Characterization of Nutrient Disorders of *Primula acaulis* 'Danova Rose'

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Keywords: calcium, magnesium, micronutrients, nitrogen, phosphorus, potassium, sulfur

Abstract

***Primula acaulis* 'Danova Rose' plants were grown in silica sand culture to induce and photograph nutritional disorder symptoms. Plants were grown with a complete modified Hoagland's all nitrate solution: (macronutrients in mM) 15 NO_3-N, 1.0 PO_4-P, 6.0 K, 5.0 Ca, 2.0 Mg, and 2.0 SO_4-S, plus μM concentrations of micronutrients, 72 Fe, 18 Mn, 3 Cu, 3 Zn, 45 B and 0.1 Mo. The nutrient deficiency treatments were induced with a complete nutrient formula minus one of the nutrients. Reagent grade chemicals and deionized water of 18-mega ohms purity were used to formulate treatment solutions. Boron toxicity was also induced by increasing the element 10× higher than the complete nutrient formula. The plants were automatically irrigated. The solution was drained from the bottom of the pot and captured for reuse. A complete replacement of nutrient solutions was done weekly. Plants were monitored daily to document and photograph sequential series of symptoms as they developed. Typical symptomology of nutrient disorders and critical tissue concentrations are presented.**

INTRODUCTION

Primula acaulis (Syn. *Primula vulgaris*), a member of the *Primulaceae* family (Dole and Wilkins, 2005) is a floriculture crop grown for use as a cool season colorful bedding plant or as a pot plant in the home (Linwick, 1996). It is not a heavy feeder (Erwin, 1999; Armitage et al., 1994) and requires a low to moderate level of fertilization (Linwick, 1996; Hartnett, 1993).

Hartnett (1993) recommended during stage 2 of germination, the period when the cotyledons emerge and first true leaves develop, the fertility regimen should include 60 $mg \cdot L^{-1}$ nitrogen (N) from potassium nitrate and calcium nitrate, 74 $mg \cdot L^{-1}$ potassium (K), balanced micronutrients, and a pH of 6.0. Linwick (1996) suggests three weeks after sowing seedlings should be fertilized with 50 to 75 $mg \cdot L^{-1}$ N from a 20-10-20 peat-lite mix or other fertilizer which has an EC of 1.25 mS/cm and a pH of 5.5. Armitage et al. (1994) recommended a lower rate of 25 to 50 $mg \cdot L^{-1}$ KNO_3 during stage 2 and 3. PanAmerican Seed (2009) suggests during stage 2 seedlings should be fed with 100 ppm of 14-0-14, and during stage 3 one should alternate between 200 ppm 14-0-14 and 20-10-20. Prior to transplanting, seedlings should be fed 200 $mg \cdot L^{-1}$ N (Dole and Wilkins, 2005; Erwin, 1999; Hartnett, 1993). The potting on mix should be high in organic matter, but starter fertilizers should be omitted to provide more control over fertility requirements (Hartnett, 1993). After transplanting, 90 $mg \cdot L^{-1}$ N with a 1 to 1 ratio of N in the nitrate form and potash fertilizer is recommended (Hartnett, 1993). Dole and Wilkins (2005) and Erwin (1999) suggested that 90 to 100 $mg \cdot L^{-1}$ N and K be used. Armitage et al. (1994) recommended a higher rate of 100 to 125 $mg \cdot L^{-1}$ N for production, and Linwick (1996) suggested a constant liquid feed rate of 100 to 150 $mg \cdot L^{-1}$ N. After the plants have developed a rosette of 6 to 10 leaves the rate can be increased to 200 $mg \cdot L^{-1}$ N and 440 $mg \cdot L^{-1}$ K to enhance bloom production (Hartnett, 1993; Linwick, 1996). In the cooler months, N should originate from calcium nitrate and potassium nitrate (Hartnett, 1993) instead of ammonium nitrate (Linwick, 1996; Erwin, 1999). Cooler temperatures slow the action of microorganisms converting ammonium into useable nitrate. The lack of

conversion results in a build up of ammonium (Holcomb, 1983) which can be toxic (Erwin, 1999). In the winter the fertilizer rate should be dropped to 75 to 100 $mg \cdot L^{-1}$ N with each irrigation (Armitage et al., 1994). PanAmerican (2009) suggests for the cold treatment lowering the 20-10-20 rate to 15-0-15 at 50 ppm. The EC of the media should be below 1.2 mS/cm (PanAmerican, 2009).

Some nutritional problems have been reported. Excess N encouraged leaf growth (Hartnett, 1993; Dole and Wilkins, 2005) and can cause root problems which results in foliar problems. Ammonium toxicity occurred during winter and causes marginal yellowing on lower leaves. This symptom can be corrected by leaching the substrate and changing to nitrate-based fertilizers (Erwin, 1999). Manganese (Mn) at toxic levels resulted in necrosis on lower leaf margins (Hartnett, 1993). Low fertility can result in small plants and premature bud set (PanAmerican, 2009). N deficiency manifested itself with chlorotic leaves (Holcomb, 1983; PanAmerican, 2009), discolored and blackening veins, pale young growth, and older growth developing marginal necrosis (Holcomb, 1983). Phosphorus (P) deficiency is reported as being a problem (Hartnett, 1993). Symptoms include bronze colored lower leaves, inward leaf curl with necrotic tips, and brown coloration in the veins (Holcomb, 1983). K deficiency appears as chlorotic lower leaves that eventually become necrotic, a reduction in young leaf expansion, and curled or rippled leaf margins (Holcomb, 1983). Calcium (Ca) deficiency is exhibited as either chlorosis, necrosis, or edge burn of upper leaves (Erwin, 1999). Pale green leaves have also been reported with Ca deficiency (Holcomb, 1983). Applying calcium nitrate will correct the problem (Erwin, 1999). Magnesium (Mg) deficiency manifested itself in older leaves with interveinal chlorosis (Holcomb, 1983; PanAmerican, 2009). This symptom progresses to necrosis of the leaf tips and margins. Younger leaves exhibit curling and backward arching (Holcomb, 1983). The most severe cases of Mg deficiency can be manifested as smaller plants, leaf canker, and burned margins (Dole and Wilkins, 2005). To help chlorosis problems and enhance growth, magnesium sulfate can be used at a rate of 3.7 $g \cdot m^{-3}$. Leaf distortion of young leaves can be caused by boron deficiency and can be corrected by applying 3.77 g/100 L Borax or 1.87 g/100 L Solubor (Erwin, 1999). Iron (Fe) chlorosis can occur when pH is above 6.5 (Hartnett, 1993; PanAmerican, 2009), when EC levels are high (Hartnett, 1993), or when insufficient Fe is provided (Erwin, 1999). Symptoms of Fe deficiency appear in young leaves (Erwin, 1999) as interveinal chlorosis (Holcomb, 1983; PanAmerican, 2009) which can develop to a white coloration and necrosis (Holcomb, 1983). Older leaves can also exhibit marginal chlorosis which progresses inward toward the midrib (Holcomb, 1983). This deficiency can be corrected by lowering pH using an acidic fertilizer that provides iron (Erwin, 1999) or applying iron chelate (Hartnett, 1993). Insufficient Mn caused bleaching in new growth (Hartnett, 1993).

While some information is provided about nutritional disorders of primula, the symptomology for nutritional problems of this crop is not complete. Thus, the goals of this study were to provide visual and tissue diagnostic values for primula so that growers will be able to accurately diagnosis nutritional problems on site.

MATERIALS AND METHODS

Single 'Danova Rose' primula plugs (3.4×3.4×5.1 cm cell size) were transplanted into 12.7-cm diameter (766 ml) plastic pots containing acid washed silica-sand [Millersville #2 (0.8 to 1.2 mm diameter) from Southern Products and Silica Co., Hoffman, NC] on 24 October 2008. This experiment was conducted in a glass greenhouse in Raleigh, NC at 35°N latitude. Plants were grown at 23°C day and 18°C night temperatures. An automated, recirculating irrigation system was constructed out of 10.2-cm diameter PVC pipe (Charlotte Plastics, Charlotte, NC). The system consisted of 24 separate irrigation lines (each 1.82 m long). Each line contained 8 openings (12.7 cm diameter) that held the 8 pots for the elemental treatment. Control plants were grown with a complete modified Hoagland's all nitrate solution: (macronutrients in mM) 15 NO_3-N, 1.0 PO_4-P, 6.0 K, 5.0 Ca, 2.0 Mg and 2.0 SO_4-S (Hoagland and Arnon, 1950), plus μM

concentrations of micronutrients, 72 Fe, 18 Mn, 3 Cu, 3 Zn, 45 B and 0.1 Mo. In order to induce nutrient deficiency treatments, the plants were irrigated with complete nutrient solution excluding one of the nutrients. The B toxicity treatment was conducted by increasing B concentration (450 μM) in the Hoagland's solution. Reagent grade chemicals and deionized water of 18-mega ohms purity were used to formulate treatment solutions (Pitchay, 2002). The plants were automatically irrigated as needed from 6:00 hours to 18:00 hours using a drip system utilizing sump-pumps (model 1A, Little Giant Pump Co., Oklahoma City, Oklahoma). The solution drained out from the bottom of the pot and was recaptured for reuse. Nutrient solutions were replaced weekly. Plants were monitored daily to document and photograph sequential series of symptoms on youngest, young, recently mature and mature leaves as they developed.

When the initial deficient symptom of each treatment occurred, three symptomatic plants were selected for sampling. The fully expanded leaves were sampled to evaluate the critical tissue concentration for each element. Harvested leaves were washed in a solution of 0.5 N HCl for 1 min and rinsed with deionized water. The remaining shoot tissue was harvested separately. Both sets of tissue were dried at 70°C for at least one week, and the weights were recorded. After drying fully expanded leaf tissue, it was ground in a Foss Tecator Cyclotec™ 1093 sample mill (Analytical Instruments, LLC, Golden Valley, MN) to pass a ≤0.5 mm sieve. Tissue analysis for N was performed with a C-H-N analyzer (Model 2400 series II, Perkin-Elmer, Norwalk, CT) by weighing 3.5 mg of dried tissue into tin cups and placed into the analyzer. Other nutrient concentrations were determined with inductively coupled plasma optical emission spectroscopy (ICP-OES; Model IRIS Intrepid II, Thermo Corp., Waltham, Mass.).

During the first harvest of nitrogen and calcium deficient plants, they were too small to obtain the necessary tissue from one plant; therefore, two plants were combined for a single sample. The experiment was terminated when the plants were in full bloom on 19 December, and non-symptomatic plants of the copper, manganese, molybdenum, and zinc plants were sampled for dry weight and nutrient levels. All the data were subjected to ANOVA using PROC ANOVA SAS program (SAS Inst., Cary, N.C.). Where the F test indicated evidence of significant difference among the means, LSD ($P \leq 0.05$) was used to establish differences between means.

RESULTS AND DISCUSSION

Plants were sampled on six separate harvest dates. Values for percentage difference in plant weights and tissue concentrations are presented.

Nitrogen

An overall pale green coloration was the initial symptom of nitrogen (N) deficiency. Dry weight was significantly different with N deficient plants being 25% smaller at harvest than the controls. The control N tissue concentration was 3.91% while the N deficient tissue was 1.80%. The control value was within the optimum range of 2.50 to 3.30%. The deficient tissue value was lower than the optimum range and greater than the deficient value of 1.51% (Holcomb, 1983). Symptoms progressed with the outer margin developing a stronger yellow coloration on the middle and lower leaves. The entire leaf then turned a lemon-yellow coloration. This symptom was followed by the leaf turning tan and becoming crispy. Our observations were similar to previous reports (Holcomb, 1983; PanAmerican, 2009) except the vein blackening and discoloration did not occur.

Phosphorus

The tips of lower leaves were necrotic for plants grown in phosphorus (P) deficient conditions. Yellow coloration surrounded this necrotic area. A significant difference occurred between the control and P deficient tissue. At sampling time P deficient plants were 45% smaller than the controls. Values for P concentrations of control and deficient tissue were 0.38 and 0.12%, respectively. The optimum range for P

is 0.36 to 0.81%. Thus, the control falls within that range; however, the P deficient tissue concentration was lower than the critical value of 0.22% (Holcomb, 1983). Symptoms progressed with lower leaves exhibiting interveinal chlorosis. Marginal browning continued to move inward on the leaf and the leaves began to curl inward. Some lower leaves became completely brown. Other than the bronze colored leaves and brown coloration in the veins, our observations match those reported earlier (Holcomb, 1983; Hartnett, 1993).

Potassium

Symptoms of potassium (K) deficiency began with marginal yellowing on the tips of both young and mature leaves. At initial sampling a significant difference did not occur between the dry weights of the K deficient tissue and the control tissue. Control plants had a control tissue concentration value of 6.23% for K while K deficient tissue had a value of 3.13%. The optimum range is 2.11 to 4.20% K (Holcomb, 1983). Thus, the control value falls above this range while the deficient value falls within this range. Within the yellow coloration of mature leaves necrotic spots formed on the margins. Some lower leaves became necrotic. At the termination of the experiment, plants were visually smaller than the controls. The symptoms reported by Holcomb (1983) were similar to those we observed.

Calcium

Initially, calcium (Ca) deficient plants developed necrotic spots on the lower leaf margins. There was no significant difference in control and Ca deficient dry weights. Values for the control and Ca deficient tissue were 0.65 and 0.51%, respectively. The control tissue value is within the optimum range of 0.62 to 1.01% while the Ca deficient tissue value was lower than this range. However, it is higher than the reported deficient level of 0.18% (Holcomb, 1983). Symptoms advanced with the lower leaves developing black veins, yellowing in the leaf margin, and lower leaf curling inward. The yellow coloration moved inward toward the midrib, and brown necrotic spots formed between secondary veins. Leaves then either retained a residual green coloration near the midrib, or they would turn brown and have a crispy texture. New leaves then developed necrotic margins. At the termination of the experiment, plants were visually smaller than the controls. Our observations were similar to Holcomb's (1983) and Erwin's (1999) observations; however, with our study additional symptoms were observed.

Magnesium

Magnesium (Mg) deficient plants exhibited a thin band of tan coloration on the very outside margin of the lowest leaves while larger mature leaves exhibited more of a yellow color in the margin. There was no significant difference in control and Mg deficient dry weights. Control Mg tissue concentration was 0.31% while the deficient tissue had a value of 0.17%. The optimum range of the values is 0.20 to 0.42%. Thus, the control value falls within this range. The Mg deficient tissue is lower than the range but higher than the deficient value of 0.09% (Holcomb, 1983). As symptoms progressed this band became wider, and bleaching began to occur within this marginal area. The margin became crispy as tan coloration progressed inward toward the midrib almost completely filling the interveinal areas. Younger leaves exhibited similar symptoms but to a lesser degree and remained mostly green. Plants were visually smaller when the experiment was terminated. Our observations match those described by Holcomb (1983) and PanAmerican (2009), but leaf cankers did not occur (Dole and Wilkins, 2005).

Sulfur

Sulfur (S) deficient plants initially developed a greenish-yellow coloration in the leaves. In older leaves it varied where it appeared either near the tip or near the petiole. In younger leaves yellowing was isolated to the margin of the leaf. Plant dry weights were not significantly different. S tissue concentrations for control and S deficient samples

were 0.54 and 0.12%. No optimal range or deficiency values have been reported for S. Symptoms progressed with the entire plant developing the greenish-yellow coloration. Necrosis then occurred on the lower leaf margins. Necrotic areas were a tan color. This symptom advanced until the entire leaf became necrotic. When the experiment ended, it was apparent that the deficient plants were visually smaller than the controls.

Boron

Boron (B) deficient plants exhibited a lack of elongation of young foliage and yellowing on the mature leaf margins. A significant difference was not observed in the dry weights. The control tissue value was 35.85 $mg \cdot kg^{-1}$ B which was similar to the reported optimum level of 35.90 $mg \cdot kg^{-1}$ (Holcomb, 1983). Deficient plants contained 4.47 $mg \cdot kg^{-1}$ B. Symptoms progressed with browning becoming prevalent in the young leaves and yellow coloration progressing inward toward the midrib. The bud then aborted and developed a black coloration, and the leaf margins became crispy with black coloration. Deficient plants were visually smaller at the end of the experiment. The initial distorted growth matches the symptoms described by Erwin (1999); however, symptoms progressed further in our study.

B toxicity plants initially exhibited tan coloration along the leaf margin of the lower leaves. Dry weights were not significantly different. Tissue B concentration for the control plants was 44.47 $mg \cdot kg^{-1}$ while it was 373.67 $mg \cdot kg^{-1}$ for the B toxicity plants. Both values were above the reported optimum value of 35.90 $mg \cdot kg^{-1}$ (Holcomb, 1983). Symptoms progressed with the tan coloration expanding inward, and the border area between the necrotic and healthy green tissue eventually developed a yellow band.

Iron

Iron (Fe) deficient plants exhibited yellowing in both young and old leaves. Yellowing was confined primarily to the margin and outer veinal areas. No significant difference was observed with the dry weights and tissue concentrations. Control and Fe deficient tissue had values of 148.11 $mg \cdot kg^{-1}$ and 95.95 $mg \cdot kg^{-1}$. Both values were within the optimum range of 78 to 155 $mg \cdot kg^{-1}$. Because of the small plant size, two plants were used per sample. One of the Fe deficient samples had a high value which resulted in a high standard deviation which was why no significant difference was observed. The mean value of the other two lowest samples was 69.24 $mg \cdot kg^{-1}$. This value is lower than the optimum range but higher than the reported deficient level of 60 $mg \cdot kg^{-1}$ Fe (Holcomb, 1983). No other symptoms were observed. In this experiment the plants manifested a yellow coloration instead of true interveinal chlorosis described by previous work (Holcomb, 1983; Hartnett, 1993; PanAmerican, 2009). Also, the plants did not progress to the white coloration and necrosis described by Holcomb (1983).

Zinc

Zinc (Zn) deficient plants initially exhibited tan or brown coloration on the leaf margins in isolated spots. A significant difference was not found between the dry weights for Zn. Control tissue had a Zn concentration of 23.57 $mg \cdot kg^{-1}$ while the deficient plants contained 13.71 $mg \cdot kg^{-1}$ Zn. Both of these values are lower than the reported optimal concentration of 38.55 $mg \cdot kg^{-1}$ (Holcomb, 1983). The coloration then began to expand inward as affected areas turned crispy.

Asymptomatic

The experiment was terminated when the plants reached full bloom after 8 weeks of growth. No visual symptoms were apparent with those plants grown under copper (Cu), manganese (Mn), and molybdenum (Mo) deficient conditions. To determine if non-visual differences due to the deficiency occurred, plants were sampled and analyzed for dry mass and tissue concentration. Of these three elements, all were significantly different for tissue concentrations, but no significant difference was observed with plant dry mass.

Control tissue Cu concentration was 5.04 $mg \cdot kg^{-1}$. The value for Cu deficient

tissue was 3.81 $mg \cdot kg^{-1}$. Both of these values are lower than the optimum reported value of 6.45 $mg \cdot kg^{-1}$ (Holcomb, 1983).

The tissue concentrations of plants grown under control and Mn deficiency conditions were 39.72 $mg \cdot kg^{-1}$ and 6.99 $mg \cdot kg^{-1}$, respectively. The optimum level for Mn is 49.70 $mg \cdot kg^{-1}$ (Holcomb, 1983). Both values are lower than this number. Hartnett's (1993) observation of new growth bleaching was not observed.

The control tissue concentration for Mo was 1.70 $mg \cdot kg^{-1}$ while Mo deficient tissue had a tissue concentration of 0.20 $mg \cdot kg^{-1}$. No value has been reported for Mo. Therefore, the value of 1.70 $mg \cdot kg^{-1}$ is considered to be an optimum value while 0.20 $mg \cdot kg^{-1}$ is a deficient value. More experiments would further elucidate these values.

Although the plants grown in Cu, Mn, and Mo deficient conditions exhibited no symptoms, the tissue concentration values will be of use to growers that need to determine if these plant nutrients are low.

CONCLUSIONS

Nutrient deficiencies were induced in *Primula acaulis* 'Danova Rose' which generated the described symptoms. Also, tissue samples taken when initial symptoms were observed provide critical tissue nutrient values for the crop. These data will allow growers to have a better grasp on diagnosing nutritional problems of this crop.

ACKNOWLEDGEMENTS

We are grateful for the funding support provided by USDA-ARS.

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Tables

Table 1. *Primula acaulis* 'Danova Rose' plant dry weight as affected by deficient or toxic nutrient treatments.

Treatment	-N	-P	-K	-Ca	-Mg	-S	-B	+B	-Cu	-Fe	-Mn	-Mo	-Zn
	Dry weight (g)												
Element	N	P	K	Ca	Mg	S	B	B	Cu	Fe	Mn	Mo	Zn
Complete control	0.35a[1]	0.57A	0.31a	0.35a	0.57a	0.31a	0.97a	3.53a	3.53a	0.31a	3.53a	3.53a	1.44a
Treatment	0.26b	0.32B	0.24b	0.24b	0.55a	0.24a	1.09a	2.93a	3.57a	0.30a	3.23a	3.81a	1.24a

[1] Significant differences between sample means based on *F* test are indicated by lower case letters if $P \leq 0.05$ or upper case letters if $P \leq 0.01$.

Table 2. *Primula acaulis* 'Danova Rose' tissue nutrient concentration as affected by deficient or toxic nutrient treatments.

Treatment	-N	-P	-K	-Ca	-Mg	-S	-B	+B	-Cu	-Fe	-Mn	-Mo	-Zn
	Tissue nutrient concentration (%)						Tissue nutrient concentration (mg kg^{-1})						
Element	N	P	K	Ca	Mg	S	B	B	Cu	Fe	Mn	Mo	Zn
Complete control	3.91A[1]	0.38A	6.23A	0.65A	0.31A	0.54A	35.9A	44.5B	5.00a	148.1a	39.7A	1.7A	23.5A
Treatment	1.80B	0.12B	3.13B	0.51B	0.17B	0.12B	4.4B	373.7A	3.81b	96.0a	7.0B	0.2B	13.7B

[1] Significant differences between sample means based on *F* test are indicated by lower case letters if $P \leq 0.05$ or upper case letters if $P \leq 0.01$.