Distribution of Nitrate Nitrogen in the Blades and Petioles of Sugar Beets Grown at Deficient and Sufficient Levels of Nitrogen[1]

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It has been generally found that N moves from older to younger tissue, especially when plants are deficient in N. Mason and Phillis (4)[4] found that N moves from the older leaves and petioles upward to the young leaves and the stem when cotton plants are deficient in N. Williams (15) found the same was true for oats. In a study of the tobacco plant Watson and Petrie (14) showed that N accumulated by each leaf subsequently underwent net export to organs higher in the acropetal series. They attributed this net export to concentration gradients between the organs and tissues involved. Walkley and Petrie (13) found this loss of N in older leaves of barley was due to a decline in the amounts of proteins and amino-acids with age. From this and other work with total N and water-soluble forms of N it has been shown that old leaves decline in total N. However, in the case of NO_3-N it has been indicated that it may be high in the older tissue even when plants are grown under N deficient conditions (2,7,12).

In sugar beets high amounts of NO_3-N are generally present in the petioles and blades. Ulrich (8,10) has used a reference point of 1,000 parts per million NO_3-N of recently matured sugar beet petioles to distinguish between N deficient and non-deficient sugar beets. Other workers (1,5) have reported the presence of high amounts of NO_3 in plants.

It has been concluded (6) that NO_3-N is apparently freely absorbed by uninjured roots and it may accumulate in the plant in enormous quantities without injury. The unequal distribution of NO_3 in the aerial portion of plants was shown by Emmert (2). He found that the younger the tissue, the lower the NO_3 concentration. It was concluded that rapid utilization in the growing points resulted in a lower NO_3 concentration. Ulrich (12) has reported that NO_3 is unequally distributed in the aerial portions of sugar beets. He found that old and mature petioles contain high concentrations of NO_3 even when the immature

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leaves are deficient in NO_3. It was concluded that if the best measure of the N status of the sugar beet plant is to be achieved, both the young and the mature petioles should be analyzed. In more recent work with sugar beets Sorensen (7) found the highest content of NO_3-N in the outer petioles and attributed this to a comparatively slow metabolism of the older leaves. He also found that the dry matter content was highest in the middle position petioles.

The purpose of this work was to investigate the distribution of NO_3-N and dry matter in the aerial portions of sugar beets grown with deficient and sufficient amounts of N.

Materials and Methods

Plant Culture Hybrid sugar beet seeds (F58-554H1 - MS of NB1 × NB4) were treated with a fungicide Phygon XL at a rate of 1% by weight and planted on October 14, 1964. The seeds were germinated in quartz sand in the greenhouse and watered as needed with 1/4 strength nutrient solution (11) prepared with C.P. salts and distilled water. On October 26 seedlings, held firmly by a dacron wool wad in the center of a polystyrene ring, were transferred at random to 3-hole masonite covers placed on 20-liter containers. The covers were painted on the upper surface with aluminum paint and the lower surface with valspar. The containers were painted on the outside with aluminum paint and the inside with Amercoat No. 33, a non-toxic plastic coating (9). Each pot supported 3 plants and contained 20 liters of nutrient solution.

At the start of the experiment the nutrient solution was half-strength modified Hoagland's nutrient solution with 4 and 8 meq of N per liter added as $Ca(NO_3)_2$ and KNO_3. The initial nutrient solution, with the exception of N, was of the following composition: 5 meq Ca^{++}, 5 meq K^+, 3 meq $SO_4^=$, 2 meq Mg^{++}, 1 meq $H_2PO_4^-$, 1 meq Na^+, 0.5 meq Cl^-, 0.5 meq $SiO_3^=$ per liter and 2.5 ppm Fe, 0.25 ppm B, 0.25 ppm Mn, 0.01 ppm Cu, 0.025 ppm Zn and 0.005 ppm Mo. Chloride and sulfate were used to balance the cation-anion content. The pH values of the nutrient solutions were adjusted as needed to maintain a pH of between 5.5 and 6.0.

A second addition of the salts, including nitrate, was made on November 25 and a third on December 17. A total of 240 meq (3.36 gm) of N were added per pot in the three salt additions for the deficient nitrogen treatment and 480 meq (6.72 gm) of N per pot for the adequate nitrogen treatment. The treatments were replicated three times and were arranged in the greenhouse in a randomized block design.

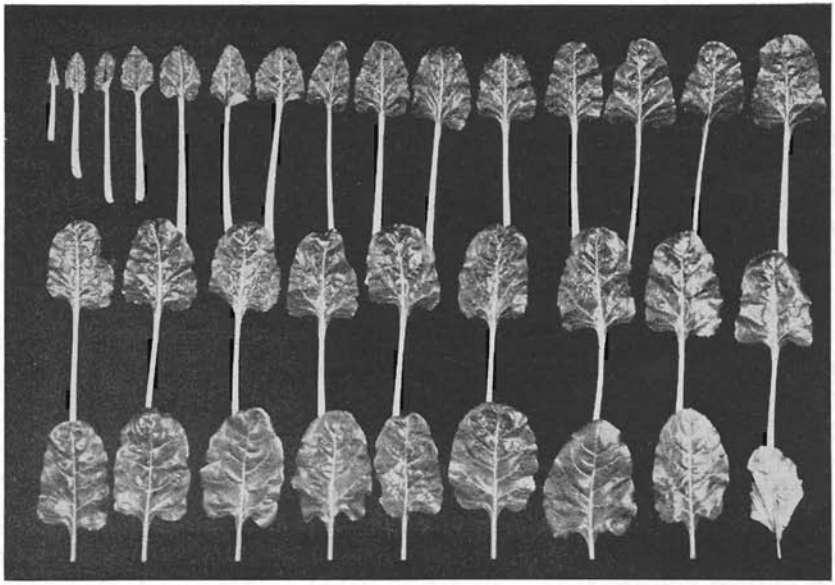

Figure 1a.—Leaf sequence of sugar beets, 96 days old, adequate N.

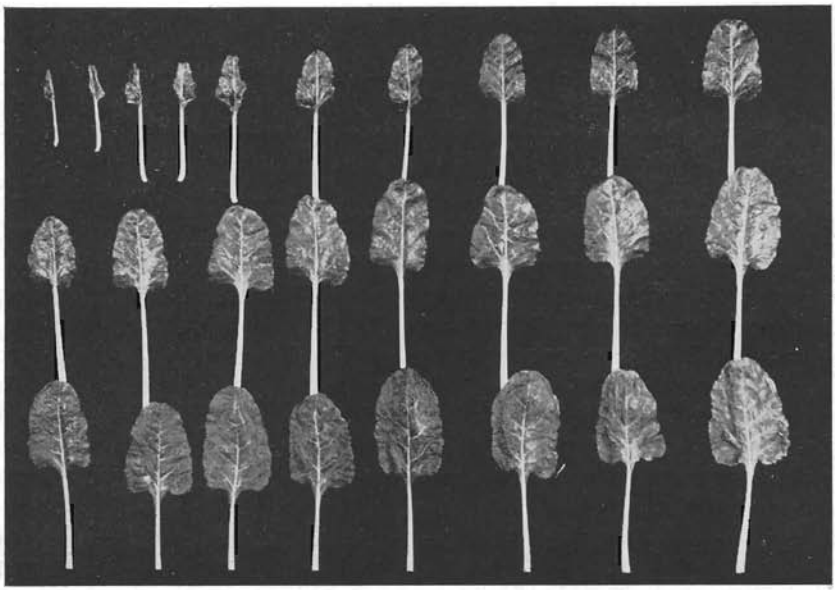

Figure 1b.—Leaf sequence of sugar beets, 96 days old, deficient N.

Harvesting After a growing period of 96 days, all plants were harvested on Jan. 18, 1965. At harvest, the tops were cut from the roots at the first leaf scar and weighed. The plant material

was separated as follows: (a) leaves that were dead or dying; (b) beet storage roots; (c) fibrous roots; (d) living leaves with blade more than one inch long; and (e) unclassified material. The smallest leaf having a blade more than one inch long was considered the youngest leaf. Leaves were removed from each crown starting with the oldest living leaf. The youngest leaf was numbered as one and the second youngest as two, etc., to the oldest living leaf on the plant forming a leaf sequence (Figure 1). Leaves were separated into blade and petiole. The blades and petioles that were taken from corresponding positions on the crown of the three plants in a pot were then combined to constitute a sample. Petioles and blades were weighed and dried.

Preparation of samples All plant parts, except the storage root, were placed in paper bags and dried at 70° C in a forced-draft oven. Dry weights were taken after 48 hours. All dried material was ground in a Wiley mill with a 40-mesh screen and stored in plastic containers until analyzed. Nitrate-N content of the tissues was determined by the phenoldisulfonic acid method (3).

Results

The effect of 4 and 8 meq N per liter in the culture solution upon growth and development of the beets is given in Table 1. Increased N increased top and root size. Maximum dry weight of tops was obtained when the culture solution contained 8 meq of N. Maximum root size was attained at the higher level of N but maximum sucrose concentration was found at the lower level of N. The net production of sucrose did not differ for the two treatments. Plants receiving the higher level of N produced more leaves than the low N plants. The number of leaves that had died on the plants increased by two leaves on the low N plants, indicating that the death rate of the older leaves was increased by a N deficiency.

Plant analysis values The NO_3 concentrations of petioles from plants receiving the two levels of N are given in Figure 2 and are expressed on the dry basis in ppm of NO_3-N. In both instances the lowest NO_3 concentration was found in the youngest petiole and the highest NO_3 concentration in the oldest petiole. Petioles gradually increased in NO_3 with age. Plants growing at the deficient N level contained about 7,700 to 8,500 ppm NO_3-N in the old petioles while the young petioles contained as little as 500 ppm. The youngest petiole from the plants receiving sufficient N had a concentration of about 10,000 ppm NO_3-N and then increased with age to a high of 45,400 ppm in the oldest petiole. The level of NO_3 in the recently matured

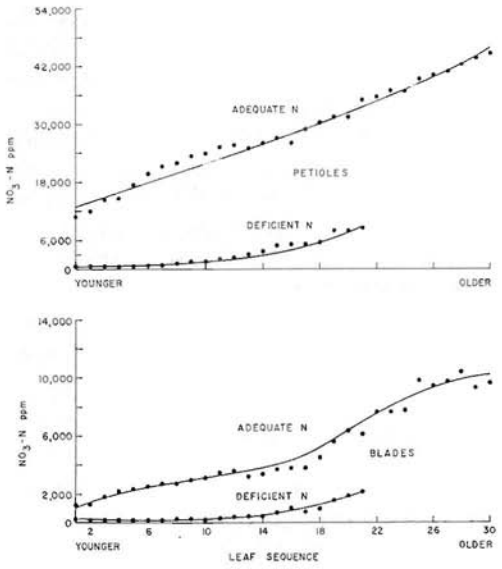

Figure 2.—Influence of N on the distribution of nitrate in the petioles and blades of sugar beets grown in culture solution.

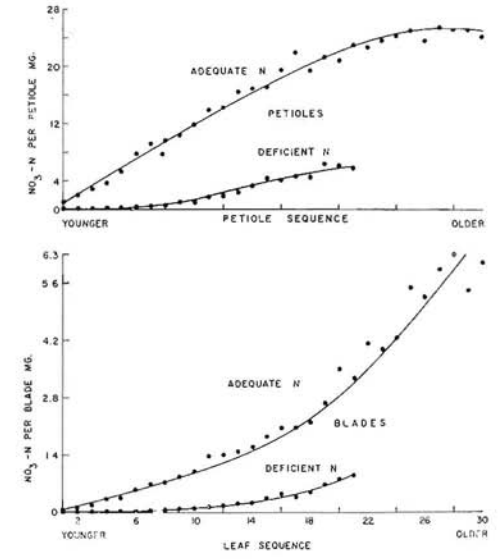

Figure 3.—Influence of N on the amount of nitrate in petioles and blades of sugar beets grown in culture solution

petioles of the low N treatment is in the critical range from 350 to 3,600 ppm NO_3-N as reported by Ulrich (12) for the growth of individual leaves.

The NO_3-N value for blades are given in Figure 2. The values are lower than for petioles but the same pattern of NO_3 distribution is found as with petioles. Plants receiving the additions of 4 meq of N per liter had a 10 fold increase in NO_3-N concentration from the youngest to oldest blades, while plants receiving the additions of 8 meq. of N per liter increased by approximately 8 fold. The NO_3-N concentration of petioles grown at the low level of N increased about 12 fold from the youngest to the oldest petiole while the relative increase for high N was only about 3 to 4 fold.

These rapidly increasing values for nitrate with leaf age emphasize the need for care in selecting leaves for determining the NO_3-N concentration of petioles from either the low or high N plants. Petioles from plants grown at a low level of N did not demonstrate such wide differences in NO_3-N concentration for adjoining petioles in the young to mature leaf categories. In the transition from the mature to the old leaf categories the values increased rapidly. Relatively little error would be made in the selection of these leaves unless an old leaf was taken inadvertently.

Total nitrate-N content In order to evaluate the distribution and accumulation of NO_3-N with leaf age, the amount of NO_3-N per petiole was calculated. These data are presented in Figure 3. Petioles of plants grown with adequate and deficient amounts of N continued to retain or even accumulate NO_3 with leaf age, even though NO_3 may have been moving in and out of the petioles at all times. The net effect was an accumulation of NO_3 in the petioles of the oldest leaves. This finding is in agreement with those of Sorensen (7). In the case of sugar beets the data suggest that the old leaves even near death may be of little or no value in supplying NO_3 to the young leaves even when these are extremely deficient in N.

Blades did not accumulate NO_3 to the same extent as petioles (Figure 3). However, as the blade ages, NO_3 values continued to increase to a greater degree than in the petiole.

Percent dry weight The percent dry weight (Figure 4) was influenced by the N status of the plant and by leaf age. Significant increases in percent dry weight occurred in the younger petioles and blades of nitrogen deficient plants. The high amounts of dry matter in petioles of beets grown with low N may be attributed to the accumulation of carbohydrates in this tissue.

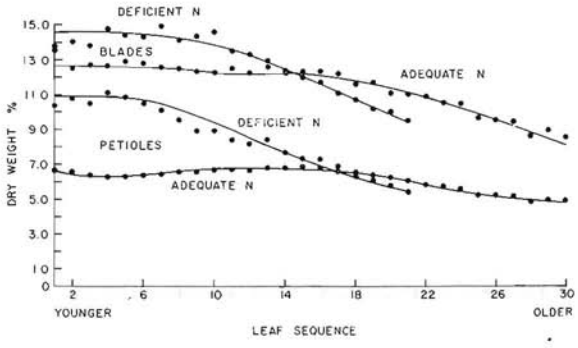

Figure 4.—Influence of N on the percent dry weight of petioles and blades of sugar beets grown in culture solution.

Younger tissues are generally believed to be high in water content and therefore low in percent dry matter, but in the case of beets the young tissues contained the greatest percent of dry matter. Plants grown at a low level of N had, therefore, the least amount of water in the youngest tissue and the greatest in the oldest petioles and blades. Beets growing at adequate levels of N had a more even distribution of dry matter from the youngest to oldest petioles and blades. The above pattern of percent dry weight distribution is unique and has not previously been reported.

Dry weight distribution The dry weight of individual petioles and blades is presented in Figure 5. At 8 meq. of N per liter of solution the largest petioles were found near the middle. This

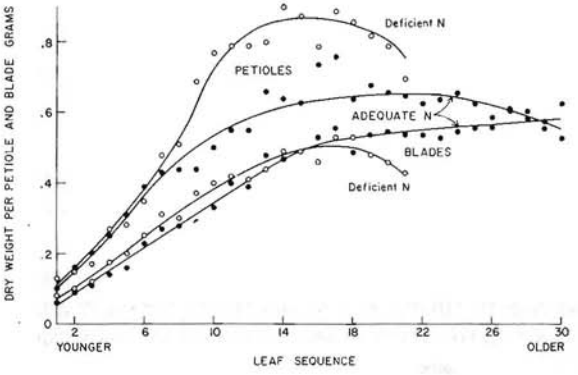

Figure 5.—Influence of N on the dry weight of petioles and blades of sugar beets grown in culture solution.

was also shown to be true by Sorensen (7). It is of interest to note that plants receiving the lower level of N had the larger petioles in terms of dry matter per petiole. This might be attributed to an accumulation of carbohydrates in these leaves due to a deficient N supply. In plants receiving 8 meq of N the total amount of dry matter produced was greater (Table 1) but was distributed among a greater number of leaves. The rapid decrease in amount of dry matter per petiole in the older leaves of N deficient plants might be attributed to the higher nitrogen status of these leaves. If it is assumed that the center leaves were developed midway through the growth of the beet, it can be seen that leaves reach a maximum petiole dry weight when approximately 35 to 45 days old, depending on N nutrition.

Discussion

Earlier studies (7,12) have been incomplete in attempting to show the relation between N nutrition and its influence on the NO_3 concentration of blades and petioles relative to age and on the dry matter concentration of leaves. The results presented here are of interest from an agronomic and physiological standpoint. It has been shown that as the petioles and blades of sugar beets age, there is a retention of NO_3 by the older leaves even when the younger leaves are suffering from N deficiency. Except for some reduction of NO_3 that may have occurred in the root tissues, it appears that a major portion of the NO_3 available from the culture solution moves directly from the roots to the leaves. It accumulates in the petioles and is reduced most likely in the blades (9,10).

The large amount of NO_3 in the older petioles of N deficient plants appears to substantiate the fact that NO_3 becomes trapped in the older petioles. Even though NO_3 may be moving both into and out of older petioles, the net effect is one of retention or possibly even of accumulation. For young expanding leaves the rate of NO_3 reduction and sugar utilization tends to be high, thus accounting for the lowest NO_3 level being in the younger leaves of the high nitrogen plants. At the outset of nitrogen deficiency nitrate apparently does not move out of the older blades and petioles readily enough to prevent a nitrogen deficiency of the younger leaves. This appears to be true even at the time of death of the older leaves. Consequently, this failure of nitrate to move rapidly from the older to the younger leaves implies that a continuous supply of nitrate must be available to the plants if a nitrogen deficiency of young leaves is to be prevented at all times.

Table 1.—Effect of two levels of nitrogen on growth and sucrose concentration of storage roots of sugar beet plants.

Treatment* N added per liter		Tops**		Storage roots**			Fibrous roots**		Old leaves** and residue	Leaf count per plant	
		Fresh	Dry	Fresh	Sucrose		Fresh	Dry	Dry	Living blades	Total
meq		gm	gm	gm	gm	%	gm	gm	gm		
4		1085	93.7	214	23.5	11.0	65	6.5	17.4	26	35
		1084	94.3	288	31.7	11.0	50	4.6	20.1	27	36
		875	94.3	263	29.2	11.1	70	6.8	36.0	25	36
	Mean	1015	94.1	255	28.1	11.0	62	6.0	24.5	26	36
8		1456	110.1	313	21.3	6.8	98	6.8	19.2	31	39
		1257	109.3	410	28.7	7.0	105	7.8	28.0	33	41
		1242	110.9	500	37.5	7.5	102	6.5	20.0	31	39
	Mean	1318	110.1	407	29.2	7.1	102	7.0	22.4	32	40

* Added October 26, November 25 and December 17, 1964.
** 3 plants per pot.

A point of physiological interest is the more or less sameness of the percent dry weight of blades and petioles of all leaf ages when an adequate N supply is maintained. At deficient levels of N the younger leaves have a significantly higher percent dry weight (Figure 4). Apparently carbohydrates accumulate in the younger leaves of N deficient plants. This accumulation of dry matter for young leaves is not apparent when one sees the dry matter per petiole but is strikingly evident for the petioles of mature leaves, numbers 9-20, (Figure 5). Quite likely, the dry matter accumulates as carbohydrate because a shortage of nitrogenous material prevents protein formation.

The rather rapid rise of nitrate with leaf age emphasizes the need for care in selecting leaves from either low or high nitrogen plants for chemical analysis and nitrogen status evaluation. Little error in status evaluation would be made in the selection of leaves from nitrogen deficient plants, since even the highest values for the young and mature leaves would still place the plants in the deficient category. With the inclusion of petiole material from the older leaves, the results would be biased in favor of nitrogen adequacy. The probability of error for high nitrogen plants is far less than for low nitrogen plants since the lowest nitrate value for the youngest leaves is still far above the critical concentration of 1,000 ppm NO_3-N and therefore the chance of calling a high nitrogen plant deficient is almost nil. The greatest amount of error in field sampling occurs, however, when unusual variability is not recognized in an irregular field. It takes only a few petioles from scattered groups of high nitrogen plants to raise the sample value above the critical concentration for a nitrogen deficient field. When plants in a field are obviously different, separate samples should be taken to represent each condition.

Summary

Sugar beet plants of the hybrid variety, F 58-554H1-MS of NB1 × NB4, were grown in 20 liter pots containing nutrient solutions with deficient and non-deficient amounts of N. After 96 days of growth, plants were harvested and the leaves removed from each plant in order of age. The leaves were separated into blades and petioles, dried, ground and analyzed for NO_3-N. Nitrate concentrations of blades and petioles increased from low values in the younger leaves to increasingly higher values in older leaves. Nitrogen deficiency occurred in the young immature leaves, even though there was an ample supply of NO_3 in the older leaves. This lack of rapid movement of NO_3 is unusual and implies that a continuous supply of NO_3 must be

available to the plant from the soil if a nitrogen deficiency of the younger leaves is to be prevented in sugar beets at all times. Nitrogen deficient petioles and blades were found to have a much higher percent dry matter than those with an adequate supply of N.

The petioles of plants were much higher in NO_3 concentration than the blades. Individual petioles of plants grown at the deficient level of N were larger than those with adequate N, but total dry weight was smaller because there were fewer leaves per plant. Leaves of a specific age must be taken if large errors due to sampling are to be avoided. Separate samples of leaves should be taken from irregular fields to represent each plant nutrient condition.

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