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Seedling Geranium Response to Nitrogen Deprivation and Subsequent Recovery in Hydroponic Culture

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Abstract. **Nitrogen (N) fertilization recommendations to achieve optimum growth are well established for many floriculture crops. Although it has been shown that plant functions can recover from N deficiency in other crops, little research has investigated the threshold beyond which a bedding plant crop is recoverable. The objective of this research was to determine the effect of N deficiency on geranium chlorophyll content and growth and then to document the degree of recovery and recovery time from N deprivation. This was determined in two experiments by monitoring chlorophyll content and growth of seedlings grown in hydroponic culture in which the N source was removed and then restored after differing lengths of time. Summarizing across both experiments, chlorophyll and foliar N levels were shown to rebound quickly after N deprivation; however, growth was reduced after just 4 days compared with plants fed constantly. Geraniums grown without N for 4 to 12 days resulted in smaller, more compact plants with lower shoot–to-root ratios. Although foliar chlorophyll and N concentration recovered from longer periods in N growth solution, geranium growth was reduced and failed to completely recover for any plant receiving more than 2 days of N-free solution.**

Plant requirements for N exceed all other mineral elements with typical tissue concentrations ranging between 2% and 5% of dry weight depending on plant species and development stage (Marschner, 1995). Plants deficient in N typically exhibit overall stunted growth and chlorotic leaves. Common causes of N deficiency are from inadequate fertilization regimes; the amount supplied is too low to meet a plant's demands at a specific developmental stage, an error occurred in the initial fertilizer mixing rate, nutrients are leached from the substrate by excessive irrigation, or an injector malfunctions. Excluding mechanical adjustments to equipment, recommendations for correcting N deficiency suggest higher rates or more frequent fertilizer applications (Dole and Wilkins, 2005; Whipker et al., 2001).

Augmenting N supply to N-starved plants has been shown to promote recovery in certain plant functions. On reintroduction, increased nitrate uptake rates were observed over 24 h, 48 h, and 3 d for wheat (*Triticum vulgare* Vill. cv. Knox), perennial ryegrass (*Lolium perenne* L.), and barley (*Hordeum vulgare* L.), respectively (Bowman and Paul, 1988; Jackson et al., 1976; Lee and Rudge, 1985). Photosynthetic rates resumed normal capacity in 10 d for corn (*Zea mays* L.), whereas chlorophyll content partially recovered over the same time period when resupplied with N (Girardin et al., 1985). Although total dry weight more than doubled after growing 10 d in N-free solution and a 15-d recovery period for soybean (*Glycine max* L.), ending shoot weight values were still only 50% of non-stressed plants (Tolley-Henry and Raper, 1986).

In commercial greenhouse production, seedling crops such as geranium follow rigid schedules for plug production. Disruption in the supply of essential elements over any time period during the production schedule may delay the readiness of a crop for shipment or may cause irreversible damage, rendering a crop unmarketable. Although it has been shown that plant functions can recover from N deficiency in other crops, little research has investigated the threshold beyond which a bedding plant crop is unrecoverable. The objective of this research was to determine the effect of N deficiency on geranium chlorophyll content and growth and then to document the degree of recovery and recovery time from N deprivation.

Materials and Methods

Geranium (*Pelargonium ×hortorum* L. H. Bailey 'Maverick Red'; Ball Horticultural Co., West Chicago, IL) seeds were sown in Oasis foam (Smithers-Oasis, Kent, OH) rinsed three times with deionized water. Seeds were germinated in a growth chamber kept at a constant temperature of 21 °C under fluorescent lights from 0600 HR to 2400 HR. Seedlings were moistened with deionized water as needed. After 14 d, Oasis cubes with an individual seedling were inserted into 2-cm holes in the lid of a constantly aerated, 4.5-L opaque bucket (Encore Industries, Sandusky, OH) and moved to a greenhouse bench with day/night temperature settings of 22/21 °C. Supplemental light was provided from a combination of high-pressure sodium (250 W) and metal halide (400 W) lights for up to 15 $h \cdot d^{-1}$ (0700 HR to 2200 HR) when light levels fell below 450 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Plants were acclimated for 7 d in a complete, modified (half-strength) Hoagland's solution, consisting of macronutrients in millimolar concentrations of 7.5 N (all nitrate), 2.0 phosphorus (as PO_4), 4.5 potassium, 2.5 calcium, 1.0 magnesium, 1.0 sulfur (as SO_4) plus micronutrients in micromolar concentrations of 71 iron, 9 manganese, 1.5 copper, 1.5 zinc, 45 boron, 0.2 sodium, and 0.1 molybdenum using KNO_3, $Ca(NO_3)_2$, KH_2PO_4, $MgSO_4$, Fe-DTPA, $MnCl_2$, $CuCl_2$, $ZnCl_2$, H_3BO_3, and $MoNa_2O_4$ adjusted to pH 5.8 ± 0.3 with KOH.

Expt. 1 consisted of 84 buckets assigned to 14 treatments, each with six replications arranged in a completely randomized design. Each bucket contained three seedling oasis plugs. After a 7-d acclimation period, solutions in all but the control buckets were replaced with an N-deficient modified Hoagland's solution on 19 Nov. 2010 supplying calcium and potassium (2.5 and 5.0 mM, respectively) with $CaCl_2$ and K_2SO_4 in place of $Ca(NO_3)_2$ and KNO_3. Treatment levels refer to the amount of time any given bucket remained in N-deficient solution. Starting at 2 d after treatment (DAT), and every 2 d thereafter, six buckets were refilled with complete nutrient solution. All treatment solutions were replaced weekly. Visual observations for deficiency symptoms and chlorophyll measurements (Minolta- 502 SPAD meter; Spectrum Technologies, Inc., Plainfield, IL) were taken with five readings on five independent recently matured leaves per bucket every other day. All plants were harvested at 33 DAT, rinsed in deionized water, and dried at 60 °C for at least 72 h. At harvest, recently matured leaves and petioles were separated for foliar nutrient analysis, rinsed with 0.1 N HCl and

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deionized water, and oven-dried to constant mass in a forced-air oven at 55 °C. Geranium tissue was ground with a mortar and pestle, and N concentration in dried plant tissue was determined by combustion using a C-H-N analyzer (Perkin-Elmer 2400 Series II CHNS/O Analyzer, Waltham, MA).

Expt. 2 was conducted similar to Expt. 1 with the following exceptions. Nine treatments with six replications each were randomly assigned across 54 buckets with six seedling oasis plugs per bucket. Plants were grown in N-deficient conditions for 0, 2, 4, 8, 12, 16, 20, 24, or 28 d starting on 14 Mar. 2011. Chlorophyll SPAD measurements were taken every 4 d. Two plants per bucket were harvested on 8 and 16 DAT, measured for root and shoot dry weight, and combined for shoot elemental analysis. One plant per bucket was harvested on 24 and 32 DAT for root and shoot dry weight and N analysis.

Data were subjected to repeated-measures analysis of variance using SAS Version 9.1(SAS Institute Inc., Cary, NC). Data for plants grown without N were compared with control plants grown with complete nutrient solution using Dunnett's one-tailed *t* test.

Results and Discussion

Expt. 1. Repeated-measures analysis indicated chlorophyll content changed over time ($P \leq 0.0001$). Chlorophyll content of all plants grown without N remained similar to controls until 14 DAT (Table 1) but visual differences in plants were not noted until 16 DAT. During the first 14 DAT, chlorophyll content of plants growing without N steadily decreased until they were returned to full N solution. Plants grown without N over 12 d maintained a reduced chlorophyll content until 4 to 8 d after full N solution was restored. Girardin et al. (1985) reported that maize (*Zea mays* L.), grown in clay-filled containers, had chlorophyll content remaining suppressed for 21 d after being returned to a the full N solution. It was also observed, but not measured, that some marginal necrosis occurred on all plants when returned to the full N solution after being grown without N for longer than 12 d. High concentrations of N have been reported to cause marginal necrosis of peace lily (*Spathiphyllum* Schott 'Sensation') (Mak and Yeh, 2001) and mango (*Mangifera indica* L.) (Singh and Saxena, 1994). In our study, chlorophyll content of plants grown without N for 26 d was lower than controls by the end of the study; however, chlorophyll content of those plants increased after being returned to N solution until the final day of the experiment. Plants grown without N for up to 10 d did not express lower chlorophyll content than control plants, suggesting that N fertilization can be skipped for up to 8 d without loss of foliar color.

At the conclusion of the study, only plants grown without N for longer than 18 d had foliar N levels lower than controls (Table 2). The sufficiency range of foliar N in geranium is 3.3% to 4.8% (Mills and Jones, 1996). Only

Table 1. Mean SPAD chlorophyll readings for geraniums (*Pelargonium* ×*hortorum* 'Red Maverick') grown in hydroponic solution in 4.5-L plastic buckets.[z]

	SPAD readings 2 to 32 d after treatment[y]								
Days N withheld	2	4	8	12	16	20	24	28	32
0–control	38.1	35.8	36.7	36.3	39.8	34.5	37.0	34.1	38.5
2		35.2	35.8	37.7	37.8	32.0	36.9	34.2	39.5
4			34.3	36.6	39.1	32.4	37.3	34.2	39.0
6			34.1	36.4	37.8	34.0	37.7	34.5	38.2
8				35.1	38.3	33.1	38.4	34.1	39.8
10				34.4	39.2	35.6	38.6	35.2	38.5
12					33.7*[x]	34.0	38.8	34.1	38.2
14					29.4*	28.6*	39.1	32.8	38.0
16						26.8*	37.0	36.1	39.5
18						26.6*	33.6*	34.8	37.1
20							27.1*	33.3	38.1
22							26.7*	29.6*	38.2
24								24.9*	37.3
26								24.3*	31.4*
Plants in 0 N solution[w]	37.6	35.5	36.4	34.9	34.1*	29.7*	27.8*		

[z]Plants were initially grown for 7 d in full nutrient solution with 7.5 mM nitrogen (N) and then N was withheld for 0 to 26 d. Every 2 d, selected buckets (n = 6) were returned to complete N solution for the duration of the experiment. All plants were harvested 33 d after starting N-free solutions.
[y]Experiment was initiated when non-control plants were switched to N-deficienct solution.
[x]Asterisk indicates mean is significantly lower than control plants by Dunnett's one-tailed *t* test.
[w]Means for plants growing in N-deficient solution were pooled, regardless of their randomly assigned treatment day.

Table 2. Final shoot mass, root mass, shoot:root ratio, and foliar nitrogen (N) concentration of geraniums (*Pelargonium* ×*hortorum* 'Red Maverick') grown hydroponically in 4.5-L plastic buckets.[z]

Days N withheld	Foliar nitrogen (%)	Shoot mass (g)	Root mass (g)	Shoot:root ratio
0–control	4.32	1.35	0.22	6.4
2	4.46	1.38	0.21	6.8
4	4.37	1.11*[y]	0.16*	7.2
6	4.43	1.06*	0.16*	7.0
8	4.33	1.25	0.20	6.5
10	4.33	1.03*	0.17*	6.0
12	4.43	0.84*	0.14*	6.2
14	4.28	0.88*	0.16*	5.8
16	4.23	0.80*	0.16*	5.3*
18	4.27	0.61*	0.13*	4.7*
20	3.89*	0.53*	0.12*	4.5*
22	3.90*	0.54*	0.14*	4.2*
24	3.74*	0.44*	0.12*	3.6*
26	3.15*	0.44*	0.12*	3.6*

[z]Plants were grown for 7 d with full nutrient solution with 7.5 mM N and then N was withheld for 0 to 26 d, after which selected buckets (n = 6) were returned to full nutrient solution. All plants were harvested and data collected 33 d after initiation of N-free solutions.
[y]Asterisk indicates mean is significantly lower than control plants by Dunnett's one-tailed *t* test.

Table 3. Mean SPAD chlorophyll readings for geraniums (*Pelargonium* ×*hortorum* 'Red Maverick') grown in hydroponic solution using plastic buckets.[z]

	SPAD readings 4 to 32 d after treatment[y]							
Days N withheld	4	8	12	16	20	24	28	32
0–control	34.0	33.7	33.4	34.8	41.2	42.4	46.0	44.0
2	33.5	32.0	33.4	37.0	40.6	41.6	45.6	46.5
4		30.3*[x]	33.8	34.9	40.2	42.3	44.0	42.5
8			26.6*	34.8	41.7	42.4	42.9	41.1
12				24.7*	37.6*	41.5	43.9	41.1
16					26.6*	39.8	43.0	41.6
20						27.5*	39.4*	40.0*
24							25.4*	34.4*
28								24.6*
Plants in 0 N solution[w]	33.0	31.3*	31.1*	29.4*	27.5*	26.1*	24.3*	

[z]Plants were initially grown for 7 d in full nutrient solution with 7.5 mM nitrogen (N) and then N was withheld for 0 to 26 d. Periodically, selected buckets (n = 6) were returned to the full N solution. All plants were harvested 32 d after initiating N-free solutions.
[y]Experiment was initiated when non-control plants were switched to N-deficient solution.
[x]Asterisk indicates mean is significantly lower than control plants by Dunnett's one-tailed *t* test.
[w]Means for plants growing in N-deficient solution were pooled, regardless of their randomly assigned treatment day.

plants grown without N for 26 d had foliar N below the sufficiency range. With these data, it is not known whether foliar N levels of geranium grown without N less than 18 d dropped below control plants sometime during the experimental period.

Only plants held in N deprivation for 2 and 8 d were similar in root mass to non-treated controls (Table 2). Root mass was reduced by a maximum of 45% for plants deprived of N for 20 to 26 d. Zhao et al. (2003) reported shoot biomass of maize grown without N for 27 d was reduced 46% to 56% compared with controls. Considering the combined results of Zhao et al. (2003) on maize biomass and Girardin et al. (1985) on maize chlorophyll content, geranium showed a pattern similar to maize for depressed growth and chlorophyll content after prolonged periods of N deficiency. Shoot mass followed a trend similar to root mass, decreasing with increasing time of N deprivation with over 67% reduction of shoot mass for plants grown without N for 26 d. Although foliar chlorophyll and N concentration recovered from longer periods in N growth solution, geranium growth was reduced and failed to completely recover for any plant receiving more than 2 d of N-free solution. Mattson and Lieth (2008) showed that rose (*Rosa* spp.) was not reduced in growth after 20 d of N-free solution.

By the end of this experiment, shoot:root ratio was lower among plants grown without N for 16 d or longer (Table 2). Tolley-Henry and Raper (1986) reported that N deficiency reduced shoot:root ratio in soybean (*Glycine max* L.) grown for 25 d in N-deficient hydroponic solution; however, Boussadia et al. (2010) reported no effect on shoot:root ratio in two hydroponically grown olive (*Olea europaea* L.) cultivars after 58 d of N deficiency. Our results, in conjunction with those of Boussadia et al. (2010), Mattson and Lieth (2008), and Tolley-Henry and Raper (1986), suggest that growth in the shorter production cycles of herbaceous plants is more sensitive to N deprivation than growth in relatively longer production cycles of woody plants.

At the conclusion of the experiment, control plants and those grown without N for 2 d appeared similar in subjective qualitative observations, corroborating their similar SPAD and growth values. Plants grown without N for 4 to 10 d appeared more compact and had slightly more zonal purple pigment development compared with control plants. Plants grown without N for 14 to 16 d had noticeably more zonation and displayed the previously mentioned marginal necrosis, making their marketability questionable. Plants grown without N for 18 d or longer were compact, had pronounced zonation, and irreversible marginal damage after N reintroduction, making these plants unmarketable.

Expt. 2. Repeated-measures analysis showed that all parameters changed over time (statistics not shown). Four d after imposing N deficiency, all plants had chlorophyll content similar to control plants (Table 3). By 8 DAT, plants grown without N for 4 d or longer had lower chlorophyll readings. Similar to Expt. 1, plants grown without N had lower SPAD readings than control plants until ≈8 d after being returned to N solution. Two exceptions occurred with plants that were grown without N for 20 and 24 d. Although SPAD values with these two treatments were still lower than control plants after being returned to the full N solution, they rebounded substantially.

Foliar N followed a trend similar to SPAD readings. By 8 DAT, only plants still growing without N had less foliar N than controls (Table 4). Similar to SPAD readings, foliar N levels rebounded in plants that were returned to the full N solution within 8 to 16 d. By 32 DAT, geranium grown without N for up to 20 d had foliar N concentrations similar to control plants and within the N sufficiency range for geranium. Plants grown without N for 24 to 28 d had less foliar N than controls, although foliar N levels increased three- to fourfold compared with what they had been when measured at 24 DAT.

Neither root nor shoot mass was affected by N deficiency at 8 DAT (Table 5). By 16 DAT, roots were still unaffected by N treatment, although shoots were smaller among plants grown 8 d or longer without N. By 24 DAT, roots and shoots of plants grown longer than 8 d without N were smaller than control geraniums that received full N throughout the experiment. Unlike foliar SPAD and N parameters, root and shoot growth across treatments did not rebound after plants were returned to N solution. Similar to Expt. 1, plants grown without N for 4 d or longer had smaller shoots than control plants at the end of the study. Le Bot et al. (2001) showed that tomato (*Lycopersicon esculentum* Mill. var. Thalis) plants grown hydroponically in a rockwool system showed reduced growth after 2 to 3 weeks of N deprivation compared with fertilized controls and had 20% reduction in shoot biomass after 6 weeks of N deprivation.

Shoot-to-root ratio was unaffected by N treatment at 8 DAT. The number of treatments with reduced shoot-to-root ratio increased with time. At 16 DAT, only plants grown without N for 12 d or longer had reduced shoot-to-root ratio; however, by 32 DAT, all plants grown without N for any period of time had lower shoot-to-root ratio compared with controls. This differs from Expt. 1 in which only plants grown 16 d or longer had reduced shoot-to-root ratio. By the end of the experiment, shoot-to-root ratio decreased with increasing time of N deficiency.

Summarizing across both experiments, geranium chlorophyll and foliar N levels rebound quickly after periods of N deficiency, although growth was reduced after just 4 d compared with plants fed constantly. This is not necessarily a negative impact because

Table 4. Foliar nitrogen (N) concentration of geranium (*Pelargonium ×hortorum* 'Red Maverick') grown in hydroponic solution in plastic buckets.[z]

Days N withheld	Days after treatment[y]			
	8	16	24	32
	---------- Foliar N (%) ----------			
0–control	4.42	3.94	4.15	3.55
2	4.50	4.84	4.08	3.57
4	4.19	5.21	4.20	3.75
8		3.04	4.19	3.78
12		2.81*[x]	3.94	3.71
16			3.53*	3.72
20			2.17*	3.52
24				3.01*
28				2.21*
Plants in 0 N solution[w]	1.91*	1.12*	0.74*	

[z]Plants were initially grown for 7 d in full nutrient solution with 7.5 mM N and then N was withheld for 0 to 28 d. Periodically, a subset of buckets (n = 6) were returned to full N solution. All plants were harvested 32 d after initiating N-free solutions.
[y]Experiment was initiated when non-control plants were switched to N-deficient solution.
[x]Asterisk indicates mean is significantly lower than control plants by Dunnett's one-tailed *t* test.
[w]Means for plants growing in N-deficient solution were pooled, regardless of their randomly assigned treatment day.

Table 5. Shoot mass, root mass, and shoot:root ratio of geranium (*Pelargonium ×hortorum* 'Red Maverick') grown in hydroponic solution in plastic buckets.[z]

Days N withheld	8 DAT[y]			16 DAT			24 DAT			32 DAT		
	Shoots	Roots	Ratio[x]	Shoots	Roots	Ratio	Shoots	Roots	Ratio	Shoots	Roots	Ratio
0–control	0.19	0.04	5.05	0.41	0.08	5.41	1.17	0.21	5.72	3.43	0.46	7.52
2	0.15	0.03	5.50	0.35	0.07	5.38	1.22	0.22	5.72	3.33	0.55	6.06*
4	0.16	0.03	5.02	0.44	0.09	4.82	1.08	0.18	6.00	2.74*	0.45	6.07*
8				0.31*[w]	0.08	5.73	0.70*	0.15*	4.81*	2.11*	0.38	5.66*
12				0.27*	0.08	3.26*	0.63*	0.16	3.95*	1.55*	0.32*	4.94*
16							0.48*	0.14*	3.59*	1.07*	0.23*	4.79*
20							0.40*	0.12*	3.30*	0.79*	0.22*	3.64*
24										0.69*	0.20*	3.49*
28										0.72*	0.24*	3.30*
Plants in 0 N solution[v]	0.15	0.03	4.49	0.23*	0.07	3.58*	0.38*	0.13*	2.93*			

[z]Plants were initially grown for 7 d in full nutrient solution with 7.5 mM nitrogen (N) and then N was withheld for 0 to 28 d. Periodically, a subset of buckets (n = 6) was returned to complete fertilizer solution. All plants were harvested 32 d after initiating N-free solutions.
[y]Days after treatments were initiated, when non-control plants were switched to N-deficient solution.
[x]Shoot:root ratio.
[w]Asterisk indicates mean is significantly lower than control plants by Dunnett's one-tailed *t* test.
[v]Means for plants growing in N-deficient solution were pooled, regardless of their randomly assigned treatment day.

smaller and more compact plants are often desired by growers to facilitate shipping. Although quality was not measured nor characterized in this study, chlorophyll content is reflective of plant "greenness" and thus a good indicator of quality. This study shows that although geranium size will be impacted by even brief periods of N-deficient growth, plant greenness can be recovered. Plants grown without N for 12 d or longer had more distinct zonation compared with plants grown without N less than 12 d. Similar to Expt. 1, marginal necrosis was observed in Expt. 2 on plants grown without N for 12 d or longer when they were transferred back to the full N solution.

This study demonstrates the reaction of geranium to changes of N level in hydroponic solution. Plants in our hydroponic system were instantly changed to an N-free solution, but in an analogous commercial greenhouse, plants would not experience an immediate change to N-free substrate solution because the media would retain some level of N through cation and anion exchange sites. Geranium response to an inadvertent switch to N-free irrigation would likely be delayed compared with the change imposed in our experiment.

These experiments demonstrate that a period of up to 20 d without N would result in a reversible reduction in geranium chlorophyll and foliar N concentration. A period without N longer than 12 d might result in marginal necrosis on reintroduction of N. Geranium grown without N for 4 to 12 d (then returned to full N solution for at least 8 d) will result in smaller and more compact plants with lower shoot-to-root ratios but otherwise similar foliar quality and N concentration to continuously fed geraniums. This information can be used by floriculture producers to determine if an N-starved plant can recover and still be marketable and could potentially be used to manipulate geranium growth while still producing high-quality crops.

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