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Investigation of Nitrogen and Utilization and Efficiency in 17 Corn Inbred Lines

Michael A. Koopman

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INVESTIGATION OF NITROGEN UTILIZATION AND EFFICIENCY IN 17 CORN INBRED LINES

BY

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MICHAEL A. KOOPMAN

A thesis submitted in partial fulfillment of the requirements for the degree Master of Science, Major in Agronomy, South Dakota State University 1977

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INVESTIGATION OF NITROGEN UTILIZATION AND EFFICIENCY

IN 17 CORN INBRED LINES

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser

Date

Date

Head Plant Science Department

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INTRODUCTION

Nitrogen (N) is considered to be one of the major nutrient elements required in obtaining maximum crop production. It has been stated that in order to keep at the same production level that we have now, two to three times as much cropland would be required if we used 1930 technology and fertilizer (16). Nitrogen is an important resource. But with price increases, shortage possibilities and ecological implications of nitrate toxicity, a new outlook on nitrogen utilization is being taken. Increases in the price of N are well known to everyone who is connected with agriculture. As for shortage possibilities, the United States, in 1950, used approximately 1 million tons. In 1974, 9 million tons were used with an expected yearly increase of 5% compounded annually (16). The world's future needs for this energy requiring product can be expected to increase at a faster rate yet. The threat to drinking water from nitrate toxicity is also being visualized where an excess of nitrates is being leached deeper into the ground (4). In spite of these problems, nitrogen utilization is and has been an important aspect in crop production improvement programs, as can be seen by the vast amount of literature published. However, even more research is needed to better understand nitrogen efficiency and the role that it can play in crop production. The purpose of this research is to 1) investigate various areas of plant metabolism for nitrogen efficiency and 2) measure the genetic

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variability present in these areas of nitrogen efficiency in inbred lines of corn.

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LITERATURE REVIEW

A large amount of research has been done on the metabolism and the physiological effects of N in plants. Discussions and reviews have been published by Black (1), Murata (17) and Viets (25). From this research it can be stated that N is an element that plays a complex role throughout the plant's metabolism and life cycle. Identifying and selecting for efficient use of N within the plant is just as complex.

In tomatoes, O'Sullivan, Gableman and Gerloff (18) utilized a nitrogen efficiency ratio which is equal to the amount of dry matter produced divided by the amount of N absorbed. They evaluated 146 strains and found a significant amount of genotypic variation for this efficiency ratio. These values, plus visual nitrogen deficiency symptoms, were used to categorize strains as efficient or inefficient in nitrogen utilization. It was found that the important factor of the nitrogen efficient strains was their ability to produce dry matter at low levels of nitrogen.

In evaluating corn hybrids, Tyner and Webb (23) suggested a formula that would be an indication of efficient nitrogen utilization. 1beir nitrogen utilization factor is equal to the amount of N applied divided by the increase in yield due to the nitrogen application.

Bruetsch and Estes (2) classified twelve corn hybrids according to nutrient efficiency. They divided the total amount of dry

matter produced after 115 days of growth in the field by the amount of the nutrient absorbed. A significant difference was found between several of the hybrids for efficient use of nitrogen.

Deckard, Lambert and Hageman (5) utilized a nitrogen efficiency ratio in evaluating six corn hybrids. Their ratio was equal to the seasonal input of reduced N (as estimated from nitrate reductase enzyme assays) divided by the total amount of reduced N found in the above ground plant. A large amount of genotypic variation was found between these hybrids, but it was stated that, at the present, nitrate reductase activity is too laborious a test to be used in screening or selecting inbreds for a practical breeding program.

Clark (4) classified thirty-six corn inbreds according to efficient use of several minor elements. He measured three seedling traits (dry matter production, degree of mineral deficiency symptoms and the mineral concentrations in the leaves) when the plants were grown in nutrient-deficient soils. Inbreds with the highest dry . matter production, least amount of deficiency symptoms and the highest mineral concentrations were rated as being highly efficient.

Smith (20) also investigated the nutrient efficiency of corn inbreds at the seedling stage. He grew four inbred lines over normal and low levels of nutrients and defined efficiency as ^a small fluctuation in a plant response when measured over varying nutrient levels.

METHODS AND MATERIALS

Seventeen elite inbred lines were selected because of their wide acceptance in public and private breeding programs in the upper cornbelt region. Experiments evaluating these inbreds were conducted at the University of Minnesota agronomy farm at Rosemount, Minnesota in 1975 and on private land near Brookings, South Dakota in 1976.

In 1975, at Minnesota, non-irrigated, silt loam soil was utilized that had been planted with corn for three consecutive years. No nitrogen fertilizer was added during those three years. The inbred lines were grown over three nitrogen levels: 0, 56 and 112 kilograms of actual N per hectare, in the form of ammonium nitrate. Two plant densities were utilized: 49,420 and 74,130 plants per hectare. Each entry was in a single row plot with a 76 centimeter row spacing. Row length was 4.57 meters with 50.8 centimeter spacing between hills for the low plant population. Row length for the high plant population was again 4.57 meters, but with a 34.8 centimeter spacing between hills. The plots were hand planted on May 22, with three kernels planted per hill and later thinned to two plants per hill.

In 1976, at South Dakota, an irrigated silt loam soil was utilized that had been seeded to wheat, with no nitrogen fertilizer applied the previous year. The same inbred lines as were used in 1975 were planted May 17 and grown over three different nitrogen

levels: 0, 67 and 134 kilograms of actual N per hectare, in the form of ammonium nitrate. The plots were again hand planted and thinned to plant populations of 49,420 and 74,130 plants per hectare. Each entry was in a two row plot at a row width of 102 centimeters. Total amount of water applied was approximately 19 centimeters with two -6 1/3 centimeter applications given before and the final 6 $1/3$ centimeters applied just-after the pollination period. Soil tests indicated that appr^oximately 90 kilograms of available nitrates per hectare were present in the top 60 centimeters of soil at Brookings. No soil samples were taken at Rosemount.

Measurements of the 17 inbreds (when grown over the different treatments) were taken on their total dry matter production, per cent leaf nitrogen, grain yield, leaf loss and on a diphenylamine tissue test. All measurements were taken from plants which were bordered on all sides by plants to assure uniform stress within the two plant populations.

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Total dry matter production was estimated from a ten plant sample per plot, taken at harvest time. The stalks were chopped, dried in a 70⁰C oven and weighed. The cob weight and the grain weight, which was corrected to 15.5% moisture, was then added to the stover weight to give a value of the total dry matter produced. In 1975, all plots were harvested at the same time due to an early killing frost. In 1976, plots were harvested over five different time intervals according to visible plant and ear maturity.

The leaf opposite and below the top ear was selected for nitrogen determination when the plants were at the mid-silk stage. The leaves from five plants per plot were dried, ground and analyzed for nitrogen by the standard Kjeldahl procedure.

Grain yield values were obtained by harvesting all the ears from the same ten plants per plot that were utilized in measuring dry matter production. The ears were weighed, dried, shelled, reweighed and the grain corrected to 15.5% moisture.

For obtaining accurate leaf loss values, the fifth and tenth foliage leaves were painted red as they emerged from the whorl. This procedure was utilized because almost all of the inbreds tend to lose the bottom five leaves through "natural senescence", regard-_ less of nitrogen stress. Therefore the number of total leaves produced by the plant and the number of green, healthy leaves (at mid-silk) were obtained by counting up from the tenth leaf. Through subtraction of these two values, the number of leaves lost and or dead was found and divided by the total number leaves, thus giving percent leaf loss. Two to three plants were utilized per plot with leaves being considered healthy if more than 50% of the leaf was green. In 1976, a second value of leaf loss was obtained by again counting leaves on the same plants, only at six weeks post-anthesis.

Plants were analyzed for available nitrates by using a diphenylamine tissue test as outlined by the Purdue University Soil and Plant Tissue Testing bulletin (12). One gram of diphenylamine

was dissolved in 100 milliliters of concentrated sulfuric acid to make an indicator-dye reagent which, when applied to the nodes of a corn stalk, would turn blue, if nitrates were present. At midsilk, two plants per plot were chopped off at ground level, $split$ longitudinally and the nodes up to and including the node above the top ear were tested. A quantitative value, suggesting the amount of available nitrates in the plant, was acquired by counting the number of nodes (starting at the top node tested and counting down the stalk) that had a negative reaction for nitrates. This value was then divided by the total number of nodes tested. This new value (representing the percent of the nodes having a negative reaction) was subtracted from one, thus giving a percent of the total number of nodes tested which had a positive reaction for available nitrates. An accurate total number of nodes tested was obtained by utilizing plants which had the tenth leaf painted red and then counting up to the node above the top ear.

The field experiments were arranged in a split-split plot design. Nitrogen levels were randomized within populations and inbreds were randomized within nitrogen levels. Two replications were used in 1975 and in 1976 three replications were utilized.

A greenhouse experiment was conducted, in October of 1975, to evaluate root density and seedling dry matter production over controlled nitrogen levels. The 17 inbreds were grown in plastic pots with a sand medium. Nitrogen levels were controlled by utilizing

three variations of Hoaglund's solution: 1) normal Hoaglund's solution, 2) a solution diluted to 50% concentration of Hoaglund's solution and 3) a normal Hoaglund's solution with only a 10% normal level of nitrogen. Four kernels were planted per pot with each being thinned later to two plants per pot. The plants were grown for a total of 28 days, removed from the pots and the sand then removed from the roots. The roots were visually rated for root density on a scale of $1 - 5$, "l" being "good" and "5" being "poor". The stems were then cut off just above the crown region, dried in a 70° C oven and weighed. This experiment was conducted in a splitplot design with two replications.

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RESULTS AND DISCUSSION

Dry Ma tter Pr oduction

Dry matter production (DMP) values for the seventeen inbreds, over the different parameters, are shown in Table 1. Comparing the mean values for the three nitrogen levels, in the 1976 data, virtually no difference is seen, whereas the 1975 means for the three nitrogen levels indicates a general trend of increased DMP with increased fertilizer application, from 0 to 56 kgs N/ha. The lack of response to increased nitrogen levels noted in 1976, is probably because of the large amount of available N which was originally present in the soil. This is also suggested by the other characteristics measured in 1976 and is affirmed by the soil test results. The effect of population levels on DMP, as shown in Table 2, is significant, as is the interaction between N and population levels.

Years had a large effect (significant at the .10 level) on DMP, as it did in much of the following data. This can be more fully understood when it is realized that this source of variation not only contains variation due to years, but also pooled within it is variation due to locations and row width.

Variation among inbreds was highly significant, indicating a large amount of genetic variability for DMP. The inbred by year interaction was also highly significant, suggesting that a comparison among inbreds may not be valid with just one year of data.

| | 1975 Minnesota) | | | | | | | 1976 (South Dakota) | | | | | |
|--|--------------------|----------------------|-------------|-----|------------------------|---------------|----------------|------------------------|---------|------------|-----------------------------|------|--|
| Inbreds | | N levels (kgs/ha) | | | Population | levels Yearly | | N levels (kgs/ha) | | | Population levels Yearly | | |
| | 0 ^a | 56 ^a | 112^a | | Lowb _{High} b | mean | 0 ^a | 67a | 134^a | | LowbHighb | mean | |
| $A547 - 2$ | 146 | 164 | 161 | 176 | 139 | 157 | 61 | 70 | 69 | 73 | 61 | 67 | |
| A632 ·· | 198 | 203 | 184 | 222 | 167 | 195 | 135 | 140 | 139 | 154 | 122 | 138 | |
| A619 | 165 | 193 | 194 | 202 | 166 | 184 | 86 | 90 | .88 | 94 | 83 | 88 | |
| A657 | 168 | 204 | 196 | 212 | 166 | 189 | 101 | 108 | 108 | 119 | 93 | 106 | |
| A654 | 101 | 120 | 119 | 124 | 102 | 113 | 77 | 79 | 82 | 87 | 72 | 80 | |
| B8(ND) | 156 | 182 | 178 | 183 | 162 | 172 | 95 | 96 | 101 | 103 | 91 | 97 | |
| B73 | 178 | 196 | 193 | 221 | 160 | 191 | 146 | 136 | 134 | 148 | 130 | 139 | |
| CO109 | 143 | 171 | 173 | 174 | 151 | 163 | 98 | 98 | 96 | 101 | 94 | 98 | |
| SDp2A | 134 | 152 | 161 | 167 | 131 | 149 | 108 | 108 | 103 | 114 | 98 | 106 | |
| SD ₅ | 111 | 128 | 117 | 125 | 113 | 119 | 90 | 84 | 83 | 92 | 79 | 85 | |
| SD10 | 145 | 146 | 154 | 163 | 134 | 149 | 85 | 86 | 82 | 88 | 80 | 84 | |
| SD15 | 177 | 221 | 205 | 235 | 167 | 201 | 127 | 105 | 119 | 137 | 96 | 117 | |
| SD24 | 140 | 181 | 161 | 169 | 152 | 161 | 97 | 94 | 98 | 106 | 86 | 96 | |
| SDp232 | 106 | 131 | 121 | 126 | 112 | 119 | 75 | 79 | 77 | 83 | 72 | 77 | |
| W64A | 161 | 213 | 192 | 222 | 156 | 189 | 122 | 122 | 125 | 138 | 108 | 123 | |
| W117 | 108 | | 139 124 136 | | 112 124 | | 101 | 95 | 107 | 104 | 98 | 101 | |
| WI53R 165 176 193 195 161 | | | | | | 178 98 96 | | | 97 | 103 | 91 | 97 | |
| mean 147 172 167 179 144 162 100 99 101 108 91 100 | | | | | | | | | | | | | |

Table 1. Dry matter production, in grams per plant, of 17 inbred lines of corn.

a averaged over populations and replications

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 b averaged over nitrogen levels and replications

Table 2. F tests from the analysis of variance for dry matter production.

a variables have fixed effects
*,** significant at the 5% and 1% levels of probability, respectively
coefficient of variability = 16.6

In comparing the DMP values of each inbred, A632, B73 and SD15 tend to produce the largest amount of dry matter, whereas A654, SDp232 and SD5 produce the least.

Percent Leaf Nitrogen

Data on the percent leaf N for the dif ferent treatments is shown in Table 3. Comparing mean values of the three nitrogen levels for each year, the percent N in the leaf tends to increase as the amount of applied N increases. This agrees with the work done in other research $(13, 21, 22)$. The F test shown in Table 4 suggest, though, that nitrogen levels are an insignificant source of variation. Population levels caused a significant amount of variation with a consistent trend of lower leaf N percentages as population levels increased. The effect due to years was small and insignificant, but the interaction between inbreds and years was highly significant. This again suggests that any comparison between inbreds for percent leaf N should be based upon more than one year of data.

Inbreds was a highly significant source of variation for leaf N content. A657, SDp2A, SD5 and SD24 had the highest percent of N in the leaf, whereas A654 and A619 had the lowest values.

Grain Yield

Grain yields of the seventeen inbreds over the different treatments are shown in Table 5. Comparing the mean yield values of the three nitrogen levels for the two years again shows a very

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Table 3. Per cent leaf nitrogen of 17 inbred lines of corn.

a averaged over populations and replications b averaged over nitrogen levels and replications

Table 4. F tests from the analysis of variance for per cent leaf
nitrogen. nitrogen.

a variables have fixed effects $*,**$ significant at the 5% and 1% levels of probability, respectively coefficient of variability = 6.6

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small and insignificant response due to nitrogen levels. The small responses noted for these inbreds differ from the large responses normally reported for hybrids. When comparing nine hybrids over high and low levels of N, Lang (15) observed a 39% average increase in yield due to increasing nitrogen levels. Even utilizing only the 1975 data, these seventeen inbreds showed only a 10% increase in yield when comparing the 0 and 56 kgs N/ha rates. Population levels were a significant source of variation, and consistently caused a slight increase in quintals of grain produced per hectare.

Between years was again a significant source of variation as shown in Table 6. This variation can also be visualized by comparing the mean yield values (Table 5) of all the inbreds for the two years -42 and 26 quintals per hectare for 1975 and 1976, respectively. In 1976, the plots were irrigated, but the amount of irrigation water appli ed, 19 centimeters, did not equal the amount of rainfall that was lack ing during the growing season, 22 centimeters (24). Also, during the pollination period, there was a long period of high, above-normal temperatures, which is felt to have been a major reason for the large difference in yield noted between the two years.

As shown in Table 6, inbreds were a highly significant source of variation. W64A, W153R, COlO9 and SD15 had the highest overall yields, whereas B73, A654 and A657 had the lowest yields over the two year period. The inbred by year interaction was also highly significant and brought to light an unusual response. Wll7 and

| | 1975 Minnesota) | | | | | | 1976 South Dakota | | | | | |
|-----------------|--------------------|----------------------|--------|------------|----|---------------------------------|----------------------|----------------------|----|-----------------------------|----|---------------|
| | | N levels (kgs/ha) | | Population | | levels Yearly | | N levels (kgs/ha) | | Population | | levels Yearly |
| | 0^a | 56 ^a | | | | 112ª Lowb Highb ^{mean} | 0^a | 67 ^a | | 134 ^a Lowb Highb | | mean |
| $A547 - 2$ | 47 | 49 | 49 | 44 | 53 | 48 | 16 | 17 | 16 | 15 | 18 | 16 |
| A632 | 46 | 45 | 42 | 41 | 47 | 44 | 26 | 32 | 28 | 27 | 30 | 29 |
| A619 | 47 | 49 | 49 | 44 | 53 | 48 | 20 | 24 | 22 | 21 | 23 | 22 |
| A657 | 34 | 42 | 43 | 37 | 43 | 39 | 17 | 13 | 12 | 15 | 13 | 14 |
| A654 | 30 | 31 | 27 | 27 | 31 | 29 | 24 | 22 | 24 | 21 | 25 | 23 |
| $BS(\text{ND})$ | 47 | 50 | 48 | 42 | 55 | 48 | 27 | 25 | 28 | 24 | 30 | 27 |
| B73 | 27 | 36 | 33 | 34 | 30 | 32 | 21 | 11 | 12 | 15 | 14 | 14 |
| CO109 | 49 | 54 | 51 | 43 | 60 | 51 | 32 | 33 | 31 | 26 | 38 | 32 |
| SDp2A | 34 | 36 | 41 | 34 | 40 | 37 | 30 | 29 | 29 | 26 | 32 | 29 |
| SD5 | 31 | 28 | 28 | 25 | 33 | 29 | 28 | 24 | 25 | $23 -$ | 27 | 25 |
| SD10 | 50 | 45 | 46 | 45 | 49 | 47 | 24 | 26 | 24 | 21 | 29 | 25 |
| SD15 | 46 | 58 | 51 | 51 | 53 | 51 | 34 | 25 | 30 | 31 | 28 | 30 |
| SD24 | 43 | 53 | 46 | 41 | 54 | 48 | 30 | 29 | 30 | 27 | 31 | 29 |
| SDp232 | 35 | 37 | 33 | 30 | 40 | 35 | 23 | 24 | 21 | 20 | 25 | 23 |
| W64A | 46 | 60 | 53 | 54 | 51 | 53 | 37 | 35 | 36 | 34 | 38 | 36 |
| W117 | 24 | 23 | 22 | 24 | 23 | 23 | 36 | 32 | 38 | 30 | 40 | 35 |
| W153R | 51 | 48 | $59 -$ | 49 | 57 | 53 | 33 | 33 | 31 | 28 | 37 | 32 |
| mean | 40 | 44 | 43 | 39 | 45 | 42 | 27 | 25 | 26 | 24 | 28 | 26 |

Table 5. · Grain yields, in quintals per hectare, of 17 inbred lines of corn.

a averaged over populations and replications

 b averaged over nitrogen levels and replications

Table 6. F tests from the analysis of variance for grain yield.

a variables have fixed effects
*,** significant at the 5% and 1% levels of probability, respectively coefficient of variability = 24.6

SD5 had fairly low yields in 1975, but in 1976, these inbreds had similar to or higher yields than in the previous year. Wll7, especially, had much higher yields in 1976, depicting its ability to to�erate high temperatures during the critical pollinating period (J.L. Geadelmann and D.T. Burmood, personal communication) .

Leaf Loss

Values for the amount of leaf loss at mid-silk are shown in Table 7. No measurements were taken at the middle nitrogen level for either year. The average effect of nitrogen levels on leaf loss could not be easily summarized due to the slight (yet statistically insignificant) amount of interaction between nitrogen levels and years. In 1975, leaf loss decreased as nitrogen levels increased, as reported by other research $(1,14)$. However, in 1976, there was an increase in leaf loss as nitrogen levels increased. As shown in Table 8, population levels were also an insignificant source of variation, but the population by year interaction was significant at the . 10 level. Years was also a significant source of variation, with more leaf loss occuring in 1975 than in 1976. One explanation for the lower amount of leaf loss in 1976 may be because of the effect of the two applications of irrigation water, plus the high levels of available N in the soil, i.e., N was more available for absorption by the plant. This complementary effect may explain the unus 1al reaction found in 1976 of increased leaf loss as nitrogen levels increased, if inbred corn plants can absorb enough N to be

| | 1975 (Minnesota) | | | | | 1976 South Dakota | | | | |
|-------------------|---------------------|----|---|--------------------------------------|------|----------------------|---------------------------------|---|----|---------------|
| | (kgs/ha) | | | N levels Population levels Yearly | | | N levels Population (kgs/ha) | | | levels Yearly |
| LEARN ROOM | 0^a | | 112ª Low ^b High ^b | | mean | 0^a | | 134 ^a Low ^b High ^b | | mean |
| $A547 - 2$ | 36 | 36 | 38 | 35 | 36 | 37 | 33 | 35 | 36 | 35 |
| A632 | 36 | 35 | 34 | 37 | 35 | 35 | 34 | 34 | 34 | 34 |
| A619 | 46 | 45 | 43 | 49 | 46 | 40 | 35 | 37 | 37 | 37 |
| A657 | 39 | 32 | .33 | 37 | 35 | 36 | 38 | 38 | 37 | 37 |
| A654 | 41 | 40 | 38 | 42 | 40 | 36 | 36 | 36 | 34 | 35 |
| B8(ND) | 35 | 36 | 36 | 36 | 36 | 35 | 34 | 38 | 33 | 35 |
| B73 | 41 | 38 | 39 | 40 | 40 | 37 | 38 | 38 | 37 | 38 |
| CO109 | 46 | 52 | 45 | 53 | 49 | 33 | 36 | 36 | 33 | 35 |
| SDp2A | 45 | 40 | 42 | 43 | 42 | 40 | 39 | 40 | 38 | 39 |
| SD ₅ | 46 | 49 | 46 | 49 | 47 | 33 | 35 | 34 | 34 | 34 |
| SD10 | 42 | 41 | 40 | 43 | 41 | 32 | 36 | 36 | 35 | 36 |
| SD15 | 40 | 43 | 39 | 43 | 41 | 35 | 34 | 36 | 33 | 34 |
| SD24 | 36 | 36 | 33 | 38 | 36 | 32 | 29 | 31 | 29 | 30 |
| SDp232 | 43 | 40 | 43 | 40 | 42 | 38 | 36 | 40 | 35 | 38 |
| W64A | 42 | 41 | 41 | 42 | 41 | 33 | 32 | 33 | 32 | 33 |
| W117 | 38 | 35 | 38 | 35 | 36 | 36 | 37 | 36 | 36 | 36 |
| W153R | 38 | 33 | 34 | 37 | 35 | 33 | 34 | 35 | 33 | 34 |
| | 40 | 39 | 39 | 41 | 40 | 33 | 34 | 35 | 33 | 34 |
| mean | | | | | | | | | | |

Table 7. Leaf loss at mid-silk, in per cent, of 17 inbred lines of corn.

a averaged over populations and replications

b averaged over nitrogen levels and replications

| Source of variation | Degrees of freedom and the state of the | Ace Friday |
|--------------------------------------|--|------------|
| Inbreds $(1)^a$ | 16 | $22.13**$ |
| Nitrogen (N) levels ^a | $\overline{2}$ | 3.85 |
| Population (P) levels ^a | I. | .58 |
| Years (Y) a | $\mathbf{1}$ | $375.39*$ |
| IXN | 32 | $2.91*$ |
| IXP | 16 | $3.89**$ |
| IXY SERIES | 16 | $21.82**$ |
| N X P | \overline{c} | 7.13 |
| N X Y | $\overline{2}$ | 5.13 |
| PXY | 1 | 92.53 |
| IXNXP | 32 | $3.78**$ |
| IXNXY | 32 | $3.02*$ |
| IXPXY 11 L S | 16 Taransa | $3.39*$ |
| NXPXY | \overline{c} STARTED | 44.40 |
| IXNXPXY | 32 ANTIFICATION | $3.62*$ |

Table 8. F tests from the analysis of variance for leaf loss at mid-silk.

a variables have fixed effects

*,** significant at the 5% and 1% levels of probability, respectively

 $coefficient of variability = 5.3$

toxic to lower leaves. Hageman (8) has discussed the improbability of nitrogen toxicity to leaves, but no information has been found that has investigated this type of leaf loss under these types of conditi ons.

Inbreds and all of the interactions of inbreds with the other treatments were ei ther significant or hi ghly significant. Very little generalization of inbred response can be made, for each inbred responds differently over different nitrogen levels, population levels and years. CO109 and A619, though, did tend to have more leaf loss than did SD24 and A632.

A second measurement of leaf loss was taken in 1976 in an attempt to better classify inbreds according to leaf loss, by including the leaves lost during the grain-filling period. These additional measurements of leaf loss, which were taken six weeks post-anthesis, are shown in Table 9. Again, nitrogen levels seemed to have little effect on leaf loss. Increasing population levels did tend to cause an increase in this leaf loss measurement at maturity and was a source of significant (at the .10 level) variation, as shown in Table 10. Inbreds are again a highly significant source of variation. CO109 and A632 have the lowest amount of leaf loss at maturity, whereas A547-2, A619, SD10 and SDp232 lost the highest amount of leaves.

The amount of leaf loss, just during the grain-filling period, is also shown in Table 9. SDp2A and B73, which tended to have the

| | N levels (kgs/ha) | | Population levels | | | maturity |
|-------------|----------------------|---------|----------------------|-------------------|------|-------------------|
| Inbreds | $\underline{\rho}^a$ | 134^a | Lowb | High ^b | mean | minus mid-silk |
| $A547 - 2$ | 51 | 56 | 51 | 53 | 52 | 17 |
| A632 | 40 | 42 | 40 | 43 | 41 | $\overline{7}$ |
| A619 | 55 | 50 | 52 | 53 | 52 | 15 |
| A657 | 44 | 43 | 43 | 44 | 44 | $\overline{7}$ |
| A654 | 46 | 43 | 43 | 44 | 44 | 9 |
| B8(ND) m | 49 | 50 | 47 | 51 | 49 | 14 |
| B73 | 43 | 45 | 44 | 44 | 44 | 6 |
| CO109 | 38 | 43 | 40 | 40 | 40 | 5 |
| SDp2A | 49 | 47 | 47 | 49 | 48 | 9 |
| SD5 | 44 | 43 | 43 | 44 | 44 | 10 |
| SD10 | 52 | 51 | 51 | 53 | 52 | 16 |
| SD15 | 50 | 51 | 50 | 49 | 50 | 16 |
| SD24 | 49 | 49 | 49 | 50 | 50 | 20 |
| SDp232 | 54 | 53 | 52 | 53 | 52 | 14 |
| W64A | 50 | 52 | 50 | 53 | 51 | 18 |
| W117 | 45 | 44 | 43 | 46 | 45 | 9 |
| W153R | 47 | 51 | 45 | 49 | 47 | 13 |
| mean | 47 | 48 | 46 | 48 | 47 | 13 |

Table 9. Leaf loss at maturity, in 1976, of 17 inbred lines of corn in per cent.

a averaged over populations and replications
b averaged over nitrogen levels and replication

averaged over nitrogen levels and replications

| Source of variation | Degrees of freedom | F | |
|------------------------------------|-----------------------|---------|--|
| Inbreds $(I)^d$ | 16 | $9.94*$ | |
| Nitrogen (N) levels ^a | 2 | .30 | |
| Population (P) levels ^a | | 55.60 | |
| IXN | 32 | .92 | |
| IXP | 16 | .85 | |
| N X P | 2 | .66 | |
| J X N X P | 32 | 1.38 | |

Table 10. F tests from the analysis of variance for leaf loss at maturity .

a variables have fixed effects ** significant at the 1% level of probability Coefficient of variability = 9.3

highest amount of leaf loss up to mid-silk, had a low amount of leaf loss during the grain-filling period. At the opposite extreme, SD24 had the lowest amount of leaf loss, up to mid-silk, but had the highest leaf loss during the grain-filling period. If this leaf loss is due to the remobilization of N out of the lower leaves this could be an indication of further N-use efficiency (3) . It has been found that, in tomatoes, nitrogen-efficient strains had less N in the lower leaves and more in the upper leaves than did inefficient strains (18). The strains in the strain strains in the strain strain strain strains in the strain s

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Grain to Stover Ratio

The grain to stover (G/S) ratios are shown in Table 11. Comparing the mean values of the three nitrogen levels of each year, the G/S ratios tended to decrease as nitrogen levels increased. This trend was especially noted in 1975, suggesting that maximum G/S ratios were occurring on the treatment without nitrogen application and a decrease in the ratio occurs as the nitrogen levels increase. This decreasing of the G/S ratio (from the maximum) as nitrogen levels increase or decrease is discussed by Black (1). As shown in Table 12, nitrogen levels were a significant source of variation, as was the N by year interaction. Population levels had a similar effect on the G/S ratios as did the nitrogen levels. As population levels increased, the efficiency of the plant to produce grain decreased. Years was also a significant source of variation with a large difference between the two yearly means, .81 and .63.

Genotypic variation was highly significant, with CO109, W153R and SD10 having the highest ratios and B73 and A657 having the lowest. The inbred by year interaction was highly significant as a source of variation and much of this variation can be attributed to W117 and SD5. Again it can be seen that some factor in the 1976 environment favored these two inbreds, whereas the other inbreds were suppressed in grain production efficiency. The inbred by population interaction was also significant and an inbred exemplifying this interaction is CO109. This inbred displayed the

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Table 11. Grain to stover ratios of 17 inbred lines of corn.

a averaged over populations and replications
b averaged over nitrogen levels and replications

Table 12. F tests from the analysis of variance for the grain to stover ratios.

 a variables have fixed effects *,** significant at the 5% and 1% levels of probability, respectively coefficient of variability = 17.9

ability to be more efficient at producing grain (per gram of dry matter) as stress due to populations increased.

Diphenylamine Tissue Test

Several difficulties were encountered while conducting the . diphenylamine tissue (DPAT) test in 1975 and 1976. In 1975, each inbred at the O nitrogen level and at the two population levels was sampled at three different dates during and after the mid-silk period. This was to evaluate the effect that sampling dates would have on the test, as no information was found in the literature on this subject. A significant amount of variation was found due to the sampling dates, but a large amount of error was incurred, as signified by a coefficient of variability value of 142.9. Inability to sample all plots at the same time of the day accounts for a large portion of this error. These results were then averaged over sampling dates and re-analyzed - coming up with a coefficient of variability value of 18.4. Data for 1976 are not shown because of a second difficulty encountered. Almost all of the inbreds, when tested over all the treatments, had a 100% positive reaction to the reagent, resulting in extremely low levels of variation. It was considered that the original levels of available N in the soil were too high to allow this test to be of any value in measuring variation .

The 1975 data for the DPAT test is shown in Table 13. Comparing the two nitrogen level means, the amount of nitrates in the

Table 13. 1975 diphenylamine tissue test values^a of 17 inbred lines of corn, in percent. we of advers the state

a the formula for these values is the number of nodes with a positive reaction for nitrates divided by the total number of nodes tested .

b averaged over populations and replications

c averaged over nitrogen levels and replications

Table 14. F tests from the analysis of variance for the diphenylamine tissue test.

a variables have fixed effects $*$, $**$ significant at the 5% and 1% levels of probability, respectively Coefficient of variability = 18.4

stalk had a large increase as nitrogen levels increased from O to 112 kgs N/ha, 74% to 93%, respectively. Other research has also shown this to be true $(9,27)$. Increased population density had an opposite effect; that of decreasing plant nitrate levels, as has been found in other research (8) . The analysis of variance for this test is shown in Table 14, and it also indicates that a significant interaction occurs between N and population levels. Inbreds exhibited a highly significant amount of variation, with A632, A654 and SDp2A showing high levels of nitrates, whereas W153R consistently had low levels.

Greenhouse Experiment

Data on experiments conducted in the greenhouse are shown in Table 15. Low levels of N tended to cause an increase in the visual amount of roots, with a compounded effect when other nutrients were also at a low level. The correlation between a visual root density rating with actual root wei ghts is not known , but Warncke and Barber (26) found that as soil nitrogen concentration decreased, root weight increased. As shown in Table 16, variation among inbreds is highly significant. Inbreds A654, A619 and SD24 exhibited a much higher root density than did BB, A657 or SDp2A .

Seedling DMP is shown also in Table 15, and nitrogen levels were a significant source of variation for this trait. Comparing the normal and 10% normal nitrogen levels, a 36% average drop in seedling DMP was noted. Variation among inbreds was again highly significant, with A654 and W117 having the highest seedling DMP and ·BB and B73 having the l owest .

Evaluation of Inbreds for Nitrogen Efficiency

As shown in the previous data, there is a large amount of genetic variation found for the plant characteristics and the responses measured over the different treatments. Large genotypic variations have been found by other researchers in the response of various plant characteristics to N $(2, 4, 5, 6, 7, 10, 11, 19, 20, 28)$. Clark (4) and Bruetsch and Estes (2) utilized these differential genetic responses as indicators of nitrogen efficiency. Efficiency being exhibited by a plant when a small fluctuation is

 $\frac{a}{1}$ 1 to 5 rating; 1 = good, 5 = poor

b normal Hoaglund solution

c 50% normal Hoaglund solution

d normal Hoaglund solution with only 10% normal nitrogen level

 Δ

Root Density Rating Was for the character books reasonal in this

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coefficient of variability = 25.6

Seedling Dry Matter Production

coefficient of variability = 20.0 . a variables have fixed effects

*,** significant at the 5% and 1% levels of probability, respectively

found when comparing responses at low nitrogen levels with high nitrogen levels.

Fluctuation values for the characteristics measured in this research are shown in Table 17. The values for each inbred were obtained by subtracting the mean values of the inbred at the O nitrogen rate from the mean values at the high nitrogen level; i.e., 112 or 134 kgs N/ha. These fluctuation values are then an indication of the response that an inbred had as nitrogen levels increased. For DMP, per cent leaf N, grain yield, G/S ratios and seedling DMP, the values were then divided by the corresponding mean value at the 0 nitrogen level to give a percentage value of the amount of response. This was done to correct any bias which might be encountered when comparing fluctuations between inbreds. For example, when evaluating DMP fluctuations, A657 has an average fluctuation between the extreme nitrogen levels of 17.8 grams per plant, while A654 has an average fluctuation of 11.5 grams per plant. Comparing these fluctuations, as percentages of their respective DMP values at the O nitrogen level, both inbreds gained almost an equal per cent of dry matter due to the increase in nitrogen levels, 13.2 and 12.9%, respectively.

In evaluating the inbreds, efficiency will be indicated by a small or negative fluctuation value, as has been done in other research (2). In other words, efficiency is when the plant can maintain normal metabolism in a certain area at low nitrogen

•

| | | ℅ | | (mid- | Leaf Loss $(matur -$ | | | (seed- ling) |
|-----------------|---------|---------------------|---------|--------|-------------------------|---------------------------------|----------------|-----------------|
| Inbreds | DMP^b | Leaf N ^b | Yieldb | silk) | rity) | G/S^b | DPAT | DMP^b |
| $A547 - 2$ | 11.1 | 1.6 | 3.2 | -2.0 | 5.0 | -13.1 | 14 | 17.2 |
| A632 | -2.9 | 1.0 | -2.8 | -1.0 | 2.0 | -1.0 | $\overline{7}$ | 82.2 |
| A619 | 12.0 | 10.0 | 6.0 | -3.0 | -5.0 | 9.9 $\overline{}$ | 27 | 63.0 |
| A657 | 13.2 | 7.2 | 7.8 | -2.5 | -1.0 | -6.2 | 40 | 48.1 |
| A654 | 12.9 | 4.4 | -5.6 | -0.5 | -3.0 | -20.5 | -3 | 64.3 |
| BS(ND) | 11.3 | 5.6 | 2.7 | 0.0 | 1.0 | -13.8 | 15 | 113.3 |
| B73 | 2.4 | 6.0 | -8.3 | -1.0 | 2.0 | -3.4 | 25 | 54.8 |
| CO109 | 11.4 | 3.9 | 2.5 | 4.5 | 5.0 | -19.1 | 14 | 55.8 |
| SDp2A | 9.4 | -1.6 | 9.4 | -3.0 | -2.0 | 0.0 | 14 | 66.7 |
| SD ₅ | -0.1 | 0.2 | -10.2 | 2.5 | -1.0 | -14.2 | 12 | 81.8 |
| SD10 | 2.6 | 1.9 | -5.4 | 1.5 | -1.0 | -13.0 | 5 | 42.5 |
| SD15 | 6.6 | 2.4 | 1.3 | 1.0 | 1.0 | -9.2 | 34 | 42.2 |
| SD24 | 9.3 | 5.3 | 4.1 | -1.5 | 0.0 | -5.6 | 17 | 45.7 |
| SDp232 | 9.4 | 5.9 | -6.9 | -2.5 | -1.0 | -24.1 | 17 | 46.7 |
| W64A | 12.2 | 6.5 | 7.2 | -1.0 | 2.0 | -7.3 | 25 | 49.5 |
| W117 | 10.5 | -1.3 | 0.0 | -1.0 | -1.0 | -11.4 | -3 | 116.7 |
| W153R | 10.4 | -0.5 | 7.1 | -2.0 | 4.0 | -6.5 | 63 | 31.0 |
| mean | 8.1 | 3.5 | 3.0 | 0.0 | 0.4 | -11.0 | 19 | 56.8 |

Table 17. Fluctuations, in per cent, of plant characteristics due to increasing nitrogen levels for 17 corn inbred l ines.

a fluctuations = high nitrogen level - 0 nitrogen level
b fluctuations as percentage of the value at the 0 nitrogen 1 fluctuations as percentage of the value at the 0 nitrogen level. levels. It is not felt that large responses to added N are undesirable, rather that these large responses suggest inefficient utilization of whatever N is available at lower levels.

Inbreds efficient in producing dry matter at low nitrogen levels are A632, SD5, B73 and SD10, whereas A657 and A654 show the largest fluctuations and less efficient use of nitrogen as levels decrease.

In evaluating the percent leaf N fluctuations, low or negative values, such as shown by SDp2A, W117, W153R and SD5, suggest that these inbreds can absorb and convert enough N at the low nitrogen level to maintain normal nitrogen metabolism. Of these four inbreds though, W117 shows tendencies to become inefficient at high levels of nitrogen application. The increased amount of DMP, due to the increased nitrogen levels (as seen in the DMP fluctuations), does not contain sufficient levels of N in the leaf and no increase in grain yield results (as seen in the yield fluctuations) . By comparing these three fluctuations, SDp2A and W153R not only appear efficient at low nitrogen levels, but at high levels as well. Because as the nitrogen levels increased, DMP is increased, the percent N in the leaf stayed constant or decreased slightly (meaning small increases in the amount of total N per plant) and yields increased. Considering A619's large fluctuation in percent leaf N and DMP, this inbred appears

to have poor absorption ability at low levels of N and requires high levels of N to maintain a normal percent of leaf N. Coupling this information with A619's high root density rating, as shown in Table 15, suggests that the visual amount of roots is not the main factor in nitrogen absorption.

In evaluating the inbreds yielding efficiency at low levels of N, a low or negative value is again considered desirable. The large decrease in grain yield exhibited by SD5 may indicate efficiency at low nitrogen levels, but from also comparing its DMP and percent leaf N fluctuations, this inbred appears unable to respond to nitrogen. Other inbreds showing yielding efficiency at low levels of N are B73, SDp232, A654, SD10, A632 and W117.

Many inbreds had an increase in leaf loss (at mid-silk and at maturity) as nitrogen levels decreased, as has been reported in \therefore other research (14) , but in terms of efficient utilization of available N, a low amount of fluctuation is desirable. In other words it is desirable to have a low amount of additional leaf loss as nitrogen levels decrease. CO109, though, exemplified a reaction where inexplicable increases in leaf loss resulted as nitrogen · levels increased. This type of response was also more prevalent with many of the inbreds in the leaf loss-at-maturity data. Therefore, the interpretation of inbred response in the area of leaf loss is uncertain with this limited data and more research is needed.

The fluctuation of the G/S ratio is a valuable measurement

of plant response to nitrogen. In itself, the ratio gives an indication of the plants efficiency in grain production per amount of dry matter produced. By evaluating the G/S fluctuation over nitrogen levels, an inbred can be monitored as to how efficiently it is responding to applied nitrogen. In other words, does the extra N go for plant maintenance or for increased grain production.. SDp2A and A632 had the greatest efficiency as nitrogen levels increased, as noted by their small fluctuations, whereas SDp232, A654 and CO109 made the least efficient use of the added nitrogen. As indicated in Table 11, CO109 had the highest grain producing efficiency per amount of dry matter at low nitrogen levels, but as levels were increased, this inbred tended to put the extra N into plant tissue. A large amount of genetic interaction appears between the actual G/S ratio and the fluctuation of the G/S ratio as nitrogen levels increased, yet inbreds like SD24 and W153R tend to show favorable, above-average values for efficiency in both grain production and nitrogen response.

For the DPAT test, a low or negative fluctuation will again indicate a desirable response as nitrogen levels decrease. A large fluctuation would suggest that the plants absorption ability may be a limiting factor at low nitrogen levels. A654, Wll7, SD10 and A632 tended to have the lowest fluctuations, whereas A657 and SD15 had large fluctuations as nitrogen levels increased.

Comparing seedling DMP fluctuations, a large amount of

variation can be seen in response to nitrogen levels. Low fluctuation, such as shown by A547-2 and W153R, indicate that in the seedling stages, these inbreds can maintain normal growth, even at low concentrations of available nitrogen. Large fluctuations, as shown by W117 and B8 suggest that these inbreds may not be able to absorb enough N at low levels in the seedling stage. Consequently, they have large decreases in their production of dry matter. However, Wll7's values for seedling DMP exemplify a difficulty found in interpreting this type of response data. As indicated above, because of its large fluctuation, W117 was considered as unable to absorb enough N at the low levels to prevent large fluctuations. But when comparing its a ctual values (from Table 15) with the corresponding means of all inbreds at both levels of N, Wll7 consistently had a large amount of DMP. Therefore from these data it appears to be efficient in DMP at both the normal and at the low nitrogen levels. In a final interpretation or application of this fluctuation data, both types of responses must be considered. For an inbred to be efficient in nitrogen utilization it should not only make maximum use of the available N at low levels, but also at normal nitrogen levels.

Efficient nitrogen utilization within the plant responses tested has been briefly discussed. The complexity of selecting for nitrogen-use efficiency can also be seen by the interrelationships of these plant responses. Correlations of these responses (in the

form of fluctuations) are shown in Table 18, with low correlation. values found between most of these traits, suggesting that each fluctuation is measuring a different aspect of the plants overall nitrogen-use efficiency. Further explanation of the interactions and relationships of these traits is beyond the scope and level of this research, but an immediate option left open is the application by plant breeders of the genetic variability found for these traits and their responses to nitrogen. Because of the complexity of the plants nitrogen metabolism system though, research should be geared to monitor multiple areas of inbred metabolism for a more complete and balanced evaluation of nitrogen-use efficiency in the corn plant .

| | | | | Leaf Loss | | | (seed- |
|---|-----------------------------|--------------------|-----------------------|---|---------|-------------------|------------------|
| | $\%$ Leaf N ^b | Yield ^b | $(mid-)$ $silk)^b$ | $(matu -$ rity ^a | G/S^b | DPAT ^a | ling) DMP^C |
| | | | | | | | |
| DMP^b | .36 | $.63*$ * | $-.31$ | $-.08$ | .29 | .21 | $-.13$ |
| % Leaf N ^b | | .08 | $-.18$ | $-.26$ | .18 | .17 | $-.14-$ |
| Yield ^b | | | $-.40$ | .11 | $-.43$ | .48 | $-.18$ |
| Leaf Loss ^b $(mid-silk)$ | | | | .32 | .38 | $-.27$ | .15 |
| Leaf Loss ^a (matrix) | | | | | $-.07$ | .28 | $-.33$ |
| G/S^b | | | | | | $-.33$ | .01 |
| DPAT ^a | | | | | | | $-.49*$ |
| a 1975 data only b C | 1975 and 1976 data combined | | | | | | |

Correlation coefficients of the fluctuations found in Table 18. 8 plant characteristics due to nitrogen levels.

greenhouse data

significant at the 5% and 1% levels of probability, $*$, $**$ respectively

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Seventeen elite inbred lines were monitored for two years for six diverse physiological and morphological traits; dry matter production, percent leaf N, grain yield, leaf loss, grain to stover ratio and a diphenylamine tissue test. A greenhouse experiment evaluated these inbreds for root density ratings and seedling dry matter production. A highly significant amount of genotypic variability was found between the inbreds for each of the traits measured. The effect of nitrogen levels was insignificant, except in the G/S ratio and seedling DMP data. The level of available N , where inbreds exhibit nitrogen deficiency, appears to be lower than levels used in this research, thus accounting for the low amount of response noted in the majority of the field experiments. Population density had a significant effect on almost all traits but statistically showed little effect in increasing the differential response between inbreds and nitrogen levels. Years and its interactions with several of the treatments were significant, emphasizing the effect that different environments has on the interpretation of these traits and their responses.

The fluctuation values shown in Table 17 indicate the re sponse that the individual inbreds had as nitrogen levels increased. Statistically, the inbred by nitrogen level interactions are insignificant for almost all of the traits, suggesting that there is a small amount of genetic variation for nitrogen response in

inbreds. But, investigation of inbreds having the extreme types of fluctuations does show a significant variation between efficient and inefficient inbreds in their responses. Efficiency within each trait is identified by a small fluctuation in plant response as levels of applied N decreased. The first order interrelationships of these areas of nitrogen efficiency are shown by the correlations in Table 18 and indicate the complexity found in evaluating for nitrogen-use efficiency.

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