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Nitrogen Fertilization as Ammonium or Nitrate-N on *Hippeastrum hybridum* Bulb Growth

Carlos Vazquez[1], Stewart T. Reed[2*], Christopher Dunn[2]

[1]School of Science, St. Thomas University, Miami Gardens, FL, USA
[2]USDA, ARS, Miami, FL, USA
Email: *stewart.reed@ars.usda.gov

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Abstract

***Hippeastrum* (*Hippeastrum hybridum*), a native of Central and South America, is a bulbous ornamental flowering plant in the Amaryllidaceae family. However, the correct balance of NH_4 to NO_3-nitrogen in a fertilizer mix for *Hippeastrum* plants is largely unknown. Nitrogen was applied 2x weekly following irrigation at either 0.6 g (high), 0.3 g (medium) or 0.15 g (low) total N every four months. Nitrogen was supplied in different combinations of NO_3 and/or NH_4. Nitrate:NH_4-N ratios were either 100% NO_3:0% NH_4 (100NO_3), 70% NO_3:30% NH_4 (70NO_3), 50% NO_3:50% NH_4 (50NO_3) (second group only), 30%NO_3:70%NH_4 (30NO_3), or 0% NO_3/100% NH_4 (100NH_4). Growth in bulb diameter after one year of fertilizer treatments not only increased from 0.15 to 0.6 g N (low to high level), but also differed with the form of N supplied to the plant. The largest diameter bulbs were produced in the 70NO_3 and 50NO_3 high N treatments. Within any NO_3/NH_4-N ratio grouping, fertilization at the high N rate resulted in larger diameter bulbs. No significant differences existed between treatments in the number of bulbs produced. Bulb growth was greater with a portion of N supplied as NO_3 than with NH_4-N alone. These results indicate that application of N as a mixture of NH_4 and NO_3 at 0.6 g per 4 months produces the largest increase in bulb diameter.**

Keywords

Hippeastrum, Amaryllis, Nitrogen Fertilization, Ammonium, Nitrate

1. Introduction

Hippeastrum (*Hippeastrum hybridum*), a native of Central and South America, is a bulbous ornamental flower-

*Corresponding author.

ing plant in the Amaryllidaceae family [1]. It features large and showy flowers that come in a variety of bright colors. *Hippeastrum* is a popular flower for Christmas and the New Year because of its combination of red petals and dark green leaves [2]. Bulbs are produced by the Netherlands, South Africa Israel, Japan, Brazil and the United States (Florida). *Hippeastrum* production in Florida for the 2012 season was valued at seven million dollars; centered mainly in Highlands County [3].

Ammonium as a sole source of nitrogen can result in a variety of negative effects to most plant species. These include but are not limited to a reduced photosynthetic rate, lower dry weights, reduced root growth, and a reduced rate of water uptake [4]-[7]. These studies were mostly on field crops where nitrification is presumed to be rapid. Nursery crops are often grown in soilless media that maybe pasteurized. The growth of ornamental shrub, "*Doublefile viburnums*" (*Viburnum plicatum* var. *tomentosum*), shoot and root was significantly lower with NH_4 (ammonium) supplied alone than that with NO_3 (nitrate) or a combination of NH_4 and NO_3 [8]. Hydroponically grown date palm seedlings had the greatest leaf area and root weight with NO_3 as the sole nitrogen (N) source but had a lower root:shoot ratio than with NH_4- or urea-N sources [9]. *Lilium* root growth was poor in liquid medium containing a high NH_4/NO_3 ratio, however, a high NH_4 content increased bulb enlargement [10]. In contrast, *Lilium* bulblets supplied with NH_4NO_3 (ammonium nitrate) depleted a hydroponic media of NH_4 very rapidly while NO_3 removal was at a steady state [11]. Uniform availability of NH_4^+ ions was thought to be important for optimum bulblet growth. A NH_4/NO_3 ratio of approximately 30:70 is thought to produce the best growth in most plants [4].

In *Hippeastrum*, bulbs grew best when fertilized with 25 mM N (as NH_4NO_3) and enriched with CO_2 (carbon dioxide) [12]. *Hippeastrum* bulbs are a strong sinks for nutrient accumulation during the initial stages of bulbil development. Once leaves begin to supply assimilates for plant growth, bulbs become a source of nutrients for the developing plant [13]. Similar results were reported for *Odontoglossum* hybrids [14]. When the N concentration was lower than that supplied to the control, the order of N content was leaves > roots > bulbs, but when the N concentration was higher than the control, the order was bulbs > leaves > roots. However, the correct balance of NH_4 to NO_3-nitrogen in a fertilizer mix for *Hippeastrum* plants is largely unknown.

2. Materials and Methods

The study was conducted at the Subtropical Horticulture Research Station (SHRS) in Coral Gables, FL (Latitude: N 25˚38.577815', Longitude: W 80˚17.545062').

Hippeastrum bulbs were grown in a greenhouse from seed collected from a self-pollinated tetraploid hybrid clone. Seed were germinated on a mist table in two groups planted approximately 30 d apart. The first (group 1) group of 150 plants was grown in the SHRS greenhouse 3 which has evaporative cooling; the second (group 2) group of 150 plants was grown in SHRS greenhouse 2 which is cooled by fans. Greenhouse 3 is approximately 10˚C cooler (mean daily temperature) than greenhouse 2. In addition, plants in greenhouse 3 received a starter fertilizer treatment resulting in larger bulbs at the onset of the study. Therefore, each group was treated separately. Soil was completely removed from plants in group 1 when bulbs were transplanted. On March 26, 2012, all plants were transplanted into 10 × 10 cm square, 450 mL pots containing a mix of 50% pine bark, 10% sand, 40% coir pith, fortified with 4.4 kg dolomite, and 3.2 kg of a micronutrient mix per cubic meter (6% Ca from calcium carbonate, 3% Mg from magnesium carbonate, 12% S, 0.10% B from sodium borate, 1% Cu from copper sulfate, 17% Fe from ferrous sulfate, 2.5% Mn from manganese sulfate, 0.05% Mo from sodium molybdate, 1.0% Zn from zinc sulfate). Phosphorus (P) and potassium (K) were supplied at a rate of 1 g triple super phosphate and 3 g K_2SO_4 per pot at planting and again every four months.

Nitrogen was supplied 2x weekly following irrigation with a syringe containing a solution with 0.241 M N for High concentration enough N to supply 0.6 g (high), 0.121 M N for Medium concentration 0.3 g (medium) or 0.060 M N for Low concentration 0.15 g (low) total N over a four month period. Nitrogen was supplied in different combinations of NO_3 and/or NH_4. Nitrate:NH_4-N ratios were 100% NO_3:0% NH_4 (100NO_3), 70% NO_3:30% NH_4 (70NO_3), 50% NO_3:50% NH_4 (50NO_3) (second group only), 30%NO_3:70%NH_4 (30NO_3), or 0% NO_3:100% NH_4 (100NH_4). Plants were irrigated with in-pot emitters 2x weekly to saturation plus a 10% leaching requirement during the hot months and once weekly when temperature cooled.

Initially measurements of bulb diameter were recorded on 17 Dec. 2012. Bulb number, diameter and weight were determined after 400 days of growth and again after 730 days. Growth is defined as the change in bulb diameter between measurements from the initial measurement to the diameter after 400 days.

A completely randomized design was utilized with five levels of ammonium-N to nitrate-N ratios, three different application rates, with eight plants per treatment for the first group. The second group had a completely randomized design with four nitrate-N to ammonium-N ratios with 12 plants per treatment. Each group was analyzed separately. Analysis of variance was performed on the data using the Mixed Procedure of Statistical Analysis System (SAS Institute, Cary, NC) and Least Squares Means values were separated by Tukey's test. Statistical tests were run of the model y = nitrogen source x application rate x source by application interaction x replication, where nitrogen source and application rate were fixed effects.

3. Results and Discussion

Table 1 shows the weight, diameter and number of bulbs produced for Group 1. Treatment 100NO_3M, followed by treatments 100NO_3H, 70NO_3M, 30NO_3H and 70NO_3H, produced the greatest bulb weights. Within a given NO_3 to NH_4 nitrogen source ratio, no significant differences between high and medium application rates occurred for bulb weight. The low application rate produced bulbs that were similar to (30NO_3L) or less than the medium rate. At both the high and medium application rate all treatments including NO_3 as a nitrogen source outperformed treatments using NH_4 as the sole source of nitrogen. Bulb diameter followed the same general trend as bulb weight. No differences existed between the high and medium application rates. Treatments receiving a portion of their nitrogen as NO_3 yielded larger bulb diameters than those receiving NH_4 as the sole nitrogen source at the high and medium application rate. Treatments receiving nitrogen only in the form of NH_4 produced the least number of bulbs.

In Group 2 the largest diameter bulbs were produced in the 70NO_3 and 50NO_3 high N treatments; however, for plants receiving a portion of their N as NO_3, no significant differences occurred between treatments at the high N rate (**Table 2**). Plants treated with 100% NH_4-N at the high rate produced bulbs similar in diameter to plants treated with a mix of NO_3 and NH_4-N supplied at the medium rate. Within any NO_3/NH_4-N ratio grouping, fertilization at the high N rate resulted in larger diameter bulbs than the medium or low application rate. At the medium (or low) application rate 100NO_3 produced larger bulbs than any other N treatment at that application level.

Bulb weight followed the same general trend as bulb diameter; at the high N rate a mixture of NO_3 and NH_4-N produced the greatest bulb weights, within any N application level, 100NH_4-N treatments produced the

Table 1. Diameter and weight of *Hippeastrum hybridum* bulbs fertilized with either 0.15 (L), 0.3 (M) or 0.6 (H) g nitrogen every four months as 100% NO_3-N, 70% NO_3/30% NH_4-N, 30% NO_3/70% NH_4-N, or 100% NH_4-N in group 1.

Treatment	Main bulb wt. (g)	Main bulb diameter (mm)	Total wt. (g)	Number of bulbs
100% NO_3-N(L)	90.3 cd[z]	47.7 cd	92.3 bc	1.9 ns[y]
100% NO_3-N(M)	161.4 a	56.1 ab	163.1 a	1.3 ns
100% NO_3-N(H)	146.3 ab	56.2 a	148.6 ab	1.4 ns
70% NO_3/30% NH_4-N(L)	91.0 cd	48.1 cd	91.0 bc	1.0 ns
70% NO_3/30% NH_4-N(M)	141.1 ab	53.7 abc	143.0 ab	1.7 ns
70% NO_3/30% NH_4-N(H)	131.8 abc	52.5 abc	131.8 ab	1.2 ns
30% NO_3/70% NH_4-N(L)	92.1 bcd	47.4 cd	92.1 bc	1.1 ns
30% NO_3/70% NH_4-N(M)	112.2 bcd	50.3 abcd	112.2 bc	1.2 ns
30% NO_3/70% NH_4-N(H)	138.1 abc	54.2 abc	138.9 ab	1.4 ns
100% NH_4-N(L)	68.7 d	44.6 d	69.1 c	1.1 ns
100% NH_4-N(M)	106.8 bcd	49.0 bcd	106.8 bc	1.0 ns
100% NH_4-N(H)	109.7 bcd	49.5 abcd	111.6 bc	1.3 ns

[z]Numbers in an individual column followed by the same letter are not significantly different at $P = 0.05$; [y]not significant

Table 2. Diameter and weight of *Hippeastrum hybridum* bulbs fertilized with either 0.15 (L), 0.3 (M) or 0.6 (H) g nitrogen every four months as 100% NO_3-N, 70% NO_3/30% NH_4-N, 50% NO_3/50% NH_4-N, 30% NO_3/70% NH_4-N, or 100% NH_4-N in group 2.

Treatment	Main bulb wt. (g)	Main bulb diameter (mm)	Total wt. (g)	Number of bulbs
100% NO_3-N(L)	41.6 cd[z]	33.8 cd	41.6 bc	1.1 ns[y]
100% NO_3-N(M)	76.2 ab	39.9 bc	77.6 ab	2.0 ns
100% NO_3-N(H)	88.5 a	43.4 ab	90.3 a	1.5 ns
70% NO_3/30% NH_4-N(L)	31.8 cd	28.6 de	31.8 bc	1.1 ns
70% NO_3/30% NH_4-N(M)	64.2 bc	30.0 bc	64.2 ab	1.5 ns
70% NO_3/30% NH_4-N(H)	96.9 a	47.1 a	96.9 a	1.3 ns
50% NO_3/50% NH_4-N(L)	35.5 cd	30.3 d	35.5 bc	1.0 ns
50% NO_3/50% NH_4-N(M)	54.4 bc	35.8 c	54.4 b	1.2 ns
50% NO_3/50% NH_4-N(H)	96.4 a	46.0 a	96.4 a	1.6 ns
30% NO_3/70% NH_4-N(L)	30.9 cd	28.8 de	30.9 bc	1.1 ns
30% NO_3/70% NH_4-N(M)	50.1 bc	34.6 cd	50.1 b	1.2 ns
30% NO_3/70% NH_4-N(H)	79.3 ab	42.5 ab	79.3 ab	1.3 ns
100% NH_4-N(L)	17.7 d	23.1 e	17.7 c	1.1 ns
100% NH_4-N(M)	32.2cd	28.5 e	32.2 bc	1.1 ns
100% NH_4-N(H)	48.9 bc	33.6 cd	48.9 bc	1.1 ns

[z]Numbers in an individual column followed by the same letter are not significantly different at $P = 0.05$; [y]not significant.

smallest bulbs, and bulb size increased with increasing N fertilization rates. Secondary bulbs were generally small and added very little to the total bulb weight; total bulb weight followed the same trend as main bulb weight. No significant differences occurred between treatments in the number of bulbs produced. However, the 100NO_3 treatments tended to produce the greatest number of bulbs and 100NH_4 the lowest within N application levels.

The number of bulb scales determines flowering in *Hippeastrum*. A flower bud is produced with every eight scales. Ephrath *et al*. [15] and Silberbush *et al*. [12] reported that bulb diameter was the best predictor of flowering capability and timing. **Figure 1** shows a box plot of growth in bulb diameter after one year of fertilizer treatments. An unfertilized treatment (zero N) is included for comparison. Zero N treatment only provided enough fertility to maintain initial bulb size with very little additional increase in bulb diameter. Since initial size of a bulb should not affect its increase in relative growth from one date to another, Groups 1 and 2 data are combined in **Figure 1**. Bulb growth responded not only to increase from 0.15 to 0.6 g N (low to high level) but also to the form of N supplied to the plant. The median increase in bulb growth was greater with a portion of N supplied as NO_3 than with NH_4-N alone. Treatments producing the five largest median bulb sizes all received a portion of their N as NO_3 applied at the high rate or with 100NO_3 treatment high and medium application rates. Bulbs from Group 1 are arranged in order of high N level treatment diameter mean (**Figure 2**). Root density was rated on visual appearance as low, medium or high. Plants receiving 50% or more of their N as NO_3 produced a denser root mass than plant receiving a majority of their N in the form of NH_4 (**Figure 2(a)**). Below 50% NO_3-N root density began to thin out. Root density was thinner at the low N fertility than at the high level (**Figure 2(b)**).

During the second year greenhouse temperatures were excessive and as a result bulb growth was inhibited. Although nitrogen source affected bulb diameter (**Table 3**) the 50NO_3L treatment was the only treatment with a significantly larger increase in diameter. All other treatments resulted in a similar change in bulb diameter from the previous year (data not shown). In all treatments except for 50NO_3L less than one cm of growth was recorded. Nitrogen source and application rate had a significant effect on changes in bulb weight.

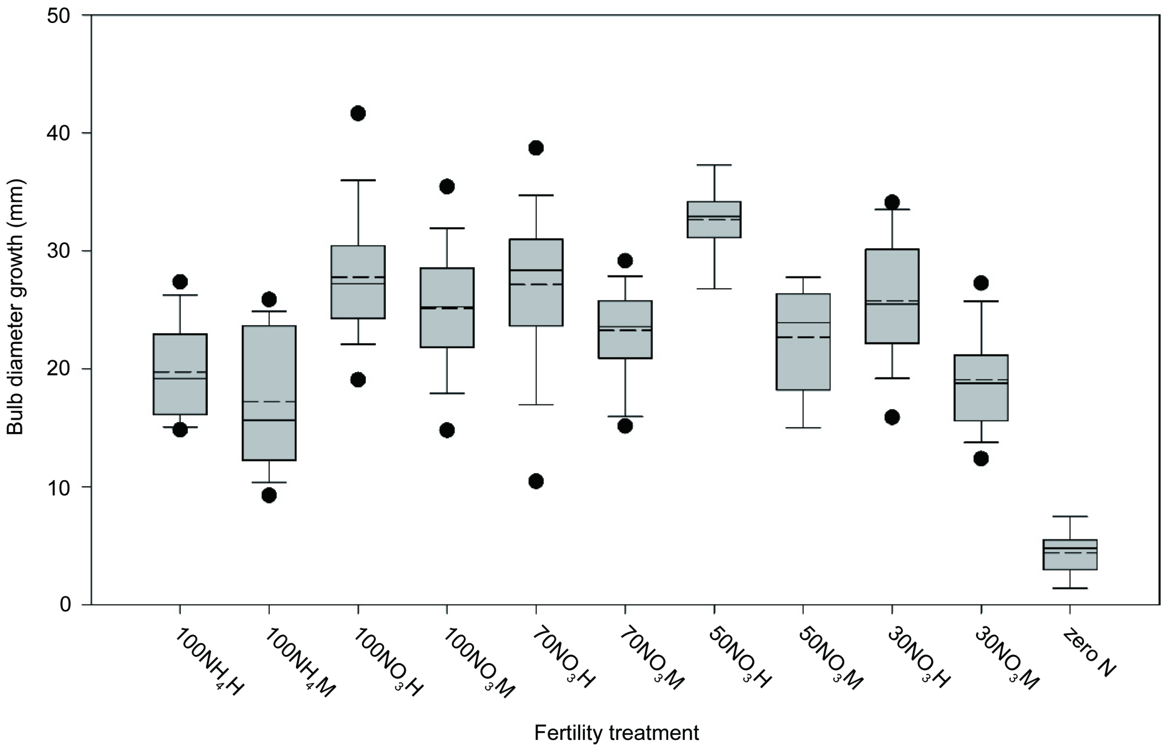
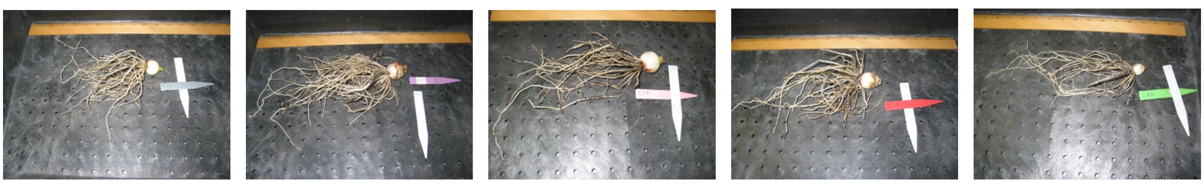


Figure 1. Box plot of *Hippeastum* bulb diameter growth from plants fertilized with 0%N, 100%NH4-N, 100% NO_3-N, or 70:30, 50:50 or 30:70 NO_3:NH_4 nitrogen ratios at 0.6 (H) or 0.3 (M) g per pot per 4-month period. Box incompases 25th through 75th percentiles, whiskers at the 5th and 95th percentiles, dash line is the mean, solid line the median, and circles represent outliers. Boxes above dotted line are from treatments producing the five largest increases in plant diameter.

70NO_3H 50NO_3H 100NO_3H 30NO_3H 100NH_4H

(a)



70NO_3L 50NO_3L 100NO_3L 30NO_3L 100NH_4L

(b)

Figure 2. *Hippeastrum* bulbs after one year of nitrogen fertilization totaling 1.8 g (a) and 0.6 g (b) applied in different ratios of nitrate to ammonium-nitrogen.

All five high nitrogen application rates and the 50NO_3M and 50NO_3L treatments were the only treatments that resulted in increased bulb weight after the second year (**Figure 3**). The reduction in bulb weight for the remaining treatments was attributed to extended periods of elevated summer greenhouse temperatures. Fertilization at the medium to low rates did not supply enough nutrients to support plant maintenance and additional growth with greater respiratory demand at high summer greenhouse temperatures. Bulb diameter and weight gain in year two were similar to that of the first year; 50NO_3H and 70NO_3H outperformed all other treatments.

Table 3. Mixed Model ANOVA for nitrogen treatment effects on bulb weight and bulb diameter.

Type 3 Tests of Fixed Effects Bulb Diameter				
Effect	Num DF	Den DF	F Value	Pr > F
Nitrogen source	5	273	2.9	0.0144
Application rate	2	273	0.91	0.4043
Source x rate	10	273	1.17	0.3077
Type 3 Tests of Fixed Effects Bulb Weight				
Effect	Num DF	Den DF	F Value	Pr > F
Nitrogen source	5	273	7.13	<0.0001
Application rate	2	273	40.3	<0.0001
Source x rate	10	273	0.93	0.5101

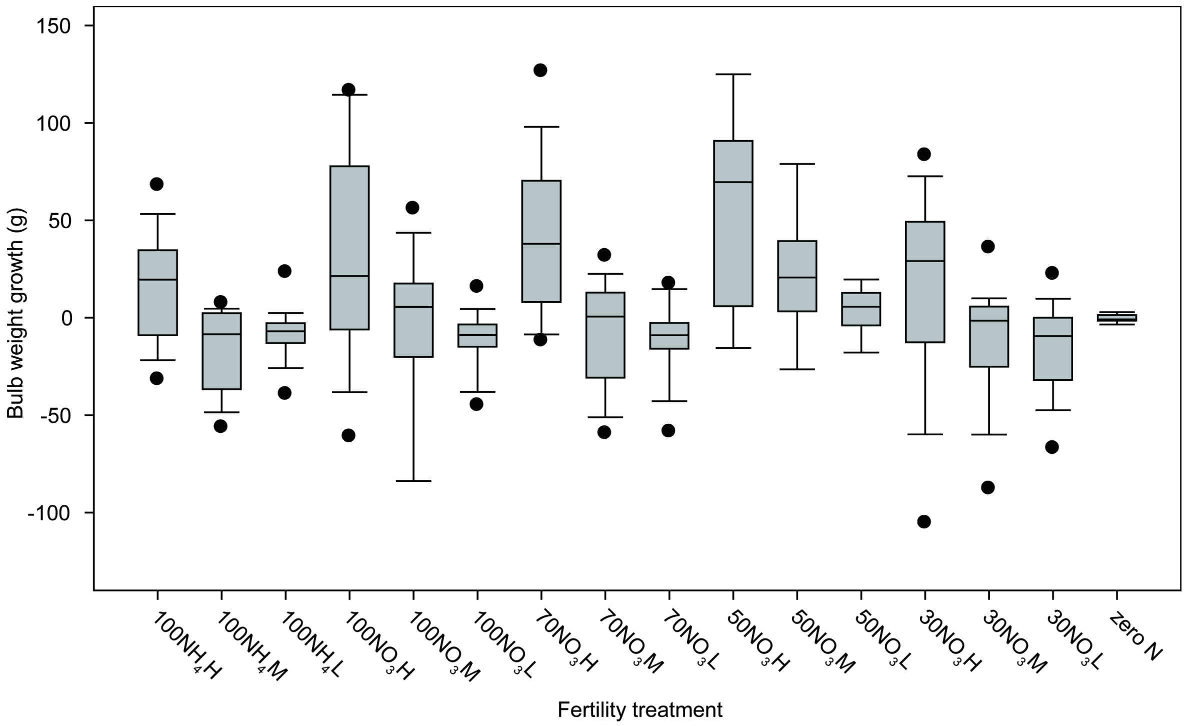


Figure 3. Box plot of *Hippeastum* second year bulb weight increase from plants fertilized with 0%N, 100%NH_4-N, 100% NO_3-N, or 70:30, 50:50 or 30:70 NO_3: NH_4 nitrogen ratios at 0.6 (H) or 0.3 (M) or 0.15 (L) g per pot per 4-month period. Box incompases 25th through 75th percentiles, whiskers at the 5th and 95th percentiles, dash line is the mean, solid line the median, and circles represent outliers. Boxes above dotted line are from treatments producing the five largest increases in plant diameter.

Nitrogen is a nutrient element that plants require in amounts larger than that of any other soil supplied element. Most plants can utilize both NH_4^+ and NO_3^-, however, NH_4^+ at high concentration can be toxic to plants [4]. Plants differ in their sensitivity to NH_4^+ toxicity. Onion, a member of the Amaryllidaceae Family, is known to be fairly tolerant to high concentrations of NH_4^+ [6] [16]. In our study *Hippeastrum*, a member of the Amaryllidaceae, suffered reduced bulb size when fertilized with ammonium as the sole source of N. The ammonium applied was not considered excessive, however, temperatures in the greenhouse were high compared to conditions that Amaryllidaceae were normally grown under in South Florida. High concentrations of ammonium reduce plant growth in a number of ways, including but not limited to: reduced stomatal conductance, increased utilization of reserve carbohydrates and the ability to neutralize reactive oxygen species [6] [17]-[19]. Each of these mechanisms can be enhanced at high temperatures. A long-term exposure to ammonium combined with elevated temperatures could easily account for the reduction in growth from ammonium as the sole source of N compared to other N sources with different nitrate concentrations. Based on these results, application of N as NH_4NO_3 at 0.6 g per 4 months produced the largest increase in bulb diameter.

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