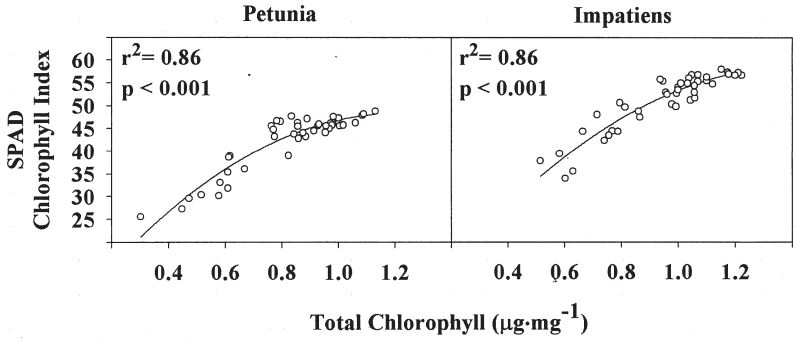

Fig. 4. Quadratic regression of chlorophyll concentration (extracted from fresh tissue using 95% ethanol and measured using a spectrophotometer) and SPAD chlorophyll index for petunia and impatiens. Data were collected 28 d after planting, from 12 replicate plants per treatment. Regression equation for petunia was SPAD = 0.07 + 79.90C – 33.11C^2, and for impatiens SPAD = –0.58 + 83.07C – 29.10C^2, where C equaled the chlorophyll content (µg·mg^{-1} of dried tissue).

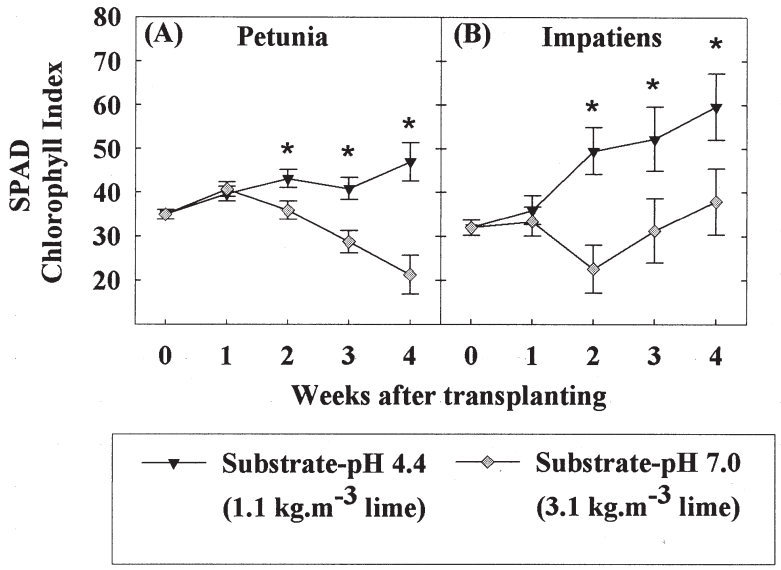

Fig. 5. SPAD chlorophyll index over time for (**A**) petunia and (**B**) impatiens at two substrate pH levels. Symbols represent least-square means across 3 micronutrient (C111) levels from 12 replicate plants per substrate pH. The substrate pH treatments shown corresponded to the lowest and highest lime rates (1.1 and 3.1 kg·m^{-3}). Asterisk symbol (*) indicates measurement dates when substrate pH significantly affected SPAD chlorophyll index ($p < 0.05$).

because commercial greenhouses usually grow many species under the same nutrient regime, as pH decreases below 6.0, the potential for micronutrient toxicity problems increases for other more Fe-efficient species (Albano and Miller, 1998).

Greater precision was obtained for SPAD measurements than for ethanol extraction of chlorophyll. Because of the efficiency of the SPAD meter (low sample time, nondestructive measurements, and highly significant relationship with chlorophyll) we found the SPAD meter a useful tool for indirectly measuring chlorophyll content, plant health, and color. The increase in SPAD over time (Fig. 4) for impatiens at all substrate pH values may have been caused by an actual increase in chlorophyll (not measured) or may have been an artifact of the SPAD chlorophyll index, which is a ratio between light transmission at red and far-red wavelengths (this ratio may differ in young versus older plants). Further research of SPAD is needed to evaluate whether the SPAD meter can be used to accurately track plant health over time.

Micronutrient deficiency and toxicity symptoms most severely affect young and old leaves, respectively. We measured ten randomly selected leaves for SPAD analysis. Because we only observed symptoms consistent with micronutrient deficiency, a random sampling of all leaves may have underestimated treatment effects on young foliage compared with sampling young leaves only.

Our results have implications for other research determining pH responses for plant species, and highlights that the acceptable pH range of any crop depends on the concentration of the applied micronutrients. At higher micronutrient concentrations, plants could be grown at a higher pH range than would be commonly recommended without negatively affecting plant growth and pigmentation. Other factors that might affect the acceptable pH range, although they were not investigated in this experiment, include medium-type (cation exchange capacity may affect available micronutrient concentration in the soil solution and Fe/Mn antagonisms (Bunt, 1988)); Fe form (ionic and chelated forms vary in solubility with pH, and stability of EDTA-chelated Fe is lower at pH 7.0 than ferric diethylenetriamine pentaacetic acid (Fe-DTPA) or ferric ethylenediaminedi(*o*-hydroxyphenylacetic) acid (Fe-EDDHA) (Norvell, 1991)); overall nutrient concentration (because complete NPK fertilizers may also contain micronutrients, and increasing applied N also increases Fe); and leaching rate (similar concentration of nutrients in the medium and plant tissue can be obtained by using high concentrations and high leaching rates of low concentrations and low leaching rates (Yelanich and Biernbaum, 1993)). Recommended pH ranges should be based on trials under a range of nutrition conditions in order to be robust to the variability in commercial practices.

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