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Department of
Experiment Station Chemistry

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Nitrogen Distribution in the Corn Plant*

EUGENE I. WHITEHEAD, FRANK G. VIETS, JR., *and* ALVIN L. MOXON†

Introduction

Sporadic outbreaks of cattle losses in cornfields of the Midwest and Great Plains areas have been reported during the greater part of the past century (1). The name "cornstalk disease" or "cornstalk poisoning" has been used to designate the cause of such livestock losses. Studies at this laboratory were begun following an extensive outbreak of cornstalk poisoning in Sully, Potter, and Walworth counties during the winter of 1942-43.

A discussion of the several theories advanced to explain cornstalk disease (or poisoning) is detailed in the Sixteenth Annual Report of the Agricultural Experiment Station of Nebraska (1903) and little progress has been made since that time toward defining the cause or causes of such cattle deaths. A recent lead has been provided by Standen (2), who has detected a toxic principle in the ether-extractable substances from several cobs of a maize *(Zea mays L.)* inbred, referred to as No. 113. This material was found to inhibit the growth of *Nigrospora oryzae* (B. and Br.) and *Diplodia zeae* (Schw.) Lev.; also, it was found to be toxic to rats. However, attempts at this station to repeat this study, using cobs of the same inbred (seed furnished by Dr. Standen), have failed so far.

While investigating cattle losses in the three South Dakota counties previously mentioned, samples of cornstalks from 12 fields in which losses had been sustained, were collected. Of these, five were found to have nitrate nitrogen contents within the range of 1.3 to 3.9 per cent (as potassium nitrate). The presence of potassium nitrate in cornstalks has been reported by Chatin, Berthelot and Andre, Schweitzer, and Mayo as noted in the Nebraska report (1). The salt was found to occur mainly as a white powder at the base of the leaf sheath and as well-defined crystals in the nodal tissues. Inasmuch as oat hay containing potassium nitrate in excess of 1.5 per cent is considered unsafe as cattle feed[1], some of the losses sustained in Sully, Potter, and Walworth counties may have been due to excess nitrates present in the cornstalks. Some cases of cornstalk poisoning may be due to nitrates, but not all such losses can be attributed to this cause as methemoglobin (characteristic of nitrate poisoning) has not been found in specimens of blood taken from cattle with cornstalk poisoning and examined in this laboratory.

Since nitrates or other nitrogen fractions (e.g. the alkaloids) are frequently associated with forage poisonings, it seemed advisable to further investigate the nitrogenous constituents of the corn plant. A review of the literature reveals that the corn plant *(Zea mays L.)* has not been subject to extensive biochemical

*This material was presented in part at the Midwest Regional Meeting of the American Chemical Society at Kansas City, Missouri, on June 24, 1947.

†Associate Agricultural Chemist, former Associate Agricultural Chemist, and Experiment Station Chemist, respectively.

[1]Personal communication from O. A. BEATH, University of Wyoming.

studies. It is the purpose of this paper to present data collected at this laboratory relative to the changes in the nitrogenous constituents of field grown corn plants, throughout the period from the seedling stage to maturation, and to note the subsequent effects of weathering upon corn stalks left standing in the field.

Review of the Literature

Since the extensive literature on nitrogen metabolism of various plants will not be reviewed here, the reader is referred to the excellent reviews of Nightingale (3,4) on the nitrogen nutrition of green plants, of Wood (5) and Steward and Street (6) on the nitrogenous constituents of plants, of Burstrom (7) on the occurrence and assimilation of nitrate nitrogen, and of Chibnall (8) on protein metabolism.

Relative to the amides of the corn plant, asparagine was found by Boussingault in 1868 (as cited by Chibnall) in corn germinated in the dark. Prianischnikow (9) demonstrated the necessity of carbohydrate for the formation of asparagine, and the crystalline amide was prepared by Jodidi (10) from etiolated corn seedlings. The role of the amides, glutamine and asparagine, in the metabolism of ammonium absorbed by corn has been investigated in this laboratory (11,12). When ammonium is supplied in excess of growth requirements asparagine, glutamine, and one or more of the amino acids (other than the glutamic or aspartic acid associated with the amides) accumulate. In studies with detached corn leaves cultured in darkness and in the light, Viets *et al.* (13) found that in percentage of total nitrogen, protein was hydrolyzed at comparable rates in both series. Residual *a*-amino acids[2], asparagine, glutamine, and ammonia (in decreasing order of magnitude) accumulated in the leaves, the ratio of residual *a*-amino nitrogen to asparagine nitrogen being 2:1. Glutamine synthesis was greater in the dark than in the light, but was not related to the amino acid level.

Klein and Taubock (14) have shown that corn seedlings in sterile culture solutions will absorb arginine and decompose it completely within the plant without urea being one of the accumulating products.

A review of the literature by Van Der Walt (15) shows that hydrocyanic acid and cyanogenic glucosides rarely occur in corn and then only in traces. Tests performed at various times at this laboratory have failed to detect even traces of cyanide nitrogen in corn plants.

Beckenbach *et al.* (16, 17, 18), in a series of nutrition studies with corn grown in culture solutions, have interpreted statistically the nutrient ion effect upon growth, and the relation between nutrient ion concentration and element content of the tissues and upon carbohydrate and nitrogenous constituents. In another study at Rutgers University, Wadleigh and Shive (19) have investigated interrelationships between the organic acid content of corn plants, pH of the substrate, and the form of nitrogen supplied; the organic acid content of the plants was found to increase with increase in pH of the substrate and with nitrate as the sole source of nitrogen.

Field studies with corn plants, aside from the common fertilizer trials, have been infrequent. Recently Ellis, Randolph, and Matrone (20), investigating diploid and tetraploid corn plants, observed that doubling the number of

[2]Residual *a*-amino nitrogen refers to the total *a*-amino nitrogen less the *a*-amino nitrogen of glutamine and asparagine.

chromosomes was accompanied by nitrogen increases of 20 to 34 per cent in the stover. Van Lanen *et al.* (21) found that the protein and vitamin (except pyrodoxine) contents of hybrid corn tassels were unusually high as compared with the corn grain and other structural parts of the corn plant.

Materials and Methods

Inasmuch as the experimental methods were altered from year to year, this discussion will be considered under appropriate yearly headings.

1943 Study

Materials and sampling procedure. In the spring a 3½ acre plot at the Experiment Station at Brookings was planted to open-pollinated Flint corn and an adjoining 3½ acre plot was planted to a variety of open-pollinated, Yellow Dent corn. Since it was observed in the outbreak of cattle losses in Sully, Potter, and Walworth counties that losses occurred most frequently in fields of Flint corn, it seemed advisable to include both Flint and Dent corn in the study. For chemical analyses samples of both types of corn were taken at six sampling dates throughout the fall and early winter. These samples[3] consisted of six stalks cut on a diagonal across the field, three of these stalks being selected with well-developed ears and three with poorly developed ears. In preparing the samples for analyses the tassels, ears, and shanks were removed and discarded; the leaves with their attached sheaths were separated from the stalks and the two fractions thus obtained were weighed and then stored in a sharp-freeze room at −10° F. When desired for analyses the samples were removed from the freezer, re-weighed in order to determine any moisture loss during storage, and then cut into fine pieces so as to render further sub-sampling as representative as possible. Leaf and stalk tissues were recombined in correct proportion (as found on the plant) to give duplicate 200 g. (fresh weigh) samples for water extraction. Toward the end of fall, as the moisture content of the plant material decreased, it was advantageous to use 100 g. samples.

Water extracts of the samples were prepared following the method described by Loomis and Shull (22), a Waring blendor being used to facilitate maceration. The insoluble residue obtained upon filtration of the extract was dried, weighed, and total nitrogen was determined by a modified Kjeldahl procedure using copper sulfate as a catalyst. The water extract was diluted to two liters and preserved under toluene at 5° C.

Methods of Analyses. Total water-soluble nitrogen was determined by the reduced-iron powder method of Pucher *et al.* (23.)

Ammonium nitrogen and amide nitrogen were determined by the methods outlined by Loomis and Shull (22) with the substitution of magnesium oxide for calcium hydroxide in the former determination. These methods and the extraction procedure are subject to some criticism (24) inasmuch as some of the ammonia may be converted to the insoluble magnesium ammonium phosphate, resulting in lower ammonia values; glutamine hydrolysis, which, if extensive, would increase the ammonia values. Considering these objections to the method, ammonium nitrogen and amide nitrogen have been summed for purpose of comparison with data from subsequent experiments.

[3]Samples consisting of six stalks may not be adequate for determining varietal differences between Flint and Dent corn; however, a series of samples were analyzed and these should suffice to show seasonal trends in nitrogen distribution in the corn plants.

a-Amino nitrogen was determined by a slightly modified Van Slyke procedure (22) upon aliquots of the water extract. The samples were not pre-treated to remove the amide nitrogen of glutamine and ammonium nitrogen, consequently the values obtained are high, since about 25 per cent of the ammonium nitrogen and 80 per cent of the amide nitrogen of glutamine are recovered in the determination.

Nitrate nitrogen was determined on aliquots of the water extract using Devarda's metal and an alkaline reduction —essentially the method of R. W. Gerdel as modified by Olson (25).

A photometric adaptation of the Griess reaction (26) was used to determine nitrite nitrogen.

The fraction designated "undetermined" soluble nitrogen (or "rest" nitrogen) would include nitrogen of non-volatile bases, diamino compounds, polypeptides, etc., and represents the difference between total water-soluble nitrogen and a summation of the values derived analytically for the separate nitrogen fractions.

1944 Study

Materials and methods. Again adjacent fields of open-pollinated Dent, and open-pollinated Flint corn at the Experiment Station at Brookings were chosen for study. An area comprised of about 15 rows, 150 rods long, in the center of each plot was selected as the sampling area. At each sampling, 30 stalks of each type of corn were selected so as to represent the entire area under study; stalks were chosen from hills containing two or more plants and only plants that had one or more ears were used in the study.

For the purpose of comparison, cut stalks were obtained by cutting off a sufficient number of plants, three to four inches above ground level, and tying them with twine to adjacent stalks in the hill until appropriate time for their harvest and analyses. The stalks were cut on September 23, for the field of Dent corn and on September 25, for the field of Flint corn, the same sampling procedure of selecting stalks being followed as for the green (or uncut) material as described above.

The reason for comparing cut with uncut cornstalks is that previous attempts to produce cornstalk poisoning in cattle at this Station, by feeding cornstalks gathered from fields in which cattle losses had occurred, were unsuccessful. A similar observation is reported by Schwarte *et al.* (27). Since it appears that the corn plant must be intact in order to cause cornstalk poisoning, we were interested in determining what differences, if any, might occur in the non-protein nitrogen constituents of cut and uncut stalks.

At the time of harvest 30 plants of each variety were cut (or in the case of the cut stalks removed from the adjacent stalks to which they were tied). Tassels, ears, shanks, and suckers were removed in the field and the remaining material was divided into stalks and leaves plus sheaths at the laboratory. Total green weights of each fraction were recorded.

The stalk and leaf plus sheath fractions were run separately through a small hand-operated ensilage cutter and the chopped material thus obtained was thoroughly mixed. Separate samples were taken for moisture determinations (air-drying at about 70° C.) and for extraction of the water-soluble nitrogenous compounds prior to analyses.

Method of extraction. The weighed fresh sample was coarsely ground by passing it through a Wiley mill (without sieve) several times. The ground sample was cytolyzed using a volume of chloroform in milliliters equivalent to the green sample weight in grams. The chlo-

roform[4] was poured off after one-half hour and distilled water was added to the cytolyzed plant material to give a total water content (water added plus that in the green sample) estimated at 15 times the dry weight of the sample. After standing two to three hours with frequent shaking, the extract was filtered through filter-paper (coated with diatomaceous silica filter-aid) on Buchner funnels. All extracts were preserved under toluene and stored at about 2° C.

Methods of analyses. Ammonium nitrogen was determined by the method of Pucher, Vickery, and Leavenworth (28) on duplicate samples of the extract the same day the tissues were extracted.

Glutamine amide and asparagine amide nitrogen were determined the following day by the differential-hydrolysis method of Vickery, Pucher, *et al.* (24) on the extract preserved overnight at 2° C. Urea nitrogen interferes in the determination of amides, but has been shown to be absent in corn (14).

a-Amino nitrogen was determined in a Van Slyke apparatus equipped with a macro-deamination chamber and microburette, using a four minute deamination time. Since ammonium and glutamine amide nitrogen interfere in the procedure (24, 29), they were removed by hydrolyzing aliquots of the deproteinized extract (3 g. of trichloracetic acid per 100 ml. of extract) for two hours with 2 M HCl at 100° C., and driving off the ammonia on a steam bath after making the solution slightly alkaline to bromcresol purple.[5] The solution was then acidified with acetic acid and diluted to volume for determination. The hydrolytic procedure leads to the formation of some humin, hence the values reported are probably low.

The difference between the determined *a*-amino nitrogen value and the *a*-amino nitrogen of glutamine and asparagine (equivalent to the amide nitrogen) is expressed for convenience as residual *a*-amino nitrogen, in common with the practice of Wood and co-workers (5,30,31).

Basic nitrogen was precipitated from the trichloracetic acid filtrates according to the procedure of Umbreit and Wilson (32). Since the trichloracetic acid filtrates contain appreciable amounts of polypeptides and higher polymers of amino acids, much peptide nitrogen is included in this determination along with small amounts of "true basic" nitrogen (32,33).

A measure of the basic *a*-amino nitrogen was attempted by determining *a*-amino nitrogen on the phosphotungstic acid filtrates, obtained in the "basic" nitrogen determination by the technique of deaminizing as previously described. By subtracting the value thus obtained from the total *a*-amino nitrogen, it was thought that a measure of the basic *a*-amino nitrogen would be obtained, but the differences though small, were sometimes positive, and sometimes negative.

Total nitrogen was determined on finely ground samples of the air-dried plant material using salicylic-sulfuric acid digestion, followed by Kjeldahl distillation.

Total water-soluble nitrogen, nitrate (plus nitrite), and nitrite nitrogen were determined as in 1943.

1945 Study

Materials and methods. Open-pollinated Flint, and open-pollinated Dent corn plants were grown in adjacent plots at the Experiment Station at Brookings and the area sampled was defined as in the 1944 study.

The sampling dates were spaced so as to include the growth period from seedling to mature plant. It seemed desirable

[4]Analysis of the chloroform layer revealed that a small amount of nitrogen, presumably of the chlorophyll-protein complex was extracted.

[5]Personal communication from T. C. Broyer, University of California.

to avoid extensive sub-sampling and since the capacity of the forced-draft oven was limited, only eight stalks were selected at each harvest, following the scheme as given for uncut stalks in 1944.

The eight plants harvested from each of the plots were divided into the following fractions (as they developed): stalks, leaves, sheaths, shanks, fertile ears, and tassels. After recording the green weights for each fraction, the material was dried for two to four hours in a forced-draft air oven at 80° C. For subsequent nitrogen determinations a dry-tissue water extract was prepared as recommended by Vickery and Pucher (34). The water extracts were layered with toluene and refrigerated at 2° C. between withdrawal of aliquots for analyses.

Methods of analyses. Ammonium, glutamine amide, asparagine amide, "basic," total water-soluble, total, and nitrate (including nitrite) nitrogen were determined as outlined for the 1944 study.

a-Amino nitrogen was determined by the Van Slyke deaminizing technique previously noted, but fresh aliquots of the extract without any pre-treatment were employed,[6] applying a correction of 25 per cent of the ammonium nitrogen and 80 per cent of the glutamine amide nitrogen (24).

Sucrose and total reducing sugars were determined on 80 per cent alcoholic extracts of the ground dry-tissue according to the procedure of Hassid (35). The residue remaining from the alcoholic extraction was exhaustively extracted with boiling water as recommended by Vickery *et al.* (36), and the nitrogen in the residue, determined by a modified Kjeldahl procedure, is reported as "true" protein nitrogen.

[6] In studies at this laboratory and based upon recoveries of known amounts of *a*-amino nitrogen, this procedure seems to give the most reliable measure of *a*-amino nitrogen.

Results and Discussion

The data collected during the three years of study are tabulated in detail and will be found in the Appendix; certain portions of this material are incorporated into the body of the discussion which follows. In order to facilitate the presentation of the data a series of area graphs (Plates I to VI) have been prepared and the reader is referred to these from time to time throughout the discussion. It is important to note that in these graphs a given nitrogenous fraction is defined (as percent of the total nitrogen) by the area between adjacent lines of the graph and not between a line and the base line, with the exception, of course, of the first fraction plotted.

Total, Soluble, and "True" Protein Nitrogen

Total nitrogen (including nitrates) was found to decrease markedly in all of the structural tissues of the corn plant throughout the growing season. It will be observed in Table I that the total nitrogen content of the entire plant continued to increase through August, although in leaves, sheaths, shanks, and tassels, total nitrogen contents had reached maxima (about the time of silking) and were decreasing. The decrease in total nitrogen of the tassels may not be entirely attributable to translocation, since the nitrogen of the nitrogen-rich pollen would be lost when the pollen is shed. The decrease in total nitrogen of leaf tissue may be due in part to shattering, although this did not appear to be extensive until mid-September. The translocation of soluble nitrogenous constituents and their deposition in the maturing kernels of the ear probably accounts for the main changes observed in

Table I. Accumulation per Plant of Nitrogen in Milligrams. Data for Flint and Dent Corn Plants Grown During the Summer of 1945

Date and variety	Entire plant	Fraction					
		Leaves	Sheaths	Stalks	Shanks	Ears	Tassels
Flint							
July 2	50						
July 13	300						
July 23	900	640	123	105			26
August 10	2700	990	219	510	620		360
August 29	3560	950	201	430	500	1350	127
Dent							
July 2	46						
July 13	237						
July 23	1020	770	144	113			
August 10	3580	1860	340	900	160		320
August 29	4640	1700	293	1090	660	800	106

the total nitrogen content of the various tissues of the corn plant and is discussed in detail by Sayre (37).

At the time of silking, the plant, though it has reached its full height, is only about half-grown on a dry matter basis (37,38). Considering the extensive vascularization which takes place during growth and concomitant increases in the structural and reserve carbohydrates, the decrease in total nitrogen is more apparent than real and may be considered in part in terms of the "dilution" of nitrogen by other plant constituents. In order to allow for this effect and also that of translocation, subsequent data is reported on a percent of total nitrogen basis.

In Table II an increase in total nitrogen content of the cornstalks is indicated in November and December, 1943. Aside from the possibility of sample variability, it seems that this increase was due to microbiological activity, resulting in the utilization of the plant carbohydrate fractions in meeting bacterial and fungal requirements. It was obvious to the observer that the stalks were in the process of disintegration and only in these two samplings (1943) of Flint and Dent cornstalks were nitrites detected, and at this time, in appreciable amounts. Nitrites were also found in the stalks collected on Nov. 13, 1944.

The water-extractable nitrogen (hereafter referred to as soluble nitrogen) of the entire plant and its component parts is recorded in Table III. The reader is also referred to Plates I through VI. It was observed that the soluble nitrogen values tabulated for the 1943 samples exceed considerably those for 1944 and 1945. It is quite possible that this is an artifact introduced by the extraction procedure used in 1943 (cf.-39).

For the entire plant it appears that the soluble nitrogen content is quite uniform throughout the season (Plate I), decreasing slightly toward mid-season (during ear development) and showing a slight increase following a frost (time of killing frost is indicated by the vertical dotted line). Whereas leaves show a marked decrease in soluble nitrogen toward mid-season and an increase during the fall (Plates II and IV), stalks exhibit trends which are higher toward mid-season followed by decreases after frost (Plates III and V). The soluble nitrogen content of the stalks on a percent of total nitrogen basis is about twice that of leaf tissue and in turn is comprised of about one-half nitrate nitrogen. On a milligrams nitrogen per kilogram of dry material basis, this relationship is apparently valid for the corn plant before it silks.

Table II. The Total Nitrogen Content of Flint and Dent Corn Plants Expressed as Milligrams of Nitrogen per Kilogram of Air-Dry Plant Tissue

Variety	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent
1943	Sept. 9		Sept. 11		Sept. 18		Sept. 25		Nov. 17		Dec. 1					
Entire Plant	9890	8970	8710	8650	7260	6500	7000	6540	9470	9710	8010	9890				
1944	Sept. 19		Sept. 25		Oct. 2		Oct. 9		Oct. 11		Oct. 16		Oct. 20		Nov. 13	
Uncut																
Entire Plant	12700	10800	13900	11200	13400	10700	14100	10700	13300	10700			14300	10900		
Leaves	17700	16500	18400	17500	16500	15100	18100	13800	16100	14800			16000	13900		
Stalks	9140	6040	9630	7030	9960	6550	10400	8500	11100	7250			12000	7380	10800	6880
Cut																
Leaves					15800	15300	17300	15200			17100	14200				
Stalks					9600	6880	7730	6650			11000	6500			8230	5750
1945	July 2		July 13		July 23		Aug. 10		Aug 29							
Entire Plant	40000	41600	32500	33300	28700	29400	17600	21000	12700	13200						
Leaves					30400	31100	29400	29000	27000	26200						
Stalks					27200	27500	12100	14300	7280	8080						
Sheaths					21900	24100	11100	13100	8200	7950						
Shanks							17600	27700	9330	10300						
Ears									13800	18800						
Tassels					42900		21600	28000	12300	12200						

Table III. The Soluble Nitrogen Content of Flint and Dent Corn Plants Expressed as Per Cent of Total Nitrogen

Variety	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent
1943	Sept. 9		Sept. 11		Sept. 18		Sept. 25		Nov .17		Dec. 1					
Entire Plant	41.5	50.6	46.9	52.5	61.1	67.5	63.1	59.9	60.6	64.3	61.4	65.1				
1944	Sept. 19		Sept. 25		Oct. 2		Oct. 9		Oct. 11		Oct. 16		Oct. 20		Nov. 13	
Uncut																
Entire Plant	34.1	26.8	37.9	26.9	39.1	30.8	38.8	29.7	43.5	35.8			37.6	30.2		
Leaves	15.8	15.0	21.4	15.9	23.5	19.4	22.9	19.6	26.4	18.9			26.2	23.2		
Stalks	59.3	53.7	67.1	45.2	67.2	55.9	64.6	48.6	63.0	64.9			57.8	45.9	41.2	36.3
Cut																
Leaves					18.7	18.0	22.6	22.6			21.9	26.1				
Stalks					54.2	51.2	60.9	50.0			69.5	58.2			40.8	46.3
1945	July 2		July 13		July 23		Aug. 10		Aug. 29							
Entire Plant	41.0	39.8	31.3	33.3	38.1	32.1	35.1	35.8	26.7	32.7						
Leaves					28.7	22.4	14.7	15.5	15.8	12.7						
Stalks					59.2	53.3	62.6	67.9	49.5	59.8						
Sheaths					72.3	67.1	50.4	54.7	29.3	24.9						
Shanks							37.1	36.5	29.1	37.2						
Ears									25.2	38.8						
Tassels					25.9		33.5	42.6	34.1	22.6						

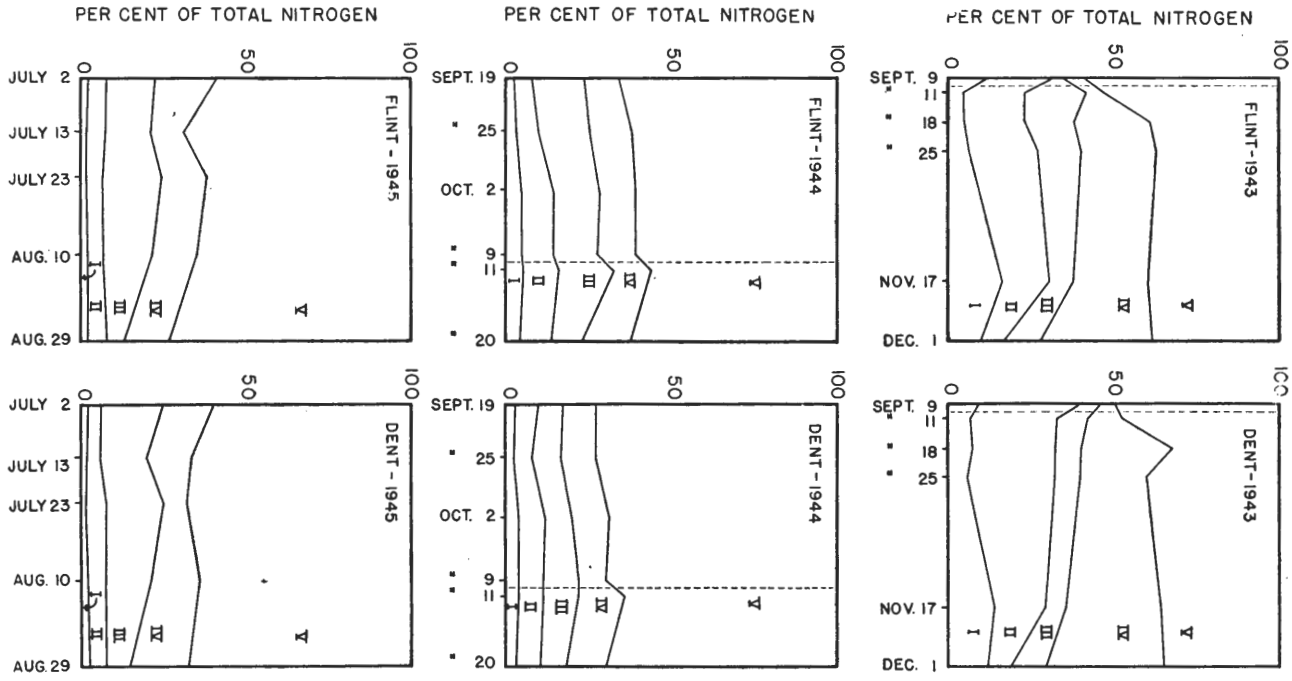

PLATE I.

Area graphs showing the changes which occurred in the nitrogen fractions of Flint and Dent corn plants during the fall of 1943 and 1944, and the summer of 1945. The area between adjacent lines denotes the amount of a given nitrogen constituent. Nitrogen fractions:

I—Ammonium plus amide. II—*a*-Amino. III—Nitrate (and nitrite). IV—Undetermined soluble. V—Insoluble (chiefly protein).

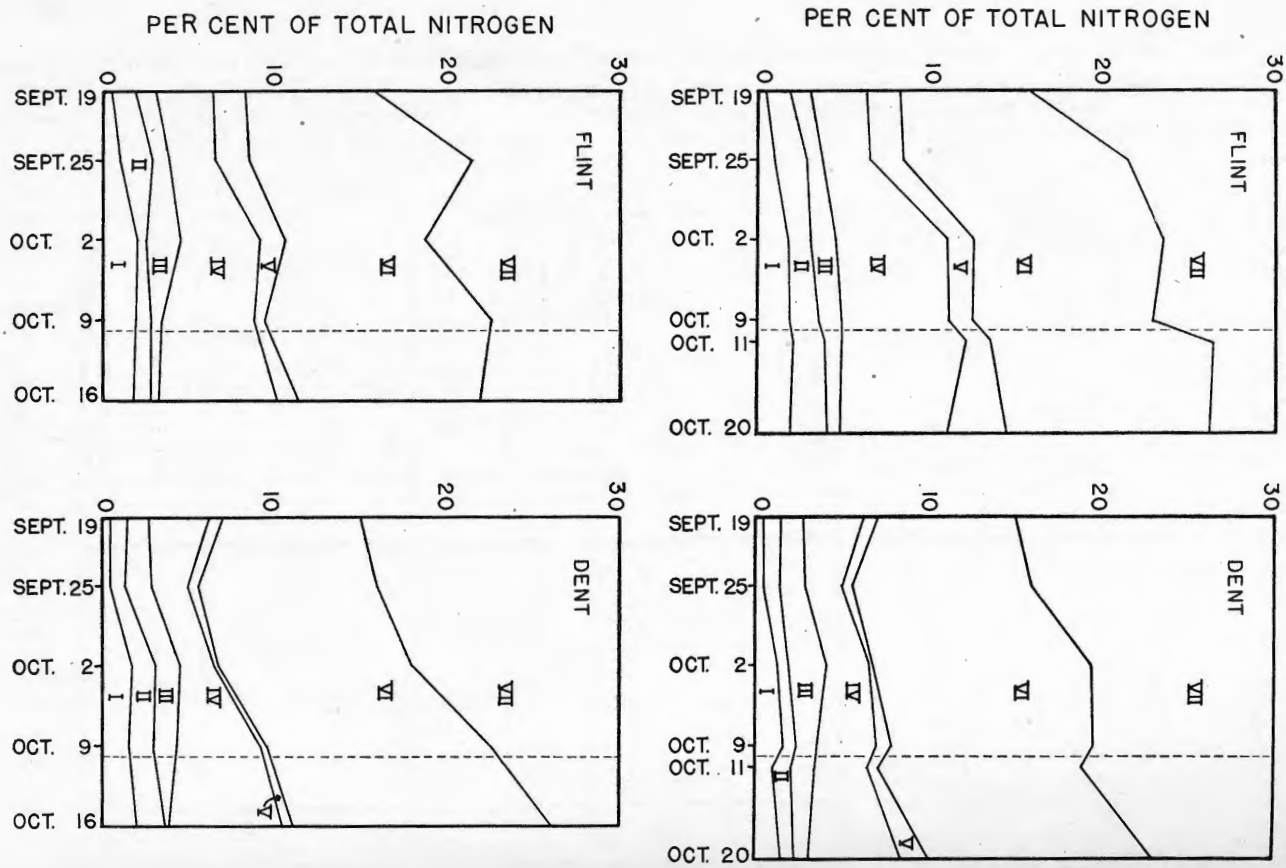

PLATE II.

Area graphs showing the changes which occurred in the nitrogen constituents of the leaves of uncut and cut (upper and lower graphs, respectively) Flint and Dent corn plants during the fall of 1944. Nitrogen constituents:

I—Ammonium. II—Glutamine. III—Asparagine. IV—Residual *a*-Amino: V—Nitrate. VI—Undetermined soluble. VII—Insoluble.

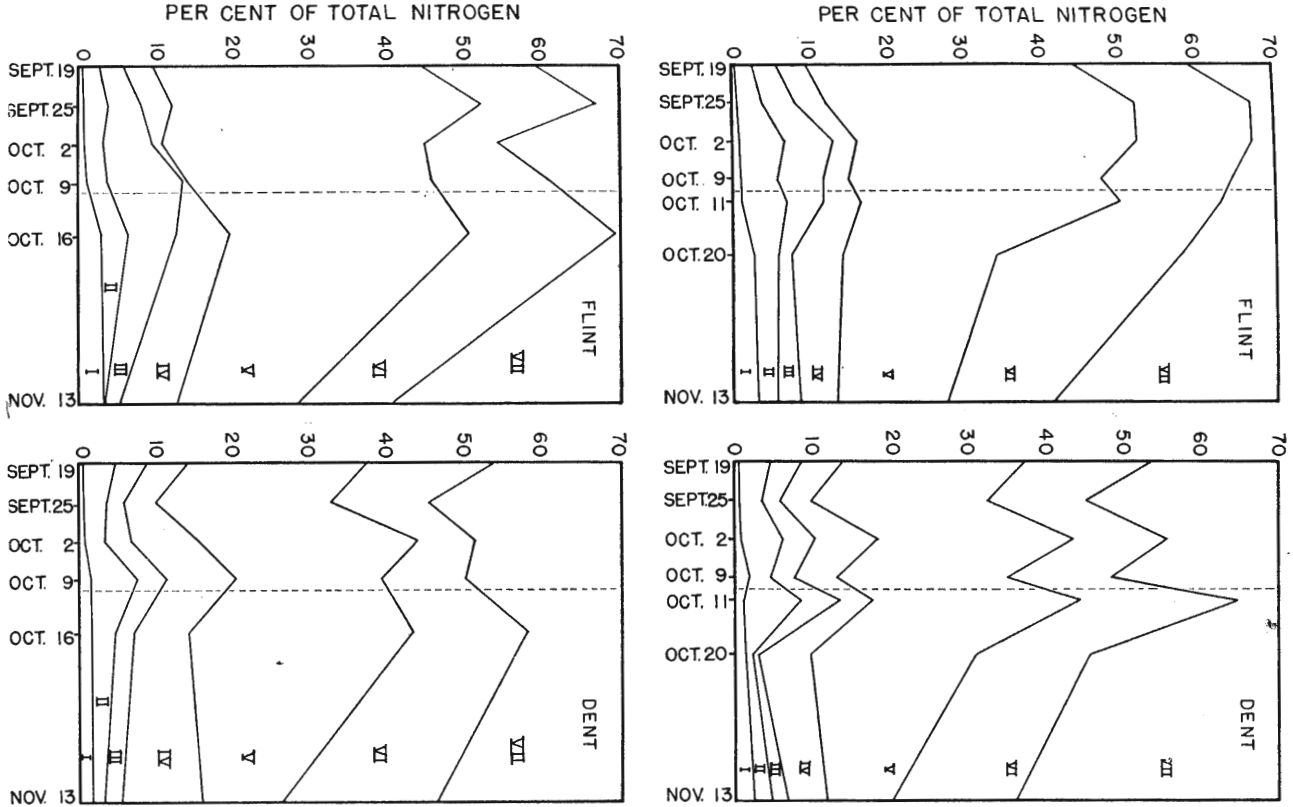

PLATE III.

Area graphs showing the changes which occurred in the nitrogen constituents of stalks of uncut and cut (upper and lower graphs, respectively) Flint and Dent corn plants during the fall of 1944. Nitrogen constituents:

I—Ammonium. II—Glutamine. III—Asparagine. IV—Residual *a*-Amino. V—Nitrate. VI—Undetermined soluble. VII—Insoluble.

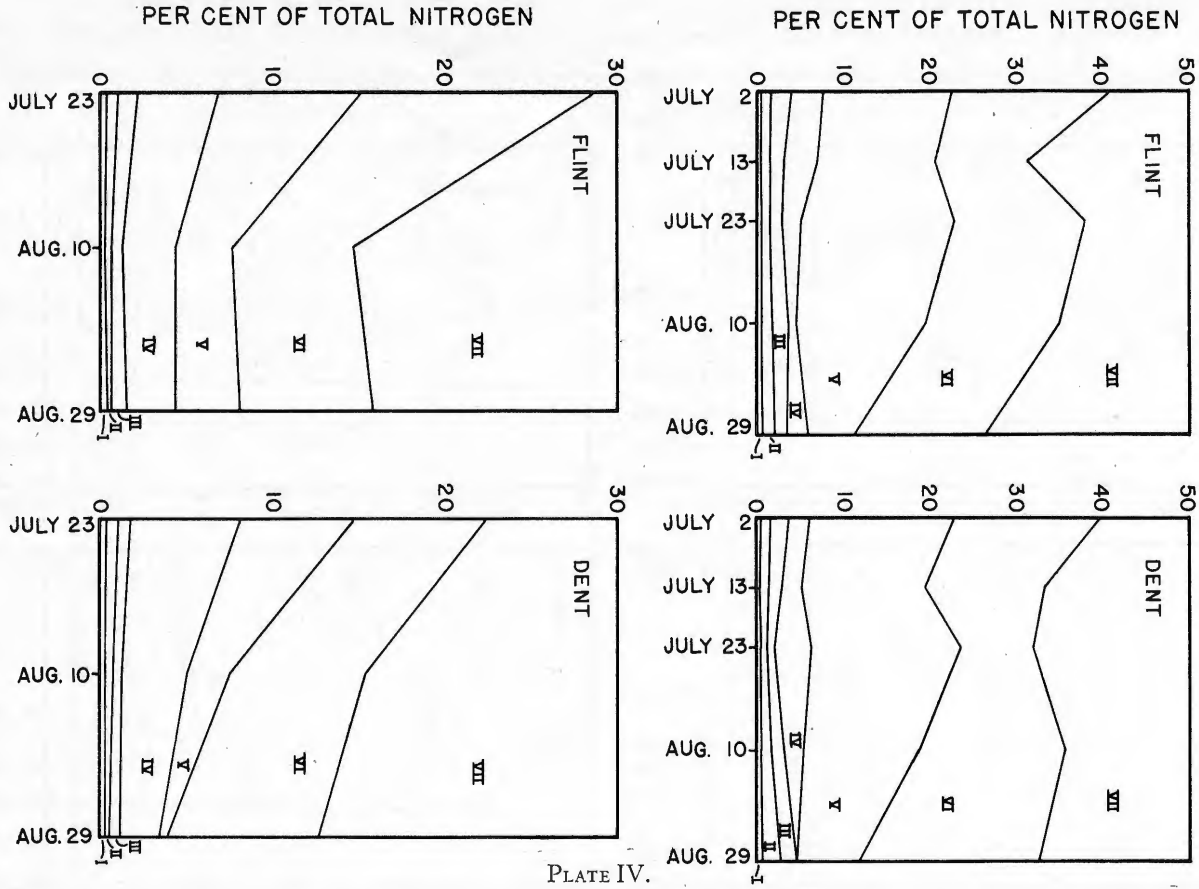

PLATE IV.

Area graphs showing the changes which occurred in the nitrogen constituents of Flint and Dent corn plants (upper graphs) and of the leaves of these plants (lower graphs) during the growing season of 1945. Nitrogen constituents:

I—Ammonium. II—Glutamine. III—Asparagine. IV—Residual *a*-Amino. V—Nitrate. VI—Undetermined soluble. VII—Insoluble.

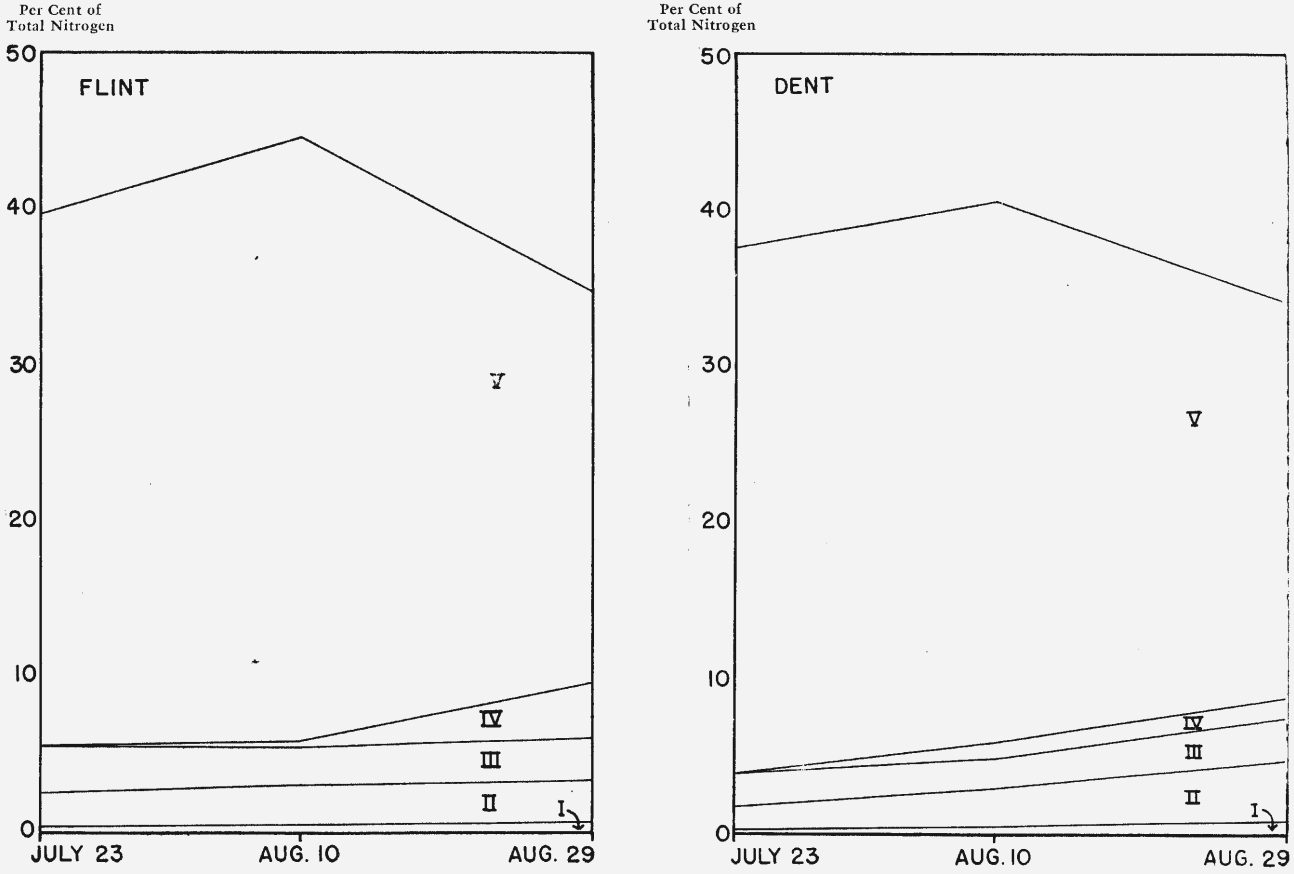

PLATE V.

Area graphs showing the changes which occurred in the nitrogen constituents of the stalks of Flint and Dent corn plants during the growing season of 1945. Nitrogen constituents: I—Ammonium. II—Glutamine. III—Asparagine. IV—Residual *a*-Amino. V—Nitrate.

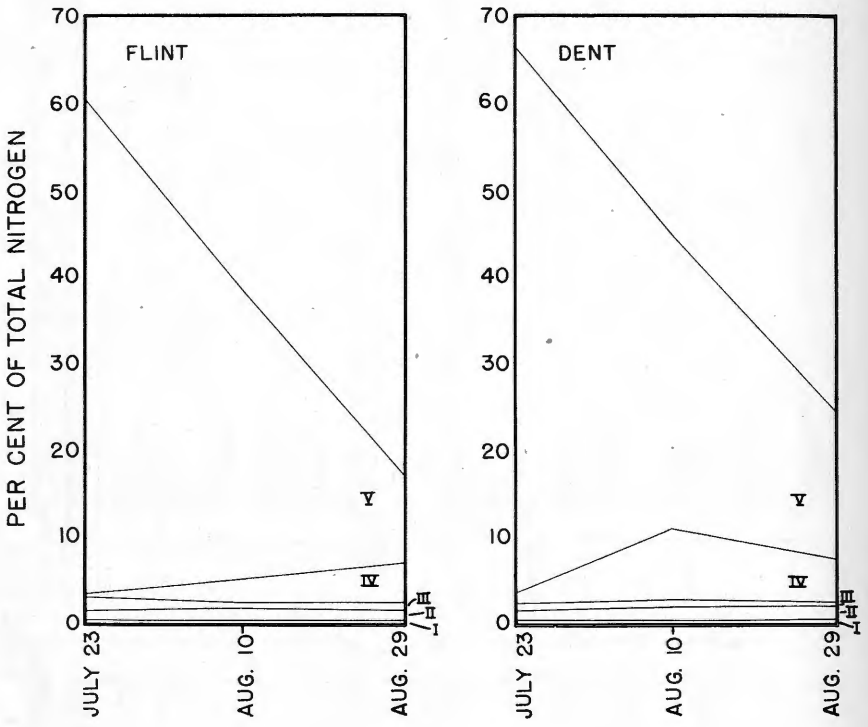

PLATE VI.

Area graphs showing the changes which occurred in the nitrogen constituents of the sheaths of Flint and Dent corn plants during the growing season of 1945. Nitrogen constituents:

I—Ammonium. II—Glutamine. III—Asparagine. IV—Residual *a*-Amino. *V*—Nitrate.

The increases in soluble nitrogen found in leaves during the fall are, no doubt, those associated with decomposition of leaf proteins and slight increases in nitrate nitrogen. In stalks, at midseason, the increase in soluble nitrogen is possibly associated with the role of the stalk as an intermediate in the translocation pathway.

Sheath tissue also has a high soluble nitrogen content early in the season; however, it decreases rapidly (Plate VI) and the decrease is directly related to a simultaneous fall in nitrate content. In the sheath the soluble nitrogen compounds, other than nitrate, represent a small fraction of the total nitrogen.

In Plates II and III it is observed that changes in the soluble nitrogen content in detached cornstalks were comparable to the changes found in the intact plant.

"True" protein nitrogen was determined only for those plants sampled during the summer of 1945 (Table IV). Protein nitrogen increased in leaf tissue (per cent of total nitrogen basis) with maturity (Plate IV). Though changes were noted in the protein nitrogen con-

Table IV. The "True" Protein Nitrogen Content of Flint and Dent Corn Plants Expressed as Per Cent of Total Nitrogen

Variety	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent
1945	July 2		July 13		July 23		Aug. 10		Aug. 29	
Entire Plant	59.0	60.2	68.7	66.6	61.9	67.9	65.7	64.2	73.3	67.3
Leaves					71.3	77.6	85.3	84.5	84.2	87.3
Stalks					40.8	46.7	37.4	32.1	50.5	40.2
Sheaths					27.7	32.9	49.6	45.2	70.7	75.1
Shanks							62.9	63.5	70.8	62.8
Ears									74.8	61.1
Tassels					74.1		66.5	57.3	65.9	77.4

tent of the stalks, these fluctuations are difficult to interpret, but with respect to "true" protein the stalk apparently maintains a fairly uniform content.

From an examination of the protein nitrogen data for sheath tissue it would appear that protein synthesis is a dominant phase in this tissue of the corn plant. However, considering the changes occurring in soluble nitrogen, particularly nitrate, in sheath tissue (Plate VI) during the July-August period, it is obvious that this tissue is not a site of extensive protein synthesis; instead, the apparent synthesis is due only to the withdrawal of a large amount of the soluble nitrogen originally present (cf. Table II).

The high protein content of the tassels is in agreement with the findings of Van Lanen *et al.* (21) as cited previously.

Nitrate and Nitrite Nitrogen

Though nitrite nitrogen determinations were made on materials representing the corn plant throughout its entire growth cycle, nitrite was not detected in the growing plant. Only during the late fall, when decomposition (bacterial and fungal) was extensive, were nitrites found (see Appendix Tables IV, V, and VI).

Soil nitrate content was determined during the summer of 1945 using phenoldisulfonic acid reagent (40). The p.p.m. of nitrate nitrogen in the 0 to 6, 6 to 12, 12 to 18, and 18 to 24 inch profile levels of the soil are recorded in Appendix Table IX and the fluctuations found are shown in Figure 1.

Considering the entire plant (Plate I) the nitrate content of the corn plant in the seedling stage accounted for more than 10 per cent of the total nitrogen and remained high until the latter part of August, when demands of the maturing plant (with developing ears) apparently drew upon the nitrate stored in the various plant tissues—at a time when the soil was no longer as well supplied with this form of nitrogen (Figure 1). An apparent increase in nitrate content of the plant tissues was observed after the frost date and is probably associated with disruption of the metabolic pattern in the green plant tissues, though the roots are still absorbing ions normally.

Leaves, shanks, ears, and tassels are relatively low in nitrate nitrogen as can be seen in Table V. In leaves, the nitrate content decreased between the months of July and September; one should note that the leaf tissue itself, as differentiated from the mid-rib, is low in nitrate nitrogen (Appendix Table X). It is of interest that the leaf tissue can maintain a low nitrate content, since the adjacent sheath tissue is very high, exceeding the stalks during early growth stages, in respect to this form of nitrogen. The assimilation of nitrate nitrogen by the leaf is apparent, therefore, as nitrate rapidly diminished in the sheath tissue without simultaneous increases in

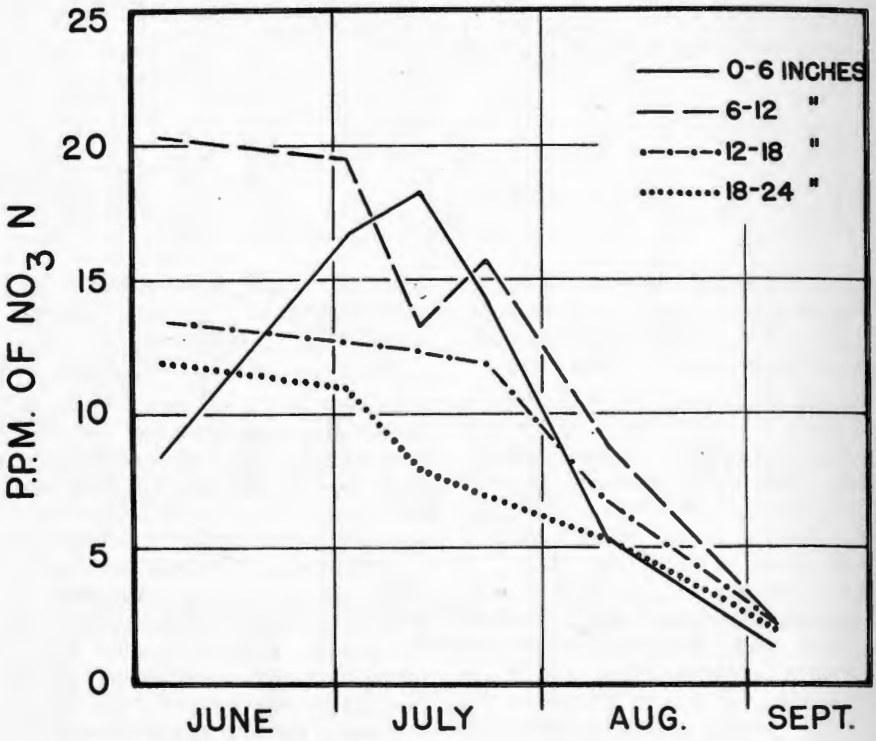

Figure 1. A graph showing the changes which occurred in nitrate nitrogen content of the various soil profile levels of the plots of Flint and Dent corn investigated during the summer of 1945.

other forms of soluble nitrogen (Plate VI). Whether nitrate nitrogen of the sheath tissue is metabolized by the leaves via ammonia to organic nitrogenous compounds or whether it is translocated to the shank and the developing ears (via the stalks) for subsequent synthesis of amino acids is a matter for speculation. Burstrom (41) in studies on the assimilation of nitrate by wheat leaves concludes that in light, nitrate is reduced (but not to ammonium) and reacts with some intermediate product of carbon dioxide assimilation (but not sugar) to form carbon-nitrogen assimilates; in this respect the assimilation of nitrate by leaves must follow a different course than in the roots.

Some evidence of the biological oxidation by plants of ammonium to nitrite nitrogen has been reported. Eggleton (42) has demonstrated the presence of nitrite in spring grass fertilized with either sodium nitrate or ammonium sulfate. Vickery *et al.* (43) in studies with *Narcissus poeticus* detected loss of nitrogen in the developing leaves and discusses this loss in terms of the hypothesis of Pearsall and Billimoria (44) according to which nitrogen is lost by the interaction of amino nitrogen and nitrous acid, the latter being derived from either ammonium or nitrate nitrogen. In detached leaves cultured in the dark (45,

Table V. The Nitrate Nitrogen Content of Flint and Dent Corn Plants Expressed as Per Cent of Total Nitrogen

Variety	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent
1943	Sept. 9		Sept. 11		Sept. 18		Sept. 25		Nov. 17		Dec. 1					
Entire Plant	4.1	6.7	18.8	9.4	15.0	8.2	13.4	7.4	6.9	6.3	10.8	10.2				
1944	Sept. 19		Sept. 25		Oct. 2		Oct. 9		Oct. 11		Oct. 16		Oct. 20		Nov. 13	
Uncut																
Entire Plant	15.8	7.5	15.6	8.9	13.9	8.0	13.3	10.7	16.5	10.3			9.3	7.7		
Leaves	1.9	0.8	1.9	0.7	1.5	0.2	1.3	0.8	1.4	0.7			3.3	1.6		
Stalks	35.0	23.3	40.1	22.8	36.2	25.3	32.7	22.1	33.7	26.8			19.9	21.5	14.3	8.5
Cut																
Leaves					1.5	0.2	0.7	0.4			1.2	0.7				
Stalks					34.1	28.6	31.8	18.9			31.0	29.1			15.8	10.5
1945	July 2		July 13		July 23		Aug. 10		Aug. 29							
Entire Plant	14.8	16.8	13.7	14.3	17.8	17.5	15.1	13.5	5.3	7.4						
Leaves					8.3	6.6	3.3	2.5	3.8	0.5						
Stalks					34.2	33.8	38.8	34.8	25.0	25.4						
Sheaths					57.1	62.9	33.5	34.1	10.1	17.2						
Shanks							1.4	0.9	4.1	1.0						
Ears									0.2	0.2						
Tassels					0.6		6.9	1.3	0.2	0.7						

Table VI. The Ammonium Nitrogen Content of Flint and Dent Corn Plants Expressed as Per Cent of Total Nitrogen

Variety	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent
1944	Sept. 19		Sept. 25		Oct. 2		Oct. 9		Oct. 11		Oct. 16		Oct. 20		Nov. 13	
Uncut																
Entire Plant	0.32	0.41	0.75	0.40	1.29	1.00	1.39	1.65	1.51	1.04			2.09	1.37		
Leaves	0.38	0.40	0.95	0.41	1.68	1.17	1.77	1.61	1.98	1.05			1.82	1.43		
Stalks	0.24	0.42	0.39	0.37	0.59	0.63	0.76	1.69	0.98	1.04			2.56	1.26	3.14	2.40
Cut																
Leaves					1.92	1.67	1.76	1.62			1.75	2.02				
Stalks					0.62	0.60	0.98	1.33			2.89	1.40			3.21	1.54
1945	July 2		July 13		July 23		Aug. 10		Aug. 29							
Entire Plant	0.30	0.25	0.21	0.22	0.33	0.32	0.33	0.33	0.36	0.45						
Leaves					0.34	0.34	0.25	0.21	0.28	0.21						
Stalks					0.28	0.22	0.49	0.47	0.65	0.83						
Sheaths					0.27	0.32	0.28	0.34	0.27	0.39						
Shanks							0.33	0.47	0.30	0.35						
Ears									0.36	0.56						
Tassels					0.29		0.42	0.52	0.42	0.55						

46) nitrate production has been noted. Though acknowledging the above evidence, it seems that the nitrate nitrogen of field-grown corn plants is for all practical considerations a storage form, representing nitrogen absorbed and in excess of the plants assimilatory powers. Should some environmental factor limit growth, it is probable that the assimilation of nitrate nitrogen will be interfered with and nitrates will accumulate in the plant tissues (27, 47).

In Appendix Table X and Figure 2 are presented data relative to the distribution of nitrate nitrogen as a function of the position of a given tissue on the corn plant. It is readily observed that the nitrate nitrogen content of a specific structural tissue decreases the higher up on a plant the sample is obtained.

Ammonium Nitrogen

The roles ascribed to ammonia in the synthesis and break down of proteins and amino acids, as postulated in the writings of Schulze and Prianischnikow, have been reviewed by Chibnall (8). As an intermediate in the classical speculations on nitrate reduction via nitrite (42) or nitrite-hyponitrous acid-hydroxylamine (8) the importance of ammonia in the primary synthesis of organic nitrogenous compounds is further defined.

Viets *et al.* (12) have shown that with luxury ammonium nutrition, ammonium nitrogen does not accumulate in the tissues of the corn plant until other soluble nitrogen has reached relatively high levels; in luxury nitrate nutrition (48) ammonium was observed to increase slightly. It appears that the level of ammonia (which is less toxic to plant than to animal tissues) is carefully regulated in the cells of the plant; marked increases in ammonium content may occur when the organic intermediates (*a*-keto acids) for assimilation have been depleted or with excessive protein breakdown.

Table VI presents the data pertaining to the ammonium content of the corn plant and its separate tissues. In the growing plant only the stalk tissue exhibited an appreciable upward trend in ammonium nitrogen (leaves decreased slightly); however, after a killing frost the level of ammonium in the entire plant increased (see Plates II, III, IV, and V); the ammonium trend is even more apparent as plotted in Figure 3. The greater share of the increase in ammonia is, no doubt, of secondary origin, i.e., arising from the breakdown of proteins and intermediate nitrogenous compound.

Glutamine and Asparagine

Data for the glutamine and asparagine nitrogen contents of the growing corn plant are given in Tables VII and VIII, respectively, and graphed in Plates IV and V. During the July-August period the amount of glutamine (on a percent of total nitrogen basis) increased slightly in the entire plant, though asparagine decreased. However, in the leaves of both Flint and Dent varieties, glutamine decreased slightly, but uniformly, suggesting that it was the simultaneous increase of glutamine in stalk tissue (which represents an increasingly larger proportion of the total plant weight as the plant matures) which accounts for the slight glutamine increase observed for the entire plant. The asparagine content of leaves and stalks did not show the uniformity of change observed for glutamine; instead, the asparagine content fluctuated more or less. It should be noted, however, that asparagine predominates over glutamine in corn plant tissues.

Asparagine has been found by Viets *et al.* (12) to be more responsive to conditions leading to amide formation (ammonium nutrition) in the corn plant than glutamine. The view that the two amides, asparagine and glutamine, have

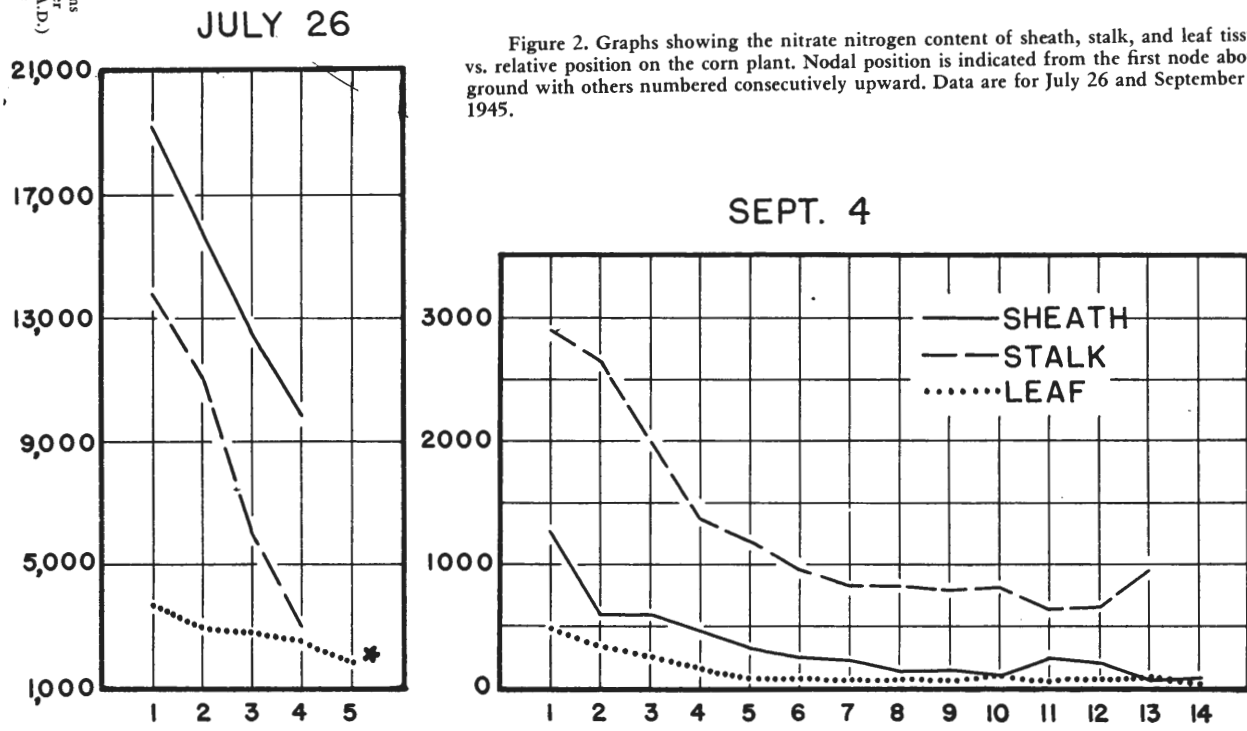

Figure 2. Graphs showing the nitrate nitrogen content of sheath, stalk, and leaf tissue vs. relative position on the corn plant. Nodal position is indicated from the first node above ground with others numbered consecutively upward. Data are for July 26 and September 4, 1945.

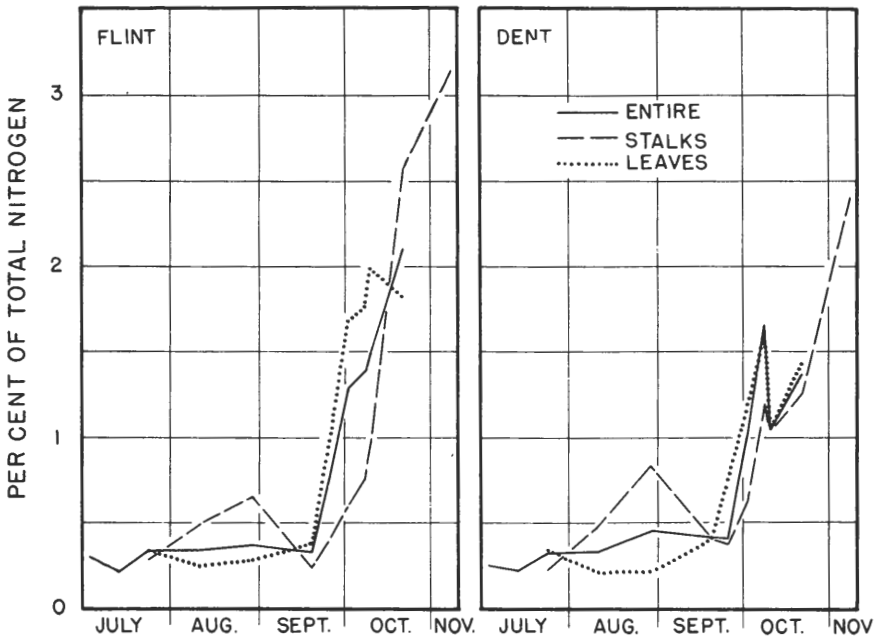

Figure 3. Graphs showing the changes which occurred in ammonium content of Flint and Dent corn plants, and leaves and stalks thereof, throughout the summer growing season and into late fall.

different roles in plants has been emphasized by Vickery *et al.* (46); and Street *et al.* (49) have found that in the potato plant both amides increased in response to ammonium supply, but when the nitrogen-enriched plants are depleted, the asparagine reserves were preferentially used. It seems that for many plants, including *Zea mays,* glutamine is more concerned with the synthetic processes than with ammonia storage and that asparagine is the preferential storage amide.

Glutamine and asparagine are discussed further in the following section.

Table VII. The Glutamine Nitrogen Content of Flint and Dent Corn Plants Expressed as Per Cent of Total Nitrogen

Variety	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent
1944	Sept. 19		Sept. 25		Oct. 2		Oct. 9		Oct. 11		Oct. 16		Oct. 20		Nov. 13	
Uncut																
Entire Plant	1.80	1.98	2.44	1.70	2.94	2.14	2.80	1.58	3.64	2.10			2.52	1.96		
Leaves	1.38	1.02	1.88	0.94	1.26	0.64	1.66	0.66	1.78	1.02			2.16	0.78		
Stalks	2.38	4.20	3.42	2.96	5.98	5.40	4.66	2.64	5.76	7.50			3.14	0.90	2.32	2.44
Cut																
Leaves					0.50	1.38	0.90	1.32			0.96	1.72				
Stalks					2.48	2.56	3.58	6.04			3.44	3.22			0.04	1.64
1945	July 2		July 13		July 23		Aug. 10		Aug. 29							
Entire Plant	1.26	1.14	1.08	0.92	0.96	0.84	1.38	1.28	1.52	2.26						
Leaves					0.70	0.72	0.42	0.52	0.34	0.34						
Stalks					2.24	1.50	2.56	2.40	2.70	3.88						
Sheaths					1.12	1.06	1.38	1.74	1.16	1.64						
Shanks							2.50	1.64	2.08	2.36						
Ears									1.88	4.42						
Tassels					1.80		1.34	1.76	1.34	1.62						

Table VIII. The Asparagine Nitrogen Content of Flint and Dent Corn Plants Expressed as Per Cent of Total Nitrogen

Variety	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent
1944	Sept. 19		Sept. 25		Oct. 2		Oct. 9		Oct. 11		Oct. 16		Oct. 20		Nov. 13	
Uncut																
Entire Plant	1.98	2.10	2.04	1.82	3.22	2.92	3.16	2.14	2.78	2.60			1.08	0.86		
Leaves	1.16	1.30	0.86	1.50	1.52	2.34	1.34	1.24	0.98	1.26			0.78	0.90		
Stalks	3.10	3.98	4.16	2.34	6.30	4.16	6.14	3.16	4.84	4.92			1.62	0.78	3.24	1.88
Cut																
Leaves					1.96	1.42	0.64	1.40			0.50	0.16				
Stalks					6.20	3.54	8.68	3.94			6.16	2.40			2.02	2.22
1945	July 2		July 13		July 23		Aug. 10		Aug. 29							
Entire Plant	2.34	2.26	1.82	1.66	1.48	0.90	1.94	1.48	1.38	1.84						
Leaves					1.12	0.74	0.60	0.54	0.86	0.56						
Stalks					3.04	2.14	2.54	0.96	2.80	2.84						
Sheaths					1.58	0.86	0.64	0.78	0.88	0.42						
Shanks							5.92	6.14	2.48	2.76						
Ears									0.90	2.88						
Tassels					3.92		2.32	3.86	1.36	1.48						

Table IX. The Residual *a*-Amino Nitrogen Content of Flint and Dent Corn Plants Expressed as Per Cent of Total Nitrogen

Variety	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent
1944	**Sept. 19**		**Sept. 25**		**Oct. 2**		**Oct. 9**		**Oct. 11**		**Oct. 16**		**Oct. 20**		**Nov. 13**	
Uncut																
Leaves	3.29	3.47	2.78	2.11	6.55	2.50	6.28	3.48	7.34	3.13			6.29	5.28		
Stalks	3.84	5.28	4.02	4.12	3.09	8.20	3.20	5.64	4.66	4.30			6.66	.....	4.64	5.07
Cut																
Leaves					4.73	2.01	5.31	4.89			6.91	6.53				
Stalks					1.40	4.33	0.56	4.45			7.04	.....			7.52	4.80
1945	**July 2**		**July 13**		**July 23**		**Aug. 10**		**Aug. 29**							
Entire Plant	.....	.....	3.80	2.28												
Leaves					4.73	6.44	3.08	3.81	2.77	2.31						
Stalks					none	none	0.33	1.05	3.50	1.17						
Sheaths					0.34	1.28	2.84	8.10	4.51	4.97						
Shanks							0.23	0.05	4.71	0.49						
Ears									4.50	1.15						
Tassels					0.76		4.54	7.04	4.80	.....						

Table X. The "Basic" Nitrogen Content of Flint and Dent Corn Plants Expressed as Per Cent of Total Nitrogen

Variety	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent	Flint	Dent
1944	**Sept. 19**		**Sept. 25**		**Oct. 2**		**Oct. 9**		**Oct. 11**		**Oct. 16**		**Oct. 20**		**Nov. 13**	
Uncut																
Entire Plant	6.1	6.0	9.1	7.7	10.4	9.7	5.1	6.4	7.0	8.2			6.5	5.8		
Leaves	5.4	5.4	8.9	7.0	9.4	8.8	5.1	6.2	8.9	6.4			7.1	4.5		
Stalks	7.1	10.5	9.3	8.9	12.0	11.7	5.1	6.5	4.9	11.3			5.4	8.8	8.5	8.4
Cut																
Leaves					7.6	7.6	7.8	7.5			9.1	8.8				
Stalks					3.4	5.3	7.0	9.5			12.6	8.7			7.7	7.0
1945	**July 2**		**July 13**		**July 23**		**Aug. 10**		**Aug. 29**							
Entire Plant	.....	.....	7.7	7.8	.....	.....	7.5	7.2	7.1	7.3						
Leaves					7.3	7.1	4.9	5.4	5.9	5.0						
Stalks					.....	.....	.....	7.9	6.1	6.2						
Sheaths					.....	.....	7.4	7.3	8.9	7.9						
Shanks							.....	14.6	9.4	7.7						
Ears									6.7	12.7						
Tassels					.....		9.0	11.7	10.2	8.3						

Amino Acids

The amino acid content, as measured by *a*-amino nitrogen, remains quite constant in the corn plant throughout the normal growing period. Following frost and particularly during weathering, the *a*-amino nitrogen content decreased slowly; during a period of intense microbiological activity (Nov.-Dec. 1943), the amino acids were apparently utilized by the microorganisms concerned in the disintegration of the stalks (see Plate I and Table IX). Not to be discounted in explanation of amino acid loss at this stage is leaching of the plant material, though total nitrogen and soluble nitrogen actually increased (cf.-Appendix Table IV).

Since the *a*-amino nitrogen of the amides is also measured by the Van Slyke procedure, a correction for it was made and the resulting value is designated residual *a*-amino nitrogen. It is discernable in Plates IV and V (and Table IX) that residual *a*-amino nitrogen decreased in the corn plant during the period of tasseling and ear development. The greatest portion of the residual *a*-amino nitrogen was found in the leaves and therefore, is intimately associated with the high protein levels (and synthesis) found therein. Stalks and sheaths showed increases in amino acids during August and these are probably associated with translocation. It is of interest that early in the growing season *a*-amino nitrogen, other than that of the amides, was not detected in the stalks, though later measurable increases in residual *a*-amino nitrogen were found. The importance of the amides as a translocation form of soluble organic nitrogen can be surmised, and is in agreement with the earlier suggestion of Schulze, 1898, (cited by Chibnall). The increase observed in the residual *a*-amino nitrogen content (part of which may be glutamic and aspartic acids, as well as other amino acids) of stalks perhaps results from increased synthesis (or secondarily, from protein breakdown) in the leaves in response to the demands of the rapidly developing ears; earlier in the growth cycle it appears that the residual *a*-amino acids are utilized about as rapidly as they are synthesized.

It should be noted in connection with this discussion on the accumulation of residual *a*-amino acids, that with ammonium nutrition (12), amides and residual amino acids accumulated, whereas, with nitrate nutrition (48) only amino acids increased. Vladimirov (50), in studies with *Nicotiana* observed that nitrate absorption and metabolism stimulates the formation of organic acids from sugars. Sideris and Young (51) suggest that the actual course of synthesis differs if nitrogen is supplied in the form of nitrate instead of ammonium, since roots receiving nitrates are relatively poor in amides and amino nitrogen and relatively rich in other nitrogen; this would be the course if some organic nitrogen complex resulted from nitrate reduction rather than ammonia being first formed.

Returning to a consideration of glutamine and asparagine, current views ascribe to the amides a more important role than only that of nitrogen translocation within the plant. Chibnall and Grover (52) have prepared asparaginase from germinating barley, and the presence of L-aspartic dehydrogenase is suggested by the studies of Damodaran and Subramanian (53). The transamination reaction:

L-glutamic acid + oxalacetic acid $\rightleftharpoons$ *a*-ketoglutaric acid + L-aspartic acid

has been demonstrated in a wide variety of plants by Leonard and Burris (54), and Virtanen and Laine (55) have shown that a similar transfer involving aspartic acid and pyruvic acid occurs. One might speculate about transamina-

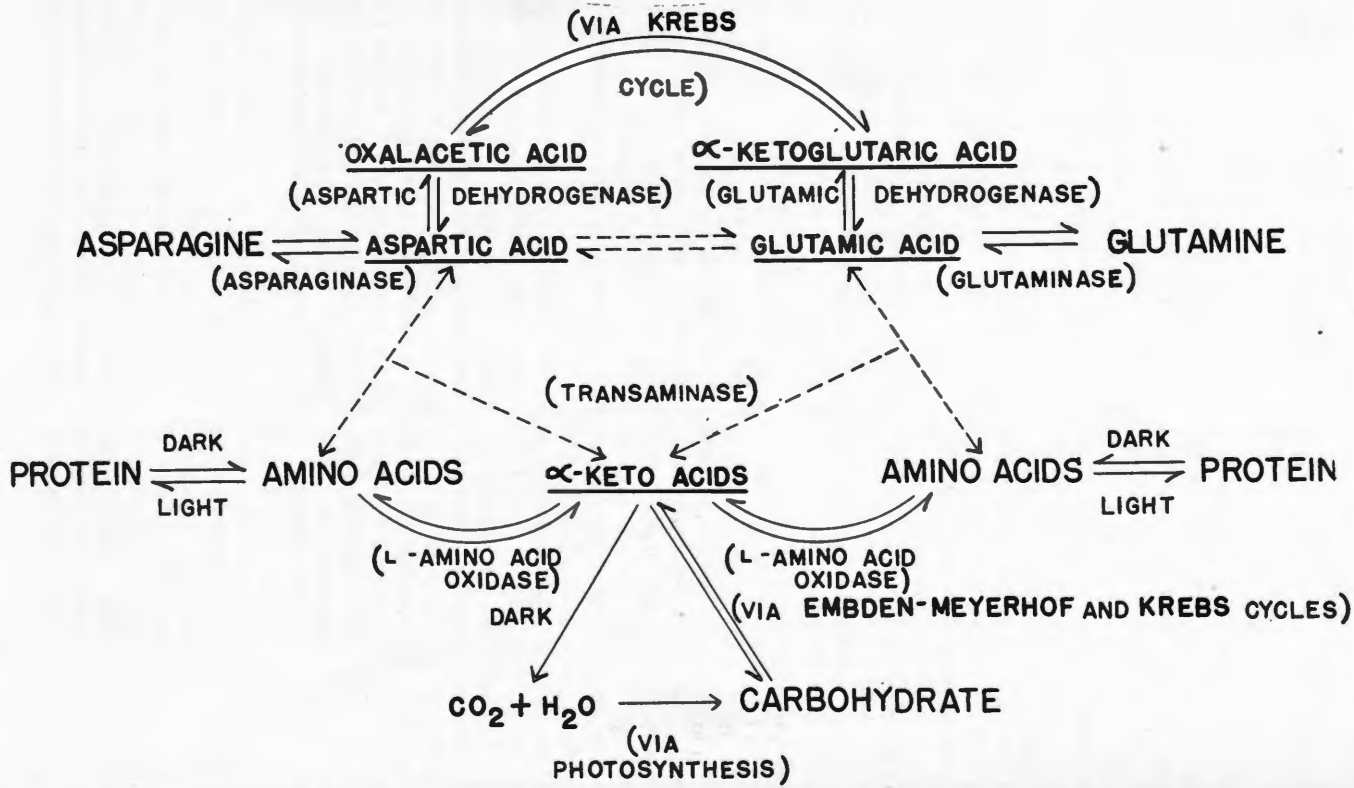

Figure 4. A scheme relating the metabolism of various nitrogen compounds of the corn plant in terms of present concepts of nitrogen metabolism in plants.

tions involving other *a*-keto acids made available through carbohydrate metabolism, or through oxidative deamination of amino acids, but it suffices for this discussion to point out that the newer knowledge ascribes to asparagine a role not only in storage of nitrogen (although, as mentioned before, it seems to be the preferential storage amide), but also in the synthesis of amino acids (other than aspartic acid) as is illustrated in Figure 4.

Vickery *et al.* (56, 57), Chibnall (8), and Archibald (58) have investigated the occurrence of glutaminase in plants, and the L-glutamic dehydrogenase of plants has been studied by Damodaran and Nair (59), von Euler (60), and Berger and Avery (61). Thus, the synthesis of glutamine and its role in amino acid synthesis parallels that of asparagine (Figure 4).

As has been observed previously, the concentration of residual *a*-amino acids in the stalk is negligible early in the season, with slight increases toward maturity. At present it is speculative to suggest that the increase noted in residual *a*-amino nitrogen is attributable to aspartic and glutamic acids, rather than to the other amino acids. However, if one considers that the other amino acids are removed by protein synthesis as fast as they are synthesized, then aspartic and glutamic acids may be regarded as a probable nitrogenous metabolic pool yielding nitrogen via transamination for the synthesis of the other amino acids. Steward and Street (6) observe that though transamination is evidently widespread, the synthesis of the aromatic amino acids appears to be outside of the normal scope of this process.

Before leaving this discussion it is of interest to recall the studies of Roine (62), and Virtanen and Czaky (63), investigating the synthesis of amino acids by low-nitrogen yeast (*Torula utilis*) fed ammonium sulfate and potassium nitrate. They observed great increases in soluble nitrogen due to aspartic and glutamic acids and their amides, as well as to alanine, with either ammonium or nitrate nutrition. Roine was unable to find other amino acids. With nitrate nutrition a rapid formation of oxime-nitrogen was observed.

Basic Nitrogen

Data for the "basic" nitrogen content of the corn plant is reported in Table X and illustrated in Figure 5. This fraction accounts for a considerable portion of the nitrogen compounds reported as undetermined soluble nitrogen and includes polypeptide and peptide nitrogen, the nitrogen of arginine, histidine, and lysine, etc. The basic amino acids constitute but a small percentage of the residual amino acids elaborated by corn plants supplied with ammonium (12).

Leaf tissue of the corn plant is high in basic nitrogen content, as is illustrated in Figure 5, and the association of high basic nitrogen content with high protein activity is implied. In late September and early October the basic nitrogen content of stalks and leaves increased sharply, reversing the downward trend observed during the normal period of growth. These increases in basic nitrogen coincide with the period of killing frosts. After the plants have been killed by frost the basic nitrogen contents returned to approximately the same levels as prior to the frost.

Appended to the foregoing discussion of nitrogen fractions is the data in Table XI relative to the distribution of sucrose and total reducing sugars in the various tissues of the corn plant. No attempt is made to correlate sugar content with amino acid metabolism or nitrate assimilation. Expressed in terms of milligrams of sugar per gram of air-dry tissue, leaves

(the photosynthetic source) are relatively low, while stalks, sheaths, and shanks are high, though the values for sheaths appear to be intermediate between leaf and stalk values. Here again the position of the stalk in the translocation pathway is important. Loomis (64) has investigated the nature of carbohydrate transport in the corn plant; diurnal variations in sugars and soluble carbohydrates in leaves of the corn plant have been studied by Miller (65), Puhr and Hume (66).

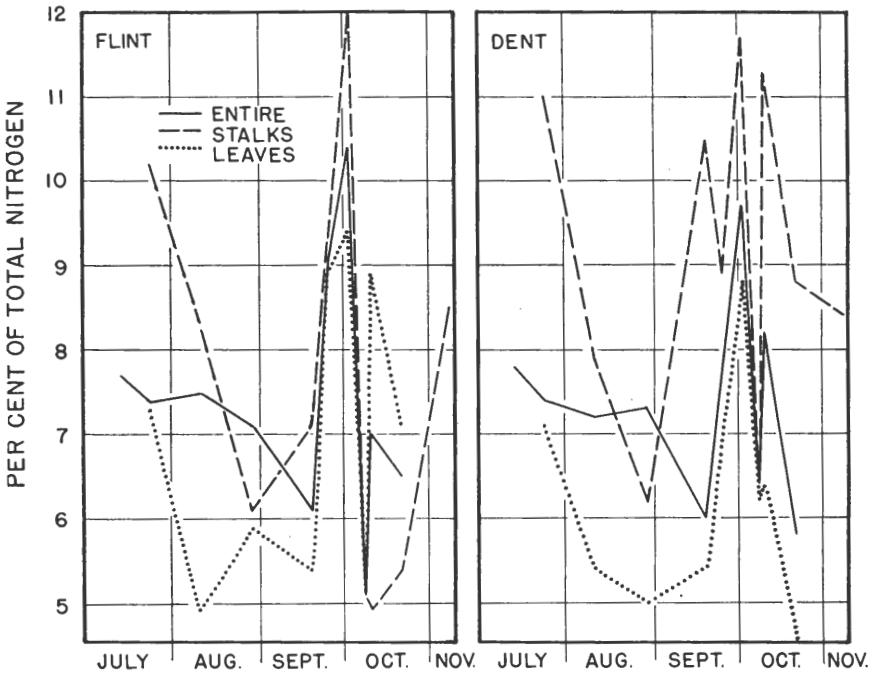

Figure 5. Graphs showing the changes which occurred in basic nitrogen content of Flint and Dent corn plants, and leaves and stalks thereof, throughout the summer growing season and into late fall.

Table XI. Total Sugar (Sucrose and Reducing Sugars) Content of Flint and Dent Corn Plants

Variety	Flint	Dent	Flint	Dent	Flint	Dent
1945	July 23		Aug. 10		Aug. 29	
Leaves						
Total Sugar (mg. per g.)	35.2	27.7	26.6	38.9	24.9	33.6
Reducing Sugars (% of Total)	69.9	78.3	65.4	56.5	87.9	91.4
Sheaths						
Total Sugar (mg. per g.)	129.3	111.5	114.5	181.1	127.0	138.5
Reducing Sugars (% of Total)	94.7	91.2	90.2	53.3	68.9	56.1
Stalks						
Total Sugar (mg. per g.)	214.3	193.5	207.6	223.5	221.6	189.8
Reducing Sugars (% of Total)	65.7	77.1	59.0	76.9	61.7	57.7
Shanks						
Total Sugar (mg. per g.)			239.0	213.0	226.6	217.2
Reducing Sugars (% of Total)			79.5	68.9	53.3	70.9

Conclusions

There is little difference between the plants of the Flint and Dent corn varieties studied, with respect to content of ammonium, glutamine, asparagine, *a*-amino, or basic nitrogen and the response of these nitrogen fractions to summer or fall environment. Nitrate nitrogen represents a slightly greater percentage of the total nitrogen of Flint corn stalks and leaves, during both summer and fall, than of Dent corn stalks and leaves.

Cutting and tying Flint and Dent corn plants to adjacent stalks left standing in the field in the fall had little, if any, effect on the nitrogen fractions studied. *a*-Amino nitrogen values (on a per cent of total nitrogen basis) seemed to remain slightly higher in the cut stalks.

Late in the fall of each of the three years of investigation, cattle were turned into the plots of corn sampled for these studies and no losses occurred, nor were there any reports of cattle losses in cornstalk fields elsewhere in the area—consequently no plant material from fields which have caused cornstalk poisoning in cattle has been available during these years for the purpose of comparison. Therefore, it seems that none of the nitrogen fractions at the levels reported in this bulletin are associated with cornstalk poisoning of cattle and that the data is representative of normal Flint and Dent corn plants.

Summary

The distribution and changes occurring in the nitrogen fractions of Flint and Dent corn plants have been studied during the fall of 1943 and 1944, and during the growing season of 1945.

Nitrogen uptake by the corn plant continued throughout the growing season, although the rate of increase diminished toward maturity. During growth the total nitrogen contents of leaves, sheaths, shanks, and tassels reached maxima and decreased, the net loss from the tissues being attributable to total nitrogen gain in the maturing ears.

Soluble nitrogen of leaf tissue, as per cent of total nitrogen, decreased toward midsummer but increased in the fall, probably due to proteolysis; in stalks, soluble nitrogen increased at midsummer coincident with tasseling. The soluble nitrogen content of stalks exceeded that of leaves, but approximately one-half of the soluble nitrogen of stalks was in the form of nitrate. Early in the growing season sheath tissue is high in soluble nitrogen, but this decreased rapidly and in proportion to diminution of nitrate nitrogen as the plant matures.

Nitrite nitrogen was detected in the corn plants only in late fall after killing frosts and during a period when microbiological activity resulted in considerable decomposition of the stalks.

Nitrate nitrogen at the seedling stage accounted for 10 per cent of the total nitrogen and remained at high levels until ear development began, but diminished in the tissues of the plant as the corn matured. Stalk and sheath tissue have a large proportion of nitrate nitrogen present. On a milligram nitrogen per kilogram dry plant material basis, Flint stalks and leaves contained about twice as much nitrate nitrogen as Dent stalks and leaves.

Ammonium nitrogen, though increasing slightly in stalks, decreased in leaves during the summer period. In the fall and during weathering of the cornstalks ammonium content of the tissues increased about four-fold.

For the entire plant and on a per cent of total nitrogen basis, glutamine content increased toward mid-season and asparagine decreased. The stalks of both Flint and Dent corn plants increased in glutamine, offsetting a decrease observed in the leaves. Asparagine predominated over glutamine in the various corn plant tissues.

a-Amino nitrogen remained quite constant in the entire plant throughout the growing season, decreasing in the fall after killing frosts and at a time coincident with intense microbiological activity. Residual *a*-amino nitrogen (see text) decreased in the plants following tasseling and during ear development. Leaves contained the highest amount of residual *a*-amino acids. In stalks, residual *a*-amino acids were negligible early in the season but increased as the ears developed.

Basic nitrogen is higher in leaf tissue than in stalk tissue. A sharp increase in this nitrogen fraction occurred subsequent to killing frost, but basic nitrogen content soon returned to previous levels. During the growing season a slight downward trend was observed for the basic nitrogen constituents in both leaves and stalks.

Cutting and tying Flint and Dent corn plants to adjacent stalks left standing in the field in the fall had little effect on the nitrogen fractions studied.

Changes observed for certain of the nitrogen fractions studied are discussed in the text in terms of current concepts of nitrogen metabolism in plants.

Though this study was made in connection with a project on cornstalk disease, no cattle deaths occurred when cattle were turned into the fields being studied; therefore, the data for the various nitrogen fractions as set forth in this bulletin are assumed to be representative of normal Flint and Dent corn plants with respect to cornstalk disease.

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Appendix

TABLE I. Harvest Weights and Per Cent Moisture of Samples of Flint and Dent Corn Plants Collected During the Fall of 1943 (Data for six plants)

Variety	Flint		Dent	
Date	Weight (grams)	Per cent Moisture	Weight (grams)	Per cent Moisture
Sept. 9				
Leaves				
Stalks				
Entire Plant*		80.6		78.9
Sept. 11				
Leaves	975		1000	
Stalks	1620		1620	
Entire Plant*		80.5		78.9
Sept. 18				
Leaves	795		795	
Stalks	1590		1515	
Entire Plant*		78.4		73.0
Sept. 25				
Leaves	395		350	
Stalks	1315		1310	
Entire Plant*		68.9		64.3
Nov. 11				
Leaves	233		247	
Stalks	563		553	
Entire Plant*		51.2		41.5
Dec. 1				
Leaves	257		221	
Stalks	413		409	
Entire Plant*		32.1		32.4

*Total value for stalk and leaf plus sheath fractions.

TABLE II. Fresh Weight and Moisture Content of Leaves and Stalks of Flint and Dent Corn Plants Collected in 1944 (Data for thirty plants)

Legend: U = green stalks (uncut)
C = cut stalks
D = Dent corn
F = Flint corn
L = leaves (including sheaths)
S = stalks (not including tassels, ears, or shanks)

Sample	Date	Fresh weight (grams)	Total fresh Weight (grams)	Per cent Moisture	Dry weight (grams)	Total dry Weight (grams)	Fraction Per cent of Total sample
UDL	9/19	5810	14,370	76.1	1390	3010	46.1
UFL	9/19	5190	14,820	76.6	1210	2920	41.6
UDS	9/19	8560		81.0	1620		53.8
UFS	9/19	9630		82.3	1700		58.4
UDL	9/25	4390	14,120	70.6	1290	3200	40.3
UFL	9/25	4670	13,140	72.5	1290	2660	48.2
UDS	9/25	9730		80.4	1910		59.7
UFS	9/25	8460		83.7	1380		51.8
UDL	10/2	6510	15,600	74.3	1670	3130	48.9
UFL	10/2	6160	14,520	74.0	1600	3070	52.2
UDS	10/2	9090		80.7	1750		51.1
UFS	10/2	8370		82.5	1470		47.8
CDL	10/2	3820	8,360	48.0	1990	3280	60.5
CFL	10/2	3460	7,900	47.8	1810	2870	62.9
CDS	10/2	4550		71.5	1300		39.5
CFS	10/2	4440		76.1	1060		37.1
UDL	10/9	2370	9,210	55.0	1060	2560	41.6
UFL	10/9	2700	9,160	60.7	1060	2190	48.2
UDS	10/9	6850		78.2	1490		58.4
UFS	10/9	6460		82.5	1130		51.6
CDL	10/9	1360	5,240	25.2	1020	2360	43.1
CFL	10/9	1920	5,140	37.0	1210	2100	57.5
CDS	10/9	3880		65.4	1340		56.9
CFS	10/9	3230		72.3	890		42.5
UDL	10/11	2300	10,000	41.0	1350	2960	45.7
UFL	10/11	1850	9,310	42.2	1070	2430	43.9
UDS	10/11	7710		79.2	1600		54.2
UFS	10/11	7470		81.7	1370		56.1
CDL	10/16	1740	3,800	22.1	1350	2430	55.6
CFL	10/16	1770	4,420	20.0	1420	2340	60.7
CDS	10/16	2070		47.8	1080		44.4
CFS	10/16	2650		65.4	920		39.3
UDL	10/20	2570	7,170	30.7	1780	3270	54.5
UFL	10/20	3710	11,450	34.0	2450	4300	56.9
UDS	10/20	4606		67.7	1490		45.5
UFS	10/20	7750		76.1	1850		43.1
UDS	11/13			35.2			31.1
UFS	11/13			50.0			
CDS	11/13			24.0			
CFS	11/13			30.4			

TABLE III. Fresh Weight and Moisture Content of the Separate Tissues of Flint and Dent Corn Plants Collected in 1945. (Data for eight plants.)

Sample and Date	Fresh weight (grams)	Total Fresh Weight (grams)	Per cent Moisture	Dry weight (grams)	Total dry Weight (grams)	Fraction Percent of Total sample
July 2						
Flint		72	86.2	10		
Dent		67	86.6	9		
July 13						
Flint		593	87.6	74		
Dent		456	87.6	57		
July 23						
Flint		2,300			250	
Leaves	1096		84.6	169		67.6
Stalks	464		93.5	31		12.2
Sheaths	688		93.5	45		18.0
Tassels	52		90.4	5		2.0
Dent		2,354			278	
Leaves	1303		84.8	197		71.0
Stalks	470		93.2	33		11.7
Sheaths	581		91.8	48		17.3
Aug. 10						
Flint		8,069			1181	
Leaves	1102		75.7	270		22.8
Stalks	2747		87.6	341		28.8
Sheaths	1124		85.9	158		13.4
Shanks	2662		89.5	281		23.8
Tassels	434		69.9	132		11.2
Dent		9,319			1366	
Leaves	2102		75.7	513		37.5
Stalks	4762		89.4	502		36.8
Sheaths	1579		86.8	210		15.4
Shanks	437		89.2	47		3.5
Tassels	439		79.1	92		6.8
Aug. 29						
Flint		10,555			2248	
Leaves	1120		74.8	282		12.5
Stalks	2530		81.2	476		21.1
Sheaths	1050		81.3	196		8.7
Shanks	2380		82.0	428		19.1
Ears	3265		76.0	783		34.8
Tassels	210		60.5	83		3.7
Dent		12,980			2814	
Leaves	1410		63.2	519		18.4
Stalks	5070		78.8	1075		38.2
Sheaths	1200		75.4	295		10.5
Shanks	2790		81.6	513		18.2
Ears	2380		85.7	342		12.1
Tassels	130		46.2	70		2.5

TABLE IV. Data for Flint and Dent Corn Plants—1943. Expressed as Milligrams of Nitrogen per Kilogram of Air-Dry Sample

Nitrogen Fraction	Total N	Soluble N	Ammonium + Amide N	Nitrate N	Nitrite N	Total *a*-Amino N
Flint Sept. 9	9890	4110	1080	410	None	1940
Sept. 11	8710	4090	380	1630	None	1590
Sept. 18	7260	4440	330	1090	None	1350
Sept. 25	7000	4420	430	930	None	1440
Nov. 17	9470	5740	1510	660	40	1380
Dec. 1	8010	4920	750	870	5	620
Dent Sept. 9	8970	4540	780	600	None	2720
Sept. 11	8650	4540	570	810	None	2230
Sept. 18	6500	4390	440	530	None	1630
Sept. 25	6540	3920	370	480	None	1730
Nov. 17	9710	6250	1310	610	20	1540
Dec. 1	9890	6440	1150	1000	20	740

TABLE V. Summary of Analytical Data on Corn Stalks Collected in 1944. Expressed in Terms of Milligrams Nitrogen per Kilogram of Air-dry Sample.

Sample and Sampling Date	Total N	Soluble N	NH_4 N	Glutamine Amide N	Asparagine Amide N	NO_3 N	Nitrite N	Basic N	*a*-Amino N (corrected for glutamine amide and ammonium N)
Flint									
Leaves (uncut)									
Sept. 19	17,720	3,080	67	123	103	340	none	960	610
Sept. 25	18,430	4,670	175	173	79	350	none	1,650	760
Oct. 2	16,500	4,570	277	104	125	254	none	1,560	1,310
Oct. 9	18,120	4,580	320	151	121	240	none	920	1,410
Oct. 11	16,050	4,800	320	144	78	220	none	1,430	1,400
Oct. 20	15,970	4,790	290	172	62	520	none	1,140	1,240
Leaves (cut)									
Oct. 2	15,780	3,310	300	39	154	243	none	1,200	940
Oct. 9	17,250	4,330	300	78	56	125	none	1,340	1,050
Oct. 16	17,100	4,110	300	82	42	203	none	1,550	1,300
Stalks (uncut)									
Sept. 19	9,140	5,470	22	109	142	3,200	none	650	600
Sept. 25	9,630	6,530	38	165	200	3,860	none	890	750
Oct. 2	9,960	6,790	59	298	310	3,610	none	1,200	920
Oct. 9	10,430	6,820	80	243	320	3,410	none	540	900
Oct. 11	11,050	7,270	108	320	267	3,720	none	550	1,100
Oct. 20	11,980	7,150	310	188	97	2,390	none	650	1,080
Nov. 13	10,830	4,600	340	126	176	1,550	87	920	800
Stalks (cut)									
Oct. 2	9,600	5,240	59	119	298	3,270	none	380	550
Oct. 9	7,730	4,750	76	131	340	2,460	none	540	510
Oct. 16	10,980	7,900	320	189	340	3,400	none	1,380	1,300
Nov. 13	8,230	3,520	264	2	83	1,300	3	630	700
Entire Plant*									
Sept. 19	12,710	4,330	410	115	126	2,010	none	780	610
Sept. 25	13,870	5,250	104	169	142	2,170	none	1,260	760
Oct. 2	13,370	5,230	173	197	215	1,860	none	1,390	1,120
Oct. 9	14,110	5,480	196	198	223	1,880	none	720	1,140
Oct. 11	13,250	5,760	200	242	184	2,180	none	930	1,230
Oct. 20	14,250	5,360	297	179	77	1,330	none	930	1,170

*Data for leaves and stalks of uncut plants.

TABLE VI. Summary of Analytical Data on Corn Stalks Collected in 1944. Expressed in Terms of Milligrams Nitrogen per Kilogram Air-dry Sample.

Sample and Sampling Date	Total N	Soluble N	NH_4 N	Glutamine Amide N	Asparagine Amide N	NO_3 N	Nitrite N	Basic N	*a*-Amino N (corrected for glutamine amide and ammonium N)
Dent									
Leaves (uncut)									
Sept. 19	16,460	2,700	66	84	107	126	none	890	760
Sept. 25	17,450	3,370	72	82	131	123	none	1,230	580
Oct. 2	15,080	3,530	176	49	177	22	none	1,330	600
Oct. 9	13,750	3,060	222	45	86	115	none	860	610
Oct. 11	14,820	3,260	155	76	94	105	none	950	630
Oct. 20	13,900	3,700	199	54	62	217	none	620	850
Leaves (cut)									
Oct. 2	15,300	3,020	256	106	109	27	none	1,160	520
Oct. 9	15,180	3,880	246	100	106	54	none	1,140	950
Oct. 16	14,230	4,110	287	123	12	102	none	1,260	1,060
Stalks (uncut)									
Sept. 19	6,040	3,300	25	127	120	1,400	none	630	570
Sept. 25	7,030	3,240	26	104	82	1,600	none	630	470
Oct. 2	6,550	3,740	41	177	136	1,660	none	770	850
Oct. 9	8,500	4,220	144	112	134	1,880	none	550	720
Oct. 11	7,250	4,880	75	272	178	1,940	none	820	760
Oct. 20	7,380	3,420	93	33	29	1,590	none	650	—
Nov. 13	6,880	2,580	165	84	65	580	trace	580	500
Stalks (cut)									
Oct. 2	6,880	3,550	42	88	122	1,970	none	360	510
Oct. 9	6,650	3,460	88	201	131	1,250	none	630	630
Oct. 16	6,500	3,830	91	105	78	1,890	none	570	—
Nov. 13	5,750	2,750	88	47	64	600	trace	400	390
Entire Plant*									
Sept. 19	10,840	2,900	44	107	114	810	none	650	660
Sept. 25	11,230	3,020	45	95	102	1,000	none	870	520
Oct. 2	10,720	3,300	107	114	156	860	none	1,040	730
Oct. 9	10,680	3,170	176	84	114	1,150	none	680	680
Oct. 11	10,700	3,830	112	113	139	1,090	none	880	700
Oct. 20	10,940	3,300	150	107	47	840	none	640	—

*Data for leaves and stalks of uncut plants.

TABLE VII. Summary of Analytical Data on Corn Stalks Collected in 1945. Expressed as Milligrams of Nitrogen per Kilogram of Air-dry Sample.

Sample and Sampling Date	Total N	Soluble N	NH_4 N	Glutamine Amide N	Asparagine Amide N	NO_3 N	*a*-Amino N (corrected)	Basic N	"True" Protein N
Flint									
July 2									
Entire Plant	40,000	16,400	119	253	470	5,910	....	.....	23,600
July 13									
Entire Plant	32,500	10,180	68	175	297	4,470	1,700	2,500	22,320
July 23									
Entire Plant	28,740	10,960	94	139	214	5,110	1,350	....	17,780
Leaves	30,430	8,740	105	107	171	2,530	1,720	2,210	21,690
Stalks	27,200	16,090	75	300	410	9,310	670	.....	11,110
Sheaths	21,900	15,830	60	122	173	12,500	370	.....	6,070
Tassels	42,900	11,100	126	390	840	260	1,550	.....	31,800
Aug. 10									
Entire Plant	18,290	6,270	62	137	221	2,290	740	1,460	12,020
Leaves	29,430	4,320	73	62	88	970	1,050	1,430	25,110
Stalks	12,100	7,570	59	155	154	4,690	350	1,010	4,530
Sheaths	11,100	5,590	31	77	36	3,720	430	820	5,510
Shanks	17,580	6,520	58	220	520	240	780	2,180	11,060
Tassels	21,600	7,240	90	144	251	1,500	1,370	1,940	14,360
Aug. 29									
Entire Plant	12,680	3,390	46	97	87	670	680	900	9,290
Leaves	27,050	4,280	76	47	116	1,020	910	1,590	22,770
Stalks	7,280	3,600	47	98	102	1,820	450	440	3,680
Sheaths	8,200	2,400	22	48	36	830	450	730	5,800
Shanks	9,330	2,720	28	97	116	380	650	880	6,610
Ears	13,780	3,470	50	130	62	30	810	930	10,310
Tassels	12,300	4,190	52	82	84	30	750	1,250	8,110

TABLE VIII. Summary of Analytical Data on Corn Plants Collected in 1945. Expressed as Milligrams of Nitrogen per Kilogram of Air-dry Sample.

Sample and Sampling Date	Total N	Soluble N	NH_4 N	Glutamine Amide N	Asparagine Amide N	NO_3 N	*a*-Amino N (corrected)	Basic N	"True" Protein N
Dent									
July 2									
Entire Plant	41,600	16,550	106	238	470	6,980	....	.....	25,050
July 13									
Entire Plant	33,300	11,100	75	153	275	4,750	1,190	2,600	22,200
July 23									
Entire Plant	29,490	9,470	95	125	133	5,170	1,730	.....	20,020
Leaves	31,130	6,970	107	111	114	2,060	2,230	2,200	24,160
Stalks	27,530	14,670	62	207	294	9,320	450	.....	12,860
Sheaths	24,130	16,190	77	127	103	15,170	540	.....	7,940
Aug. 10									
Entire Plant	21,030	7,520	69	134	155	2,830	1,060	1,510	13,510
Leaves	28,990	4,480	60	75	78	740	1,260	1,570	24,510
Stalks	14,340	9,730	68	172	140	4,990	460	1,140	4,610
Sheaths	13,080	7,160	44	114	51	4,460	1,220	950	5,920
Shanks	27,680	10,090	131	227	850	260	1,090	4,030	17,590
Tassels	28,000	11,940	146	246	540	360	2,750	3,290	16,060
Aug. 29									
Entire Plant	13,200	4,320	60	149	122	980	500	960	8,880
Leaves	26,150	3,310	54	44	77	130	720	1,310	22,840
Stalks	8,080	4,830	67	157	115	2,050	360	500	3,250
Sheaths	7,950	1,980	31	65	17	1,370	470	620	5,970
Shanks	10,300	3,830	36	122	142	100	310	790	6,470
Ears	18,750	7,280	106	410	271	30	900	2,390	11,470
Tassels	12,200	2,760	67	99	90	80	175	1,010	9,440

TABLE IX. Moisture and Nitrate Nitrogen Content of Soil Samples from Cornfield—1945.

Depth	Per Cent Moisture						p.p.m. NO_3N					
Date	6/4	7/2	7/13	7/23	8/10	9/4	6/4	7/2	7/13	7/23	8/10	9/4
0-6 inches	29.0	21.6	20.8	21.1	20.9	14.0	8.5	16.8	18.4	14.3	5.4	1.3
6-12 inches	25.6	32.2	18.7	19.5	18.7	13.3	20.3	19.6	13.3	16.2	8.9	2.3
12-18 inches	23.5	19.2	17.9	18.4	19.2	11.8	13.5	.....	12.3	11.9	6.8	2.2
18-24 inches	21.8	17.9	16.9	17.8	18.6	10.9	.....	11.0	8.0	6.9	5.4	2.0

TABLE X. Nitrate Nitrogen Distribution in the Corn Plant in Relation to Position of the Tissue—Data for Dent Corn (1945). Expressed as Milligrams of Nitrogen per Kilogram of Air-dry Sample.

Tissue Analyzed	Nodal Position from Base to Top of Plant													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
July 26														
Leaf-blade	3700	2870	2790	2520	1790*									
Leaf-blade†	560													
Leaf mid-rib‡	8550													
Internode	13810	11000	6000	3000										
Sheath	19190	15800	12500	9800										
Sept. 4														
Leaf-blade	480	330	240	140	70	70	60	71	50	90	60	70	90	30
Leaf mid-rib	810	620	520	470	310	300	40	40						
Internode	2900	2660	1990	1380	1190	960	820	820	790	810	630	650	940	
Sheath	1250	590	590	430	320	230	210	120	130	100	230	190	50	80
Shank		840	460	480	270	190	150							
Ears (fertile)					80	110	100							
Tassels														200

*Remainder of plant tissue above node 4.
†Leaf-blades 1 through 4, but minus mid-rib tissue (Av. value).
‡Leaf mid-ribs 1 through 4 (Av. value).