The Effect of Altering Nitrogen and Sulfur Supply on the Growth of Cut Chrysanthemums

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ABSTRACT. ‘Dark Yellow Fuji Mefo’ chrysanthemums (*Dendranthema grandiflora* Tzvelev.) were grown hydroponically with either 64, 127, or 254 mg·L⁻¹ N and either 0, 1, 2, 4, 8, 16, 32, or 64 mg·L⁻¹ S in a randomized complete block. Time to flower was measured and symptoms of S deficiency were observed on root, stem, and leaf systems. New leaves and inflorescences were analyzed for S, and lower leaves were analyzed for N concentration. There were four sampling dates and two experiments. Flower diameter was measured when flowers were present, while stem length was measured every sampling date. Nitrogen application could be reduced by half to 127 mg·L⁻¹ as long as some S, 4 mg·L⁻¹ in the fall and 8 mg·L⁻¹ in the spring, was applied. Sulfur deficiency symptoms observed included branchless roots, which aged earlier, overall yellowing of new leaves, and reddening on the leaf abaxial starting from older leaves and moving acropetally. Plants receiving no S had smaller leaves, shorter stems, delayed inflorescence initiation, and restricted inflorescence development. Without S, plants did not produce flowers suitable for commercial sale.

Chrysanthemums are believed to originate in China (Crater, 1992; Kawata, 1992; Kofranek, 1992) and require large amounts of nutrients especially during the first 7 or 8 weeks of growth to ensure quality foliage and blooms (Boodley and Meyer, 1965; Laurie et al., 1969; Lunt and Kofranek, 1958). It has been proposed that high levels of N will promote early root growth, which will support the quality growth of the aboveground parts later (Laurie et al., 1969). Joiner and Poole (1967) indicated that high levels of N during early growth stages were necessary for chrysanthemum production, as a high frequency of fertilizer application during the later growth stage did not increase nutrient content of the tissues. Additionally, Boodley and Meyer (1965) showed that there was no radical change in leaf N concentration throughout a growth cycle. If the plants are fertilized adequately, the N level in leaves is from 4.0% to 4.5%. Levels of 2.25% to 2.75% cause slight deficiency. The level for serious deficiency is <2.0% (Lunt and Kofranek, 1958). Waters (1965) reported that N concentration of 3.5% to 4.5% in young mature leaves was optimal for quality chrysanthemum yields.

Adequate S level in leaf tissue of chrysanthemums was reported to be 0.3% to 0.75% with the critical S level at 0.25%; 0.07% to 0.19% would cause moderate to severe deficiency (Kofranek, 1992; Lunt et al., 1964). Winsor and Adams (1987) reported that 0.07% S was a deficient level and 0.19% was normal.

Laurie and Wagner (1940) studied the visual symptoms of S deficiency on chrysanthemums. Results included a dramatic reduction in plant height, chlorosis in younger and newly matured leaves, and delayed blooming. They also found that roots were abundant and branched if the plants received low amounts of S. Lunt et al. (1964) showed that roots of S-deficient plants were longer, unbranched, and had a yellow pigment present.

Previous studies with other crops have indicated that N and S metabolism were closely related (Adams, 1995; Friedrich and Schrader, 1978; Paparozzi et al., 1994). When S was absent, plants

reacted by reducing N metabolism. When an adequate level of S was used, less N was required for satisfactory plant growth. Reducing N applications could not only reduce groundwater contamination but may reduce plant susceptibility to disease (Chase, 1991).

The objectives of this research were to determine if the amount of N applied to chrysanthemums could be reduced by applying S and to typify S deficiency effects. To typify S deficiency effects, the minimum and sufficient amounts of S needed, growth parameters such as stem length and flower diameter, growth stages before and during flowering, and seasonal effects were investigated.

Materials and Methods

Rooted cuttings of ‘Dark Yellow Fuji Mefo’ chrysanthemum (Anderson, 1987) were obtained from Yoder Brothers Inc. and held under mist for no more than 2 weeks before being graded by leaf number, stem height, and root appearance. Plants were then grown hydroponically in 3.78-L black plastic pots sprayed white as described by Dale (1988). Pots were lined with two plastic bags and then covered with a 20.3 × 20.3 × 1.5-cm polystyrene bead-board lid. The lids were drilled with one or two holes, then small pieces of bead board were inserted into the holes to support the cuttings. The aboveground parts of cuttings were fixed to a support system, which was composed of wood strips and bamboo sticks, with twist ties to prevent stem distortion. Roots were gently washed with distilled-deionized water to remove any medium. Fifty cuttings, selected randomly, were separated into five groups to serve as initial samples for N and S concentration.

A randomized complete block with 3 benches each divided into 2 blocks (replications) was used to account for a temperature difference. Each block contained all 24 treatment combinations; 144 pots total. To allow for an additional early sampling, 8 pots per block (64 pots, 2 replications of each treatment) were selected randomly to be planted with 2 plants; all others held only 1.

Treatments were arranged factorially with three N levels—64, 127, and 254 mg·L⁻¹—combined with eight different S levels—0, 1, 2, 4, 8, 16, 32, and 64 mg·L⁻¹—for 24 N and S combinations. Nitrogen sources were potassium, magnesium, and/or sodium nitrate. The S source was magnesium sulfate. The concentrations of all remaining macronutrients and micronutrients were based on

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Fig. 1. Stem length measured at the fifth week after short day initiation (SDI), spring experiment. The statistical breakpoint is at 1 mg·L⁻¹ S.

Hoagland's solution (Dale, 1988; Hoagland and Arnon, 1950). Solution pH was initially adjusted to 5.5 to 6.5 with 85% phosphoric acid. Electric conductivity (EC) was 0.14 to 0.23 dS·m⁻¹ initially. Nutrient solutions were all changed at the same time either once or twice per week (if level dropped appreciably). At the end of the week, the pH of old solutions ranged from 5.6 to 7.2; EC was 0.13 to 0.26 dS·m⁻¹.

There were four sampling dates. One of the two plant pots was selected randomly for sampling at the end of vegetative growth (3 weeks after planting) from all six blocks. Then two whole blocks (replications) were sampled for the next three dates: the fifth week after short day initiation (SDI), the eighth week after SDI, and the ninth week after SDI.

Air temperature was ≈25/20 °C (day/night). A 2200 to 0200 HR night break of 100-W incandescent lights was supplied to keep plants vegetative during the first 3 weeks. Short days were imposed by pulling black cloth from 1700 to 0800 HR until all the buds showed color.

Data from spring (March to June) and fall (September to December) experiments are presented here. Data recorded included visual observations of roots, stems, and leaves; days to flower; stem length; and flower diameter. Flower diameter and stem length at the ninth week after SDI were compared only with the minimum saleable criteria for flower diameter (10 cm) and stem length (60 cm, yellow or grade 2) commercial standards for standard chrysanthemums (Floral Marketing Association and Society of American Florists, 1994). Timing of floral initiation was also recorded.

Newly expanded leaves and young stem tissue or inflorescences were harvested (Biddulph et al., 1958), air dried, ground, and digested as per Dale (1988), which was modified from Huang and Schulte (1985). The product was then measured for total S concentration using inductively coupled plasma emission spectroscopy (Dale, 1988; Dale et al., 1991). Lower leaves, not leaves of the original cutting, were oven dried, ground, and digested for N analysis using a micro-Kjeldahl method; an ammonia electrode was used to determine total N (Nelson and Sommers, 1980).

Analysis of variance was done by using SAS-GLM (SAS Institute, 1992) and SAS-IML (SAS Institute, 1985). Once short

days were initiated, sampling date was significant for all variables. Thus, each variable was analyzed by date. Contrasts were generated using SAS-GLM to determine significance and generate regression coefficients and response surfaces. When lack of fit was significant, which indicated that typical polynomial models could not adequately describe the surface, profile analysis on N and S levels was done. This analysis determined the breakpoint; that is the point at which further increases in N or S resulted in no significant change in the response. Coefficients for testing contrasts were used to generate a prediction model for stem length and were computed using the ORPOL function of IML (Huang, 1994). Graphs were generated using SAS-GRAPH (SAS Institute, 1988).

Results and Discussion

In both experiments, plant vegetative growth had the same overall appearance and growth pattern. All plants had healthy aboveground parts, regardless of treatment. However, roots of plants receiving no S were branchless regardless of N level applied. All plants receiving any S looked similar, with comparable heights and healthy, branched roots as per Laurie and Wagner (1940).

Once short days were started, plants receiving no S with any amount of N were yellow (new leaves) to pale green (older leaves), had smaller leaves, stunted height, brownish-red lesions on the

Table 1. Actual and predicted stem lengths at the ninth week after short day initiation (SDI), fall experiment. Using the prediction equation additional treatments for inclusion in future research were identified.

Nitrogen (mg·L ⁻¹)	Sulfur (mg·L ⁻¹)	Stem length (cm)	Predicted stem length ² (cm)
64	2	59.7 ^y	60.0
64	2	65.5 ^y	60.0
127	2	62.2 ^y	58.8
64	4	54.6	62.9 ^x
64	4	69.8 ^y	62.9
127	4	59.8 ^y	61.7
127	4	62.3 ^y	61.7
254	4	59.5 ^y	59.2
64	8	61.0 ^y	59.8
64	8	63.5 ^y	59.8
127	16	63.5 ^y	56.8
254	16	61.0 ^y	54.4
64	32	59.4	60.3 ^x
64	32	63.7 ^y	60.3
127	32	70.0 ^y	59.1
254	32	51.0	66.7 ^x
64	64	56.8	60.5 ^x
64	64	65.5 ^y	60.5
254	64	66.3 ^y	56.8
254	64	61.8 ^y	56.8

²Predicted stem length (cm) by the equation $YHAT = 51.785 - 0.019N + 6.9S - 1.25^2 + 0.082S^3 - 0.0021S^4 + 0.0000169S^5$.

^yTreatment combinations resulting in commercially acceptable stem lengths of ≈60 cm or greater.

^xAdditional treatment combinations that were identified using the prediction equation.

Fig. 2. Flower diameter measured at the ninth week after short day initiation (SDI), spring experiment. The statistical breakpoint is at 4 mg·L⁻¹ S.

stems, and reddening on the leaf abaxial. This reddening had started after 3 weeks in hydroponics and moved acropetally as the experiment progressed. In the spring study, lower leaves of plants receiving 1 or 2 mg·L⁻¹ S started to redden when the inflorescence was developing. This started on the original cutting leaves and gradually progressed to the younger leaves, increasing in severity over time. This anthocyanin production could actually be a S-induced N deficiency, as reddening has been reported as a typical N deficiency symptom in chrysanthemums (Lunt et al., 1964).

Any plant receiving S continued to increase in height after SDI. Symptoms of S deficiency, which had been obvious at vegetative growth stage, continued and became accentuated; e.g., branchless roots of plants receiving no S aged earlier and remained branchless as in Lunt et al. (1964).

STEM LENGTH. In the fall study, during vegetative growth, stem lengths of the plants receiving 0 mg·L⁻¹ S were shorter than those of plants receiving at least 1 mg·L⁻¹ S ($p > 0.0006$) (response similar to Fig. 1). However, in the spring experiment, treatments had no significant effect on stem length (14.5 to 22.5 cm) and all plant heights were about the same at the end of vegetative growth (data not shown; in Huang, 1994).

After SDI, however, stem length was significantly affected by sampling date; thus, separate analyses were run for each date. In fall, plants that received no sulfur regardless of N supplied were shorter at the fifth (Fig. 1) and eighth week after SDI ($p > 0.0001$). Thus, at least 1 mg·L⁻¹ S was needed for final stem lengths to approach commercial standards of 60 cm (Floral Marketing Association and Society of American Florists, 1994). However, by the ninth week after SDI, plants that were receiving either 0 or 1 mg·L⁻¹ S were still too short for the commercial market ($p > 0.0501$). Thus, at least 2 mg·L⁻¹ S is needed.

Applications of either 64 or 127 mg·L⁻¹ N, compared to 254 mg·L⁻¹ N, produced stem lengths that were as tall or taller than the commercial grade (60 cm, yellow or grade 2) (Floral Marketing Association and Society of American Flo-

rists, 1994). There was no difference between 64 and 127 mg·L⁻¹ N. Thus, N application can be reduced without affecting stem length. This response to N was the same for the eighth and ninth week after SDI (Table 1). Combinations of N and S that produced acceptable stem lengths are given in Table 1. Since some of the actual stem lengths were <60 cm, we also used a prediction equation to identify additional N and S combinations that should be included in any future research.

In spring, for all three sampling dates, stem lengths were significantly shorter if plants received no sulfur (data not shown). At the fifth and eighth week after SDI, plants receiving 64 or 127 mg·L⁻¹ N were taller than plants receiving 254 mg·L⁻¹ N. By the ninth week after SDI, plants receiving 127 mg·L⁻¹ N were taller than all others. Plants receiving 64 mg·L⁻¹ N were taller than plants receiving 254 mg·L⁻¹ N. Since all actual stem lengths were <57.6 cm, a model equation was again used. However, this equation did not

identify any additional N and S combinations.

FLOWER DIAMETER. Flower buds became visible during the fourth week after SDI. Flower buds on all plants receiving some S were visible on the same day. Inflorescence initiation of plants receiving no S was delayed at least 2 to 3 d. Inflorescence development of plants receiving 0 mg·L⁻¹ S was strongly inhibited and buds had not opened by the ninth week after SDI. In the spring study, inflorescences never showed color.

In fall, flower diameter at the eighth week after SDI was significantly larger for plants receiving 2 mg·L⁻¹ S or more ($p > 0.0153$; data not shown; response similar to Fig. 2). By the ninth week after SDI, plants receiving 4 mg·L⁻¹ S or more were larger ($p > 0.0361$). All plants receiving 1 mg·L⁻¹ S or more had flower diameters that met commercial standards of 10 cm (Floral Marketing Association and Society of American Florists, 1994).

In the spring, by the eighth and ninth weeks after SDI, plants receiving 4 mg·L⁻¹ S or greater produced flowers that were larger than the other treatments and were at commercial minimum diameters of 10 cm ($p > 0.0001$; data not shown; response similar

Fig. 3. Leaf S concentration measured during vegetative growth, spring experiment. The statistical break point is at 2 mg·L⁻¹ S.

Fig. 4. Leaf S concentration measured at the fifth week after short day initiation (SDI), fall experiment. The statistical breakpoint is at 2 mg·L⁻¹ S.

to Fig. 2). There was a linear effect of N on flower diameter, such that plants receiving more N had larger flowers ($p > 0.0501$). This trend changed at the ninth week after SDI, however, when plants receiving 127 mg·L⁻¹ N had flowers that had a significantly larger diameter than all others ($p > 0.0075$) (Fig. 2). Again, as in the fall, by the ninth week after SDI, all plants receiving any S produced commercially acceptable diameter flowers.

LEAF S CONCENTRATION. Leaf S concentration at the end of vegetative growth varied from 1600 to 4263 mg·kg⁻¹ in the fall study, and 1400 to 3370 mg·kg⁻¹ in the spring study (Fig. 3). In both experiments, leaf S concentration was affected by S level applied such that the break point in the profile analysis was at 2 mg·L⁻¹, regardless of N level applied (seen graphically as a plateau; $p > 0.0001$; Fig. 3). Thus, 2 mg·L⁻¹ S or more needs to be applied to obtain adequate leaf S concentration. We define the adequate leaf S as the concentration at which increasing amounts of N do not significantly increase the leaf S concentration. Interestingly, these leaf S concentrations are cited as deficient (0.07% to 0.19%) (Kofranek, 1992; Windsor and Adams, 1987). However, no deficiency symptoms occurred at this time. This may be due to high initial S concentration in leaves. Thus, as with hydroponically grown vegetative poinsettias, the aboveground parts had similar height and did not show any symptoms of S deficiency even though the foliage levels were deemed deficient (Dale, 1988). There was no S × N interaction. This again indicates that N may be reduced to 127 or 64 mg·L⁻¹ without effecting leaf S concentration.

For the fall and spring studies, samples taken at the fifth week after SDI had the largest concentrations of S. In the fall, at the fifth week after SDI, S applied needed to be at least 2 mg·L⁻¹ ($p > 0.0001$) (Fig. 4). At the eighth week after SDI, S applied needed to be >2 and 8 mg·L⁻¹ ($p > 0.0509$; cubic response; data not shown), and, at the ninth week after SDI, S applied needed to be at least 4 mg·L⁻¹ for adequate S concentration ($p > 0.0146$; response similar to Fig. 4 except breakpoint at 4 mg·L⁻¹). This was regardless of N

level applied. Thus, again, N application may be reduced as long as S is supplied.

In the spring study at the fifth week after SDI, there was a N × S interaction ($p > 0.0108$) such that, as N and S applied increased, there was an overall increase in S concentration (Fig. 5). The response approached 8 mg·L⁻¹ S applied before beginning to plateau. At the eighth week after SDI, not only were the levels of S concentration dramatically lower, but there were no differences in S concentration due to N or S applied. This is probably due to the small sample size of lowest S treatments. At the ninth week after SDI, for adequate S concentrations at least 8 mg·L⁻¹ S needed to be applied ($p > 0.0023$; data not shown; response similar to Fig. 4 except breakpoint at 8 mg·L⁻¹). Skewed values at 0 mg·L⁻¹ S applied were obtained due to extremely small sample sizes. Thus, during the spring, S applied may need to be greater than during the fall.

LEAF N CONCENTRATION. In the fall, leaf N concentration ranged from 3.4% to 6.3% and was affected by S level applied. Sulfur needs to be applied at least at 2 mg·L⁻¹ to keep leaf N concentration at a sufficient level during vegetative growth stage ($p > 0.0357$; Fig. 6). In the spring experiment, leaf N concentration at the end of vegetative growth was 37,649 to 64,792 mg·kg⁻¹ and was also affected by S and N level applied, the interaction and gave a response similar to Fig. 6 (except breakpoint at 1 mg·L⁻¹; data not shown). Sulfur applied should be at least 1 mg·L⁻¹ to ensure adequate growth ($p > 0.0024$). The contrast N linear was significant ($p > 0.0010$); as N level increased, N leaf concentration decreased such that either 64 or 127 mg·L⁻¹ N applied produced more N in the foliage than 254 mg·L⁻¹ N applied. There was no difference between 64 and 127 mg·L⁻¹. Adequate N level in leaves of chrysanthemums was stated as 3.5% to 5.0% (Boodley and Meyer, 1965; Lunt and Kofranek, 1958; Waters, 1965). Nitrogen values in these experiments, 3.4% to 6.3% (fall) and 3.9% to 6.5% (spring), fell within the adequate range. Thus, reducing N applied did not cause N deficiency.

In the fall, at the fifth, eighth, and ninth weeks after SDI, the N

Fig. 5. Leaf S concentration measured at the fifth week after short day initiation (SDI), spring experiment.

Fig. 6. Leaf N concentration measured during vegetative growth, fall experiment. The statistical breakpoint is at 2 mg·L⁻¹ S.

and S levels applied affected leaf N concentrations such that 127 mg·L⁻¹ N produced greater leaf N concentrations than 254 mg·L⁻¹ N ($p > 0.0100$; data not shown; similar to Fig. 6). There was no difference in leaf N concentrations between 64 and 127 mg·L⁻¹ N applied. Applications of at least 4 mg·L⁻¹ S ($p > 0.0248$) gave similar N leaf concentrations for the fifth week after SDI. At the eighth week after SDI, 2 mg·L⁻¹ S was needed and at the ninth week only 1 mg·L⁻¹ was needed to ensure adequate N concentration ($p > 0.0002$; data not shown; response similar to Fig. 6 except breakpoint at 2 and 1 mg·L⁻¹).

In the spring, at the fifth week after SDI, there was a N × S interaction such that, at 64 mg·L⁻¹ N applied, regardless of S applied, leaves contained less N than 127 or 254 mg·L⁻¹ ($p > 0.0001$; Fig. 7). Overall, S levels of at least 4 mg·L⁻¹ ($p > 0.0014$) were needed for optimal N concentration.

At the eighth and ninth weeks after SDI, N levels of 64 mg·L⁻¹ consistently provided less leaf N concentration than either 127 or 254 mg·L⁻¹, despite S addition. There was no difference between 127 and 254 mg·L⁻¹ N applied at the eighth week after SDI; but, at the ninth week after SDI, the combinations 127 mg·L⁻¹ N and 16 mg·L⁻¹ S stood out (Fig. 8). At the eighth week after SDI, S applied needed to be at least 8 mg·L⁻¹ to give optimal leaf N concentration ($p > 0.0566$; data not shown). At the ninth week after SDI, S applied needed to be at least 4 mg·L⁻¹ ($p > 0.0193$; Fig. 8).

Empirically, it has been shown that, during vegetative growth, applying 1 to 2 mg·L⁻¹ S gave adequate N and S concentration in chrysanthemums. However, during the spring, at 5 weeks after SDI, visual deficiency symptoms occurred at these levels. Thus, a minimum of 4 mg·L⁻¹ S is recommended. The actual S concentration values (fifth week after SDI) associated with plants receiving 0, 1, and 2 mg·L⁻¹ S put the deficient range below 1780 mg·kg⁻¹ (spring). In the fall, 2700 mg·kg⁻¹ leaf S concentration caused deficiency symptoms when no S was applied. The spring data concur with previously published levels (Kofranek, 1992; Lunt et al., 1964; Windsor and Adams, 1987). The discrepancy in the fall

data may be due to higher initial S concentration of cuttings, higher N levels associated with treatments containing any S, different sampling date, or season.

Over all, these results also indicate that the vegetative growth stage is indeed a critical nutrient time, particularly for S, as deficiency will not become apparent until after SDI. It should be noted that, during the vegetative stage of hydroponically grown chrysanthemums, S deficiency can only be diagnosed by looking at the roots or destructively sampling the new leaves. Additionally, in these experiments, the fifth week after SDI (just as buds were forming) was also an important time for plants to receive N and S. This is recommended as the best sampling time for N and S concentration, as this was when the S concentrations were the highest and the N × S interaction tended to be significant.

From this data, there also appears to be a relationship between the N and S applied, plant tissue N and S concentration, and seasonal growth. Future research is needed to include more levels of applied N and S to clarify this relationship. Also, nutrient levels applied could change when plants are grown in a potting mix. However, as evidenced by our work with poinsettias, N and S hydroponic experiments can be good predictors of N and S application rates for soilless mixes (Adams, 1995).

In conclusion, the amount of N applied to hydroponic chrysanthemums can be reduced at least by half to 127 mg·L⁻¹ N, possibly lower during the fall, without causing reduced stem length, delayed inflorescence initiation, or reduced flower diameter. During the vegetative and flowering growth stages, to obtain commercially acceptable stem lengths, at least 2 mg·L⁻¹ S and any level of N at or above 64 mg·L⁻¹ N must be applied. During the vegetative and flowering growth stages, to obtain commercially acceptable flower diameters, at least 4 mg·L⁻¹ S and any level of N at or above 64 mg·L⁻¹ N must be applied. For adequate S and N leaf concentrations during vegetative growth, at least 2 mg·L⁻¹ S with either 64 or 127 mg·L⁻¹ N was needed. At 5 weeks after SDI (flower bud initiation), generally, fall-grown plants will need at least 4 mg·L⁻¹ S

Fig. 7. Leaf N concentration measured at the fifth week after short day initiation (SDI), spring experiment. The statistical breakpoint is at 4 mg·L⁻¹ S.

Fig. 8. Leaf N concentration measured at the ninth week after short day initiation (SDI), spring experiment. The statistical breakpoint is at 4 mg·L⁻¹ S.

and spring-grown plants will need at least 8 mg·L⁻¹ S combined with 127 mg·L⁻¹ S. These amounts will continue to provide enough S and N through the ninth week after SDI. Sulfur-deficient plants had shorter stems and were slower to flower and had inadequate leaf S concentrations. Plants receiving no S did not flower.

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