The Effect of Root-zone Nitrogen concentration on Corn

Jason Clark

Abstract

Nitrogen (N) is a major enzyme constituent making it a determining factor in plant yield and health. When a plant is deficient in N the shoot:root ratio typically decreases, chlorosis occurs, and growth is reduced. The objective of this study was to determine the level of nitrogen needed for optimal growth of corn. Plants were grown in containers and watered with 0, 0.3, 1.0, 3.0, and 10 m*M* N for 34 days. Chlorophyll content, fresh, and dry mass measurements were then taken. Chlorophyll content increased with increasing N. Fresh and dry weight also increased rapidly up to three mM N and increased gradually up to 10 m*M N*. The 3 mM treatment supplied enough N to nearly optimize growth. The chlorophyll produced above three mM N did not significantly increase growth.

Introduction

Nitrogen is the fourth most abundant element in plants and composes 1-5% of total plant dry matter (Hawksford et al., 2012). N is a major part of amino acids, proteins, enzymes, nucleic acids, cell walls, chlorophyll, phytohormones, and secondary metabolites (Hawksford et al., 2012; Schrader, 1984). Nitrogen is involved in both structural components and metabolic reactions making it a large factor in determining a plants yield and health. If too much N is added to the soil the excess N can be leached out of the rooting zone and contaminate ground water.

When a plant has a sufficient amount of N most of its resources will be moved to the shoot. When N is deficient the plant will move more of its resources to the roots in order to explore more of the soil environment to find the N needed. This lowers the shoot to root biomass ratio (Anandacoomaraswamy, 2002).

When the plant is not able to resupply itself with the needed N through increased root production it will start to hydrolyze nucleic acids, proteins, chlorophyll, and other N containing compounds leading to leaf senescence (Hortensteiner and Feller, 2002). Since chlorophyll contains N, the plant will also begin to be chlorotic. Plants naturally breakdown proteins and move them from the older plant parts to the younger ones, so chlorosis will usually appear first on the older leaves and then move to the younger leaves (Schrader, 1984). Around 75% of the N in mesophyll cells is located in the chloroplasts where that N is used mainly in the enzyme Rubisco, which plays a large role in photosynthesis (Peoples and Dalling, 1988). When Rubisco is broken down, the plants ability to photosynthesize decreases which inhibits growth (Hawksford et al., 2012). For these reasons chlorosis and stunting are the two main visual symptoms of N deficiency.

Since N is largely found in chlorophyll, a chlorophyll meter can be used to estimate the amount of N in the plant. A chlorophyll meter measures the amount of N in the leaf by comparing 650 nm wavelenghts that relate to chlorophyll activity and 940 nm wavelengths that calibrate the instrument by helping it compensate for leaf thickness and water status (Hawkins et al., 2007; Rambo, 2010). Studies by Girardin et al. (1985), Wolfe et al. (1988), Lohry (1989),

and Wood et al. (1992) have shown a positive correlation between leaf N concentration and chlorophyll content validating the use of a chlorophyll meter to measure N in plants. Rambo (2010) used a chlorophyll meter, chlorophyll fluorescence, leaf area, and canopy reflectance to measure chlorophyll in the plant and determined that measuring leaf chlorophyll with a chlorophyll meter is the best way to measure the necessity of corn N, and that chlorophyll meters work best when they are referenced with a plant that is known to have sufficient N.

The objective of this study is to determine the level of N needed by an individual corn plant to produce the highest yields. Because N is needed for structural and metabolic components in the plant it is hypothesized that chlorophyll content, wet weight, and dry weight will increase with increasing N levels until there is excess N. When N becomes excess, the factors mentioned will level out meaning that any additional N will not increase any of the factors significantly. The plants that have an insufficient amount of N will be chlorotic and stunted, and these two symptoms will increase in the plants that have a higher deficiency of N.

Materials and Methods

This study was conducted at the Research Greenhouse located in Logan, UT, on Utah State University's campus. The experiment was set up as a completely randomized design with N level being the randomized variable. N levels were 0, 0.3, 1.0, 3.0, and 10 mM. $CaNO_3$ was used to supply the N to the nutrient solution.

A 4 x 4 x 5 inch pot was filled with a peat/vermiculite soil mixture and a corn seed was planted two inches deep on October 16, 2012. The greenhouse temperatures were set at 25°C day and 20°C at night. Each pot was watered thoroughly with a nutrient solution containing all of the essential nutrients except N (Table 1). N was added to the nutrient solution at the above rates. Plants were watered with the nutrient solution and the added N approximately twice a week for the first five weeks and three times the sixth week.

Table 1. Nutrient solution (Bugbee, 2004)

Salt	Stock Concentration	mL per 100 L	Final Concentration
K_2SO_4	0.5 M	200	2 mM
KH_2PO_4	0.5 M	40	0.2 mM
$MgSO_4$	1 M	100	1.0 mM
K_2SiO_3	0.1 M	100	0.1 mM
HEEDTA	100 mM	25	25 uM
$FeCl_3$	50 mM	20	10 uM
$MnCl_2$	60 mM	10	6 uM
$ZnCl_2$	20 mM	50	10 uM
H_3BO_3	40 mM	50	20 uM
$CuSo_4$	20 mM	20	4 uM
Na_2MoO_4	1 mM	10	0.1 uM

On November 19, 2012 an Opti-sciences chlorophyll content meter was used to determine the amount of chlorophyll in each plant by taking three readings on the last fully expanded leaf (Rambo, 2010). These numbers were averaged to determine the mean chlorophyll content index for each plant. Each plant was then harvested by cutting the stem right above the soil surface and weighed to determine fresh weight. Dry weights were determined after each plant had been dried in an 80°C force air dry oven for 48 hours. Percent dry weight was calculated by dividing fresh weight by dry weight.

Results

The chlorophyll content index (CCI) increased with each level of N as shown in Table 2 with the 0 m*M* level being the lowest and 10 m*M* being the largest. The three lower levels showed a smaller difference between each level where as the difference between one and three is larger. The difference between three and ten is even larger (Figure 1).

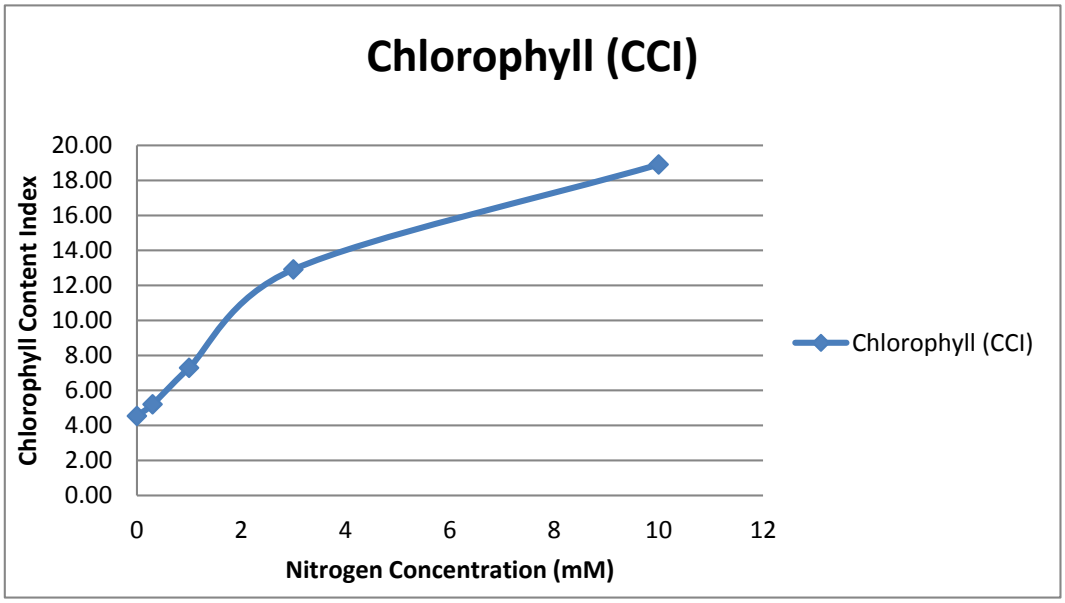


Figure 1. The effect of N concentration on CCI

The fresh weight of the plants followed a similar pattern of fresh weight increasing with N level (Figure 2; Table 2). The 0 mM level was the lowest weight and 10 mM was the largest. Dry weight also increased with N level. Percent dry weights were all within one percentage point (Table 2).

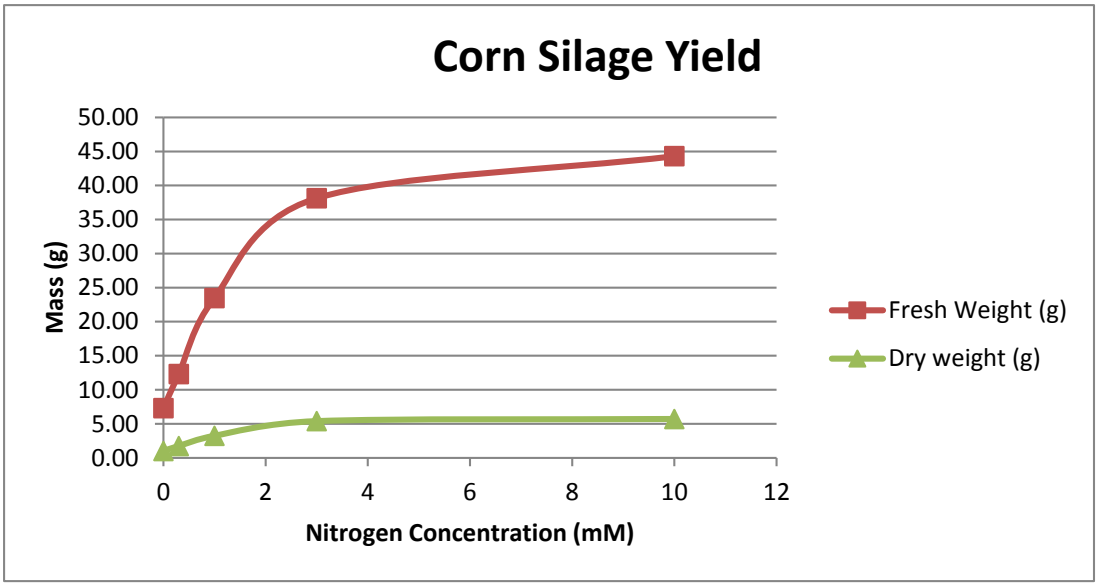
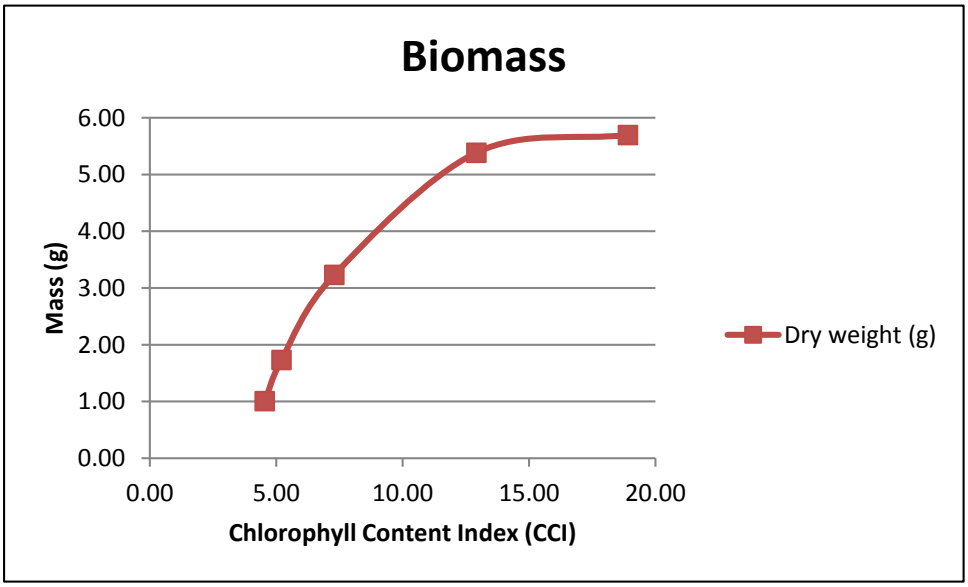


Figure 2. The effect of N concentration on fresh and dry weight of corn.



s CCI
t the Mass
ee mM N

Table 2. The effect of N concentration on CCI, fresh weight, dry weight, and % dry weight.

Concentration	Chlorophyll (CCI)	Fresh Weight (g)	Dry weight (g)	% Dry weight
0	4.54	7.29	1.01	14%
0.3	5.21	12.30	1.73	14%
1	7.29	23.47	3.23	14%
3	12.92	38.13	5.38	14%
10	18.91	44.30	5.70	13%

Chlorosis of the older leaves is strongly evident in the 0, 0.3, and 1.0 m*M* N levels as shown in Figure 1. The 3.0 m*M* N level does not have leaves that are extremely chlorotic, but it is a lighter green color than the 10 mM N level plant. Figure 4 clearly shows that as N level decreases there is an increase in the amount of stunting of each plant.

Figure 4. Corn plants on day of harvest (day 34). The treatments from left to right were 0, 0.3, 1, 3 and 10 mM. Note the increasing chlorophyll concentration with increasing N level.

Discussion

The chlorosis of the older leaves of the plants in this study supports the theory put forth in Hawksford et al. (2012) that as the N supplied to the plants was no longer sufficient to produce the needed proteins and enzymes the plants began to break down these molecules in the older leaves and transport the needed N to the younger leaves. The younger leaves were then able to produce the proteins and enzymes to create chlorophyll, which is evident by the greenness of these younger leaves while the older leaves turned chlorotic. Even with the transporting that was done in each of the plants as N levels decreased the CCI also decreased (Table2; Figure 1) showing that the plants still did not have enough N to produce the amount of chlorophyll as the highest N level plants that showed no or only slight symptoms of chlorosis in the leaves.

The pattern of increased fresh and dry mass as N level increased is likely associated with the fact that N is a large component of the pigment complexes, light harvesting proteins, and

the enzyme Rubisco (Evans, 1988). As N level increased more of these metabolic compounds were produced increasing the photosynthetic ability of the plant. These changes likely increased photosynthesis, which would provide the energy needed to produce the structural and metabolic compounds needed for plant growth. This resulted in plants that were taller with larger leaves whereas the plants with lower N levels were stunted with smaller leaves.

In this study, fresh weight, dry weight, and CCI index increased up to the three mM level of N. Then fresh and dry weight (Figure 2) began to level out suggesting that the optimal N level is around three mM and anything past that is excess. This is further supported by the results shown in figure 3 where biomass increased rapidly up to the CCI produced at the 3 mM N level and then only slightly increased. The fact that CCI and biomass continued to slowly increase between three and ten mM (Table 2; Figure 1) indicates that if N is present the plant will produce more chlorophyll and increase in biomass, but in very low amounts after an optimal point that was shown to be three mM N in this study supporting our hypothesis that that increased N will increase yield rapidly until N becomes excess then only slight increases will be made. Future studies should include more N levels between three and ten mM concentrations to further refine the optimal N concentration. Higher levels of N will need to be used in further research to determine at what point the plant will have enough N so that CCI will no longer increase but level out showing that past that point the plant can no longer use the N to make chlorophyll. Measurements of plant height and leaf area would also be useful to further quantify the effects of increased N and help refine the optimal N level.

Literature Cited

Anandacoomaraswamy, A., W.A.J.M. Decosta, P.L.K. Tennakoon, and A. VanDerWerf. 2002. The physiological basis of increased biomass partitioning to roots upon nitrogen deprivation in young clonal ea (*Camellia sinensis* (L.) O. Kuntz). Plant and Soil 238: 1-9.

Bugbee, B. 2004. Nutrient Management in Recirculating Hydroponic Culture. In: Proceedings of the South Pacific Soilless Culture Conference. M. Nichols, ed. Acta Hort 648: 99-112

Evans, J.R., 1988. Acclimation by the thylakoid membranes to growth irradiance and the partitioning of nitrogen between soluble and insoluble proteins. Aust. J. Plant Physiol. 15: 93-106.

Girardin, P., M. Tollenaar, and J.F. Muldon. 1985. The effect of temporary N starvation on leaf photosynthesis rate and chlorophyll content of maize. Can. J. Plant Sci. 65:491-500.

Hawkins, J.A., J.E. Sawyer, D.W. Barker, and J.P. Lundvall. 2007. Using relative chlorophyll meter values to determine nitrogen application rates for corn. Agron. J. 99:1034-1040. doi:10.2134/agronj2006.0309.

Hawksford, M., W. Horst, T. Kichey, H. Lambers, J. Schjoerring, I. Skrumsager Moller, and P. White, 2012. Functions of Maronutrients. P. 135-149. In: P. Marschner (ed.) Marschner's

Mineral Nutrition of Higher Plants. doi: 10.1016/B978-0-12-384905-2.00006-6. Elsevier, San Diego, CA.

Hortensteiner, S., U. Feller. 2002. Nitrogen metabolism and remobilization during senescence. J. Exp. Bot. 53: 927-937.

Lohry, R.D. 1989. Effect of N fertilizer rate and nitrapyrin on leaf chlorophyll, leaf N concentration, and yield of three irrigated maize hybrids in Nebraska. Ph.D. Dissertation University of Nebraska, Lincoln, NE.

Peoples, M.B., M.J. Dalling. 1988. The interplay between proteolysis and amino acid metabolism during senescence and nitrogen reallocation. P. 181-217. In L.D. Nooden and A.C. Leopold (ed.) Senescence and aging in plants. Academic Press, San Diego, CA.

Rambo, L., B. Ma, Y. Xiong, and P. Regis Ferreira da Silvia. 2010. Leaf and canopy optical characteristics as crop-N-status indicators for field nitrogen management in corn. J. Plant Nutr. Soil Sci. 173: 434-443.

Schrader, L.E., 1984. Functions and transformations of nitrogen in higher plants. P. 55-66. In R.D. Hauck (ed.) Nitrogen in Crop Production. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI.

Wolfe, D.W., D.W. Handerson, T.C. Hsiao, and A. Alvino. 1988. Interactive water and nitrogen effects on senescence of maize. II. Photosynthetic decline and longevity of indiviueal leaves. Agron. J. 80: 865-870.

Wood, C.W., D.W. Reeves, R.R. Duffield, and K.L. Edmisten. 1992. Field chlorophyll measurements for evaluation of corn nitrogen status. J. Plant Nutr. 15: 487-500.