

Relationship between Nitrate Uptake, Flux, and Reduction and the Accumulation of Reduced Nitrogen in Maize (*Zea mays* L.)

II. EFFECT OF NUTRIENT NITRATE CONCENTRATION¹

Received for publication November 9, 1979 and in revised form July 17, 1980

ANDREW J. REED² AND RICHARD H. HAGEMAN
Department of Agronomy, University of Illinois, Urbana, Illinois 61801

ABSTRACT

Two maize hybrids were grown under growth chamber conditions on solution or vermiculite medium that contained 2.5, 7.5, or 15 millimolar nitrate. The objectives were to determine: (a) the effect of nitrate supply on N metabolism and growth and (b) the interrelationship between nitrate uptake, flux, and reduction on the accumulation of reduced N and nitrate by the various plant parts and for the whole plant.

Increases in nutrient nitrate concentration caused increases in (a) shoot, but not root, dry weight and (b) nitrate uptake, flux, and reduction and accumulation of nitrate and reduced N by the aerial plant parts of both hybrids. Increases in nitrate supply resulted in decreases in nitrate reductase activity and negligible increases in reduced N in the roots of both hybrids. At 2.5 and 15 millimolar, but not at 7.5 millimolar, external nitrate, hybrid B had higher rates of nitrate uptake and flux. Hybrid B also had lower nitrate reductase activity at all levels of external nitrate and accumulated less reduced N than did hybrid C, except when the plants were grown at 2.5 millimolar nitrate. Correlation values between nitrate uptake and flux were significant for each hybrid and for both hybrids, whereas correlations between nitrate reductase activity and flux and nitrate reductase activity and uptake were significant only for a given hybrid. The correlation values (pooling of all data) between (a) nitrate uptake, flux, or reduction and the accumulation of reduced N by the whole plant, and (b) flux \times nitrate reductase activity and accumulation of reduced N were all positive and statistically significant. Because nitrate uptake, flux, and reduction (as measured) were all closely associated with the accumulation of reduced N by the plant, all three parameters should be measured in attempts to estimate the genetic potential of a maize genotype to accumulate reduced N.

Studies with wheat where nitrate was not limiting have shown that NRA³ (integrated over time with samples representing the entire leaf canopy or plant) was highly correlated (r in excess of +0.9*) with the actual accumulation of reduced N (2, 6). With field-grown wheat (cv. Arthur) provided with varying amounts of fertilizer N, the correlation between NRA (integrated over time for the leaf canopy) and the increase in reduced N (total shoot) were initially high ($r = 0.894^*$) but decreased with successive samplings during vegetative development (8). When six field-grown maize hybrids were supplied with varying amounts of fertilizer N, Deckard *et al.* (7) found that NRA (integrated *in vitro* seasonal level of the leaf canopy) accounted for only 35% of the variability in reduced N accumulated by the above ground vegetation, grain, or vegetation plus grain at maturity. Differences in nitrate uptake (4, 13, 24), nitrate accumulation (4, 11, 12), and distribution of NRA (16) have been observed among maize genotypes. Other studies (10, 19, 22) indicate that nitrate uptake and flux and the availability of reductant, as well as NRA, affect the assimilation of nitrate. It is probable that the accumulation of reduced N in response to N fertility is a function of all of these parameters.

The objectives of the study here were to determine: (a) how variations in external nitrate supply affect uptake, flux, and accumulation of nitrate, NRA, plant growth, and accumulation of reduced N; and (b) if accumulation of reduced N can be related to uptake, flux, accumulation, or reduction of nitrate.

MATERIALS AND METHODS

Plant Culture. Maize kernels (*Zea mays* L.) hybrids C123 \times B14A (hybrid C) and B37 \times H96 (hybrid B) were germinated and the seedlings were subsequently grown hydroponically or in vermiculite (pans) as previously described (19). For both studies, the treatment was variation in nitrate supply (2.5, 7.5, and 15 mM). Hoagland No. 1 was used as the basic medium and K₂SO₄ and CaCl₂ were substituted on an equimolar basis for the nitrate salts. The pH of all solutions was adjusted to pH 4.0 with H₂SO₄. Plants grown in the nutrient cultures were harvested after 6 h illumination when the plants were 25 days old. The plants were subdivided into roots, stalks, leaf blades, and midribs for determination of fresh and dry weight, reduced N, and nitrate accumulation. Transpiration and nitrate uptake were measured during the 3- and 24-h period prior to harvest, respectively. Transpiration was expressed as ml g⁻¹ total fresh leaf weight h⁻¹ and nitrate flux was calculated by multiplying nitrate concentration of xylem exudate ($\mu\text{mol ml}^{-1}$) by transpiration rate. Leaf blade and root samples representative of the leaf canopy and root system, respectively, were assayed for NRA. Vermiculite-grown plants were harvested after 6 h of illu-

Current estimates show that more than two-thirds of the edible dry matter and half the protein produced in the world are contributed by the cereal plants (9). The increased use of N fertilizer has been a significant factor in increasing productivity of these crops, especially maize (10). Because the energy required for fertilizer N is costly (5), there is an obvious need for knowledge of the effects of external N supply on the factors affecting nitrate assimilation and plant growth.

¹ This research was supported by Grant 5901-0410-8-144-0 from the Science and Education Administration of the United States Department of Agriculture and by grants from the Frasci Foundation and Hatch Funds.

² Present address: Department of Biology, Queen's University, Kingston, Canada K7L 3N6.

³ Abbreviations: NRA, nitrate reductase activity; NR, nitrate reductase; *, **, statistically significant at 1 and 5% level, respectively.

mination when the plants were 12, 17, or 24 days old. Leaf blades representative of the leaf canopy were assayed for NRA. Dry weights and reduced N were determined for the entire shoot. In both studies, plant parts from three plants were composited for a sample, and triplicate samples were taken at each harvest.

Assays. The methods for measurement of NRA, N components, xylem exudate, and transpiration were as described (19). Average values of the three samples were used for each observation in calculation of correlation values.

RESULTS AND DISCUSSION

Dry Weight Accumulation. The effect of increased nutrient nitrate on dry weight accumulation of the whole plant or plant parts was nearly identical for both hybrids (Table I). Similar changes of dry weight in response to increases in N supply have been observed with maize (23) and other plant species (15, 17). Such data show that availability of external nitrate has a major effect on shoot growth.

Because shoot, but not root, growth was increased in response to added nitrate, the increase in shoot to root ratio was essentially a reflection of the increase in shoot growth. The differential growth response of shoot and root to added nitrate has been observed with other plant species (15, 18, 21, 23, 26). One possible explanation for this response is that the increase in nitrate supply increases nitrate assimilation in the shoot and carbohydrate levels in the plant are decreased (18). Because the shoot is a more effective competitor than the root for photosynthate, shoot growth exceeds root growth.

Nitrate Uptake, Flux, and Accumulation. The profiles of nitrate uptake and flux and nitrate concentration in the xylem exudate as a function of nitrate supply were similar for both hybrids (Fig. 1). Because transpiration rates (ml g^{-1} total fresh leaf weight h^{-1}) were comparable for all measurements, nitrate flux values reflect the nitrate concentrations of the xylem exudate.

By extrapolation of the curves back to zero nitrate supply, the uptake patterns (Fig. 1) are indicative of saturation kinetics as previously reported for excised maize roots (14). K_m values were not estimated for nitrate uptake because of the limited number of observations and the confounding effects of nitrate flux.

When the plants were supplied with 2.5 mM nitrate, hybrid B was more effective in absorbing and transporting nitrate than was hybrid C. Because both hybrids had equal root mass, these data indicate that differences in root morphology and/or uptake mechanisms may account for the different uptake rates. At 7.5 mM nitrate, both hybrids had identical rates of uptake and flux, in

agreement with previous findings (19). At 15 mM nitrate, hybrid B had higher (about 10%) rates of uptake and flux than did hybrid C. Why hybrid C would be as effective as hybrid B in uptake and flux only at 7.5 mM nitrate is open to question.

Rates of nitrate uptake and flux were correlated (r values: hybrid B, +0.997*; hybrid C, +0.997*; combined, +0.99*, data of Fig. 1). External nutrient nitrate concentrations and flux rates (values from both hybrids) were also related ($r = +0.88^*$).

For both hybrids, nitrate accumulation in all plant parts increased in response to increased nitrate supply. Hybrid B accumulated more nitrate in all plant parts for all nitrate treatments, except roots at 7.5 mM nitrate, than did hybrid C (Table II). Allowing for experimental variation, the increase in accumulated nitrate was linear or near linear for leaf blades and midribs and roots in response to increased nitrate supply. In contrast, the accumulation of nitrate by the stalks was nonlinear (parabolic like).

Of the four plant parts, the nitrate concentration of the roots was least affected by the increased nitrate supply. The 6-fold increase in nutrient nitrate caused only a 13 to 14% increase in root nitrate. Hybrid B had higher concentrations of nitrate in the roots than did hybrid C at 2.5 and 15 mM, but not at 7.5 mM, external nitrate, which is similar to the effect of nitrate supply on nitrate uptake. The lack of difference in uptake rates and root nitrate concentrations between the hybrids when grown on 7.5 mM nitrate were also observed in a previous study (19). The differences in root nitrate concentrations between the hybrids at 2.5 and 15 mM nitrate (Table II) indicate that genetic traits as well as external nitrate supply could affect root nitrate concentrations.

The accumulation of nitrate in the various aerial parts (Table II) was numerically related to rates of nitrate flux (Fig. 1). Considering mean values (six observations) for both hybrids, the correlation values (r) between nitrate concentration and nitrate flux rates were +0.61, +0.88**, and +0.91** for leaves, midribs, and stalks, respectively. Differences in leaf NRA levels would have a more direct effect on nitrate concentrations in the leaf blades and midribs than on the stalk nitrate.

NRA. Increases in the external nitrate supply resulted in increases in leaf blade NRA of both hybrids (Fig. 2), as expected, because NR is substrate-inducible (1), and the level of activity is associated with nitrate flux (22). Hybrid C had more NRA as measured by both *in vivo* and *in vitro* assays than hybrid B for all three nitrate treatments. Although the *in vitro* activity was 5 to 6 times greater than the *in vivo* activity, its response to nitrate supply was similar to that of the *in vivo* activity, especially for hybrid B. We infer that the increase in *in vivo* activity reflects an increase in amount of enzyme rather than an increase in reductant (energy) supply (1).

Hybrid C had higher leaf blade NRA than did hybrid B for all nitrate treatments (Fig. 2), as was previously reported for 25-day-old plants (19). In contrast, hybrid C had lower rates of nitrate uptake and flux than did hybrid B, except at 7.5 mM nitrate (Fig. 1). Assuming that nitrate flux regulates the level of NRA (19), these results indicate genetic differences in the requirement for nitrate (flux) for induction and/or maintenance of the enzyme. The correlation values (r) between nitrate flux (Fig. 1) and *in vivo* NRA (Fig. 2) were +0.84 for hybrid B, +0.99 for hybrid C, and +0.52 for both hybrids. These correlation values are consistent with the conclusions that, for a given maize genotype, nitrate flux regulates NRA (22) but that a given flux rate will not result in the same amount of NRA among genotypes (19).

The two hybrids did not differ in root NRA, although hybrid C had higher average values than did hybrid B, especially at 2.5 mM nitrate (Fig. 2). For both hybrids, root NRA decreased with increased external nitrate, as was previously observed with cotton (18) and peas (25). Whether the decrease in root NRA reflects a lower level of NR (25), a lack of reductant (18), or other factors

Table I. Effect of Increasing Nitrate Concentration in Nutrient Media on Dry Weight of Two Maize Hybrids

Plant Part	Hybrid	Effect of Following Nutrient Nitrate Concentrations		
		2.5 mM	7.5 mM	15 mM
		g		
Whole plant	C	2.12 ± 0.15	2.64 ± 0.12	2.87 ± 0.11
	B	2.23 ± 0.12	2.72 ± 0.12	2.98 ± 0.10
Shoot	C	1.63 ± 0.10	2.10 ± 0.08	2.29 ± 0.05
	B	1.68 ± 0.11	2.15 ± 0.08	2.38 ± 0.05
Root	C	0.49 ± 0.05	0.54 ± 0.05	0.58 ± 0.06
	B	0.55 ± 0.04	0.57 ± 0.05	0.60 ± 0.05
Shoot/root ratio	C	3.30 ± 0.15	3.80 ± 0.07	3.95 ± 0.10
	B	3.10 ± 0.15	3.75 ± 0.08	3.92 ± 0.10

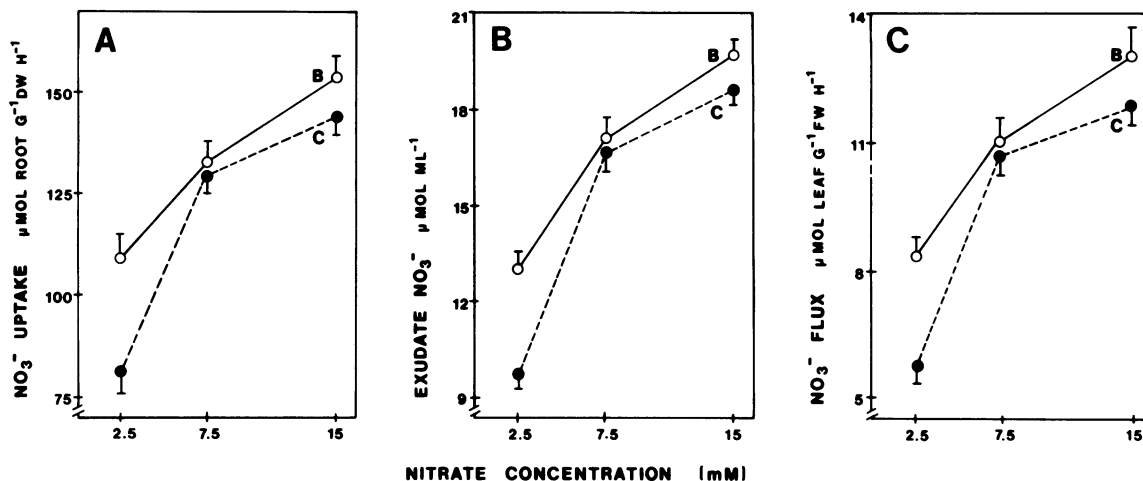


FIG. 1. The effect of nutrient nitrate concentration on (A) nitrate uptake (B) nitrate content of the xylem sap, and (C) nitrate flux of two maize hybrids. The plants were 25 days old at harvest. Transpiration rates were $0.64 \text{ ml g}^{-1} \text{ leaf weight h}^{-1}$ for all measurements except hybrid C at 2.5 mM nitrate (0.58) and hybrid B at 15 mM nitrate (0.66). Bars around points indicate 1 SD.

Table II. Effect of Nutrient Nitrate Concentration on Nitrate Concentration of Leaf Blade and Midrib, Stalk (Includes Leaf Sheaths), and Roots of Two Maize Hybrids

Plants were 25 days old at harvest. Standard deviations are given.

Plant Part	Hybrid	Effect of Following Nutrient Nitrate Concentration		
		2.5 mM	7.5 mM	15 mM
<i>μmol nitrate g⁻¹ fresh wt</i>				
Leaf blade	C	8.3 ± 0.3	10.8 ± 0.4	12.8 ± 0.6
	B	12.9 ± 0.6	14.9 ± 0.8	16.8 ± 0.3
Midrib	C	45.0 ± 1.0	50.0 ± 2.3	57.3 ± 2.2
	B	51.8 ± 2.4	61.0 ± 2.9	68.4 ± 2.0
Stalk	C	53.2 ± 1.4	62.0 ± 1.0	67.8 ± 2.4
	B	56.1 ± 1.8	71.9 ± 1.1	77.2 ± 2.0
Root	C	37.5 ± 1.0	42.0 ± 1.5	44.0 ± 2.1
	B	42.0 ± 1.1	43.5 ± 1.6	48.0 ± 0.9
<i>mmol nitrate plant⁻¹</i>				
Whole plant	C	1.06	1.51	1.80
	B	1.25	1.74	2.15

was not investigated.

Reduced N Accumulation. The reduced N (mg g^{-1} dry weight) in the shoots of both hybrids increased with increased nitrate supply (Fig. 3). For hybrid B, the increase in reduced N was linear or near linear, as was the increase in NRA (Fig. 2) and in nitrate uptake and flux (Fig. 1). For hybrid C, the increases in all of these parameters was less linear and more parabolic in response to increases in nitrate supply.

A possible explanation for the lower level of shoot-reduced N in hybrid C than in hybrid B at 2.5 mM nitrate (Fig. 3) is that the flux of nitrate in hybrid C is inadequate to meet the substrate requirements of the NR. The ratio of nitrate uptake to flux for hybrid C was higher at 2.5 mM nitrate than for hybrid B (Fig. 1). At the higher concentrations of nutrient nitrate, the ratio values were more comparable.

Collectively, these data provide some evidence that accumulation of reduced N by the shoot is influenced by both nitrate flux and NRA. Further support for this view is provided by the correlation values calculated from the data of Figures 1 to 3. The

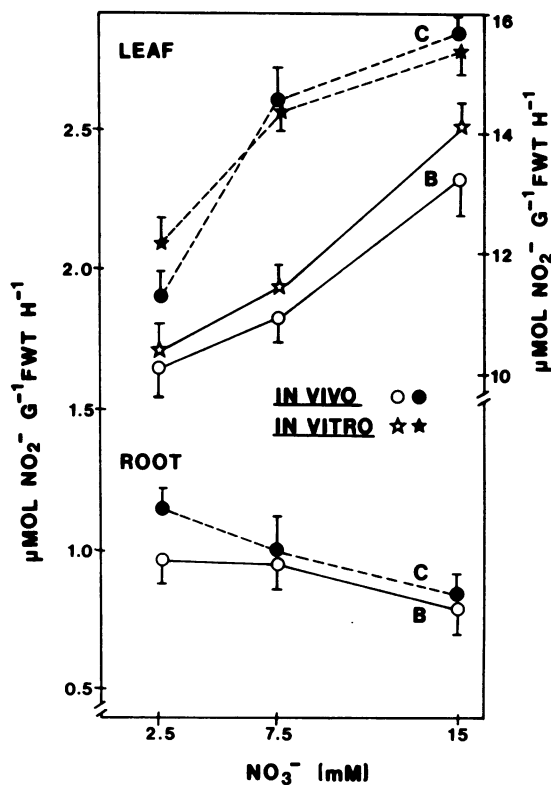


FIG. 2. The effect of nutrient nitrate levels on nitrate reductase activity of leaf and root of two maize hybrids. Leaf blades representative of the entire canopy were assayed for both *in vivo* and *in vitro* NRA. Root samples, representative of the entire root were assayed for *in vivo* NRA. Plants were harvested when 25 days old. Bars around points indicate 1 SD.

r values between nitrate flux and shoot reduced N were: hybrid B, +0.999**; hybrid C, +1.00**; and both hybrids, +0.91*. The *r* values between NRA of leaf blade and shoot reduced N were: hybrid B, +0.95; hybrid C, +0.997**; and both hybrids, +0.87. The *r* values between product of nitrate flux times NRA and shoot reduced N were: hybrid B, +0.98; hybrid C, +0.997*; both hybrids, +0.993**. The correlation equation of reduced N (*y* axis) versus NRA was $y = 9.32x + 8.81$. This indicates either that the *in vivo* leaf activity is underestimating the *in situ* nitrate reduction or that the root is supplying reduced N to the shoot. We favor the latter

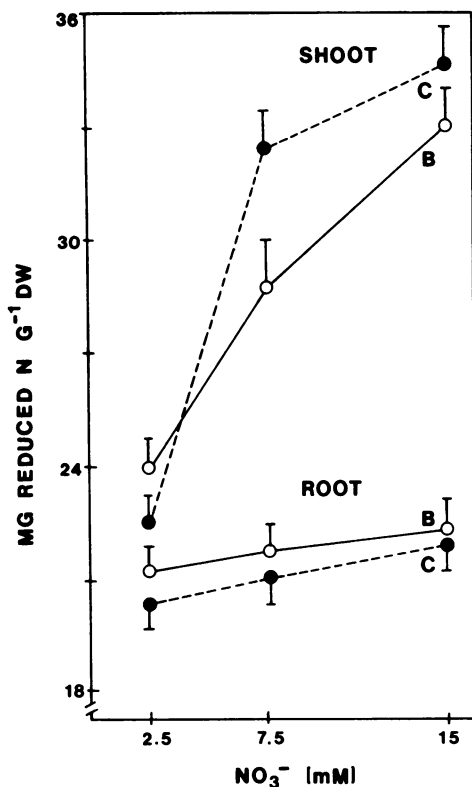


FIG. 3. The effect of nutrient nitrate concentration on the accumulation of reduced N in the shoot and root of two maize hybrids. Plants were 25 days old when harvested. Bars around points indicate 1 SE.

view because the intercept value 8.81 mg reduced N accounts for 37, 31, 27% of the shoot reduced N at 2.5, 7.5, and 15 mM nitrate, respectively. The decreasing proportion of reduced N supplied by the root with increasing external nitrate supply is consistent with the increase in shoot to root ratio (Table I) and the increase in the shoot and decrease in the root NRA (Fig. 2). Assays of xylem exudate of several maize genotypes (comparable plants) indicated that from 25 to 50% of the nitrate is reduced in the root (16).

Although interesting, these and all subsequent correlations need to be viewed with caution, as all parameters are directly affected by the variable nitrate treatment, and the number of observations were limited. The correlation values (r) between nutrient nitrate concentrations and shoot reduced N were: hybrid B, +0.988; hybrid C, +0.892; and both hybrids, +0.903*.

In response to increased nitrate supply, the increase in concentration in reduced N of the roots of both hybrids was linear and limited relative to that of the shoots (Fig. 3). The roots of the two hybrids did not differ in reduced N concentrations, although hybrid B had slightly higher average values than did hybrid C, especially at 2.5 mM nitrate. The higher rate of flux for hybrid B than for hybrid C (Fig. 1) may be a possible reason for the higher level of reduced N in the roots of hybrid B. The accumulation of reduced N by the root was negatively related to root NRA. This could indicate either that (a) the *in vivo* assay was not representative of the *in situ* nitrate reduction or that (b) as the external nitrate supply increased, less photosynthate was available to the roots. The latter view is supported by the accelerated growth and nitrate reduction by the shoot and negligible root growth (Table I; Fig. 1). If the *in vivo* NRA is a reasonable reflection of *in situ* reduction, the decrease in NRA (Fig. 2), could indicate that less reduced N is exported to the shoot with increase in nitrate supply. With cotton, Radin (17) found that reduced N was transported from shoot to root as nutrient nitrate level was increased.

Whole Plant Comparisons. The close relationships previously

noted among these various parameters when the values were expressed on a per unit weight basis were also found when the values were expressed on a per plant basis (Table III). The correlation equation between reduced N (whole plant) and NRA (shoot plus root) was $y = 0.267x - 1.63$. The negative intercept value would indicate that the *in vivo* activities of the root and shoot are overestimating the *in situ* nitrate reduction as measured by the accumulation of reduced N by the whole plant. The correlation value (r) between reduced N (whole plant) and shoot NRA was +0.926** (both hybrids) and that between reduced N (whole plant) and root NRA was -0.678. The respective equations were y (red N) = $0.249x + 1.28$ and $y = -1.62x + 21.20$. Our tentative interpretation of these last two equations is that the shoot assumes an increasing, and the root a decreasing, role in supplying reduced N to the entire plant as nutrient nitrate is increased. At the onset of the growing season, 15 mM nitrate would be an average level of nitrate in fertile fields used for corn production.

The total N (nitrate plus reduced N) of the hybrid B plant was greater than that of hybrid C at 2.5 and 15 mM nitrate, but not at 7.5 mM nitrate (Table III). These patterns of N accumulation are consistent with the rates of nitrate uptake (Fig. 1).

If flux of nitrate is the only factor that affects the accumulation of reduced N by the plant, hybrid B should have accumulated more reduced N than did hybrid C. Hybrid B had higher reduced N only when the plants were grown at 2.5 mM nitrate (Table III). At 7.5 mM nitrate, where nitrate flux rates were comparable, hybrid C, with its higher level of NRA, accumulated significantly more reduced N per plant than did hybrid B. We concluded that both flux and NRA levels are factors affecting accumulation of reduced N.

Vermiculite Experiment. This experiment was designed to show the relationship between the input of reduced N as estimated by leaf-blade NRA and the actual accumulation of reduced N by the

Table III. A Comparison of Rates (A) of Nitrate Uptake, Flux, and Reduction and Content of Nitrate and Reduced N of Two Maize Hybrids Grown at Three Levels of Nutrient Nitrate and Correlation Values (B)

In vivo nitrate reduction was measured with samples representative of the entire leaf canopy and root system. Each value is the average of three samples.

A. Rates						
Hybrid	Nutrient Nitrate	Nitrate			Component Contents	
		Uptake	Flux	Reduction	Nitrate	Reduced N
	mM	$\mu\text{mol plant}^{-1} \text{h}^{-1}$			mmol plant^{-1}	
C	2.5	39	32	21	1.1	3.3
	7.5	70	76	28	1.5	5.8
	15.0	83	96	32	1.8	6.5
B	2.5	60	50	20	1.3	3.7
	7.5	76	81	24	1.7	5.3
	15.0	92	107	28	2.2	6.6

B. Correlation Values			
Between	Hybrids		
	C	B	C + B
	r		
Reduced N versus uptake	+0.997*	+0.998*	+0.929**
Reduced N versus flux	+0.994	+0.999*	+0.973**
Reduced N versus NRA	+0.989	+0.998*	+0.910*
Reduced N versus flux \times NRA	+0.982	+0.997*	+0.983**
Uptake versus flux	+0.999*	+0.999*	+0.985**
Uptake versus NRA	+0.998*	+1.0**	+0.725
Flux versus NRA	+0.999*	+0.999*	+0.80

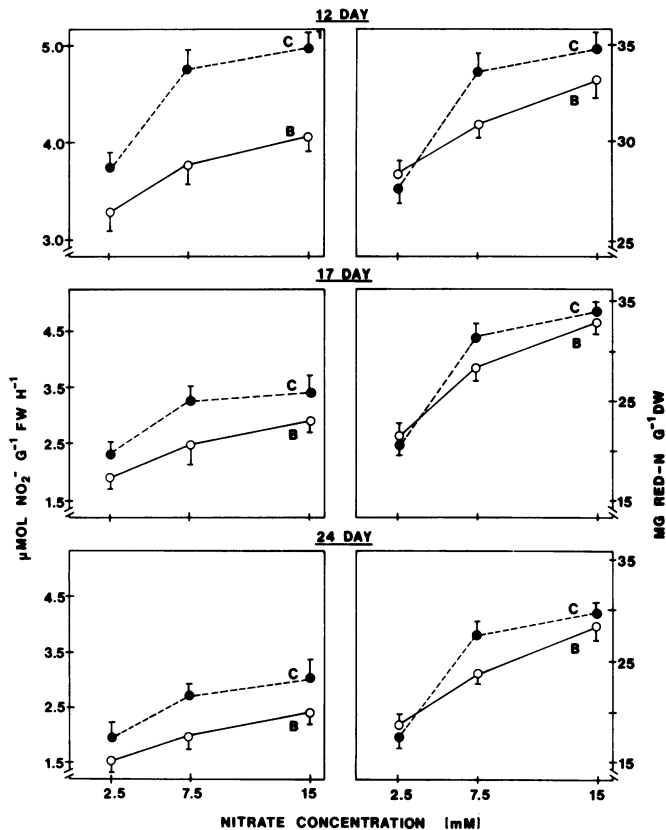


FIG. 4. The leaf *in vivo* nitrate reductase activity and shoot reduced N accumulation of two maize hybrids grown on vermiculite and supplied with nutrient solution containing different levels of nitrate. Plants were harvested when 12, 17, or 24 days old. Bars around points indicate 1 SD.

entire maize shoot. Comparable studies with wheat had shown a high degree of correlation (2, 6). With Vermiculite-grown plants, leaf-blade NRA of hybrid C was higher than that of hybrid B at all nitrate levels and all harvests (Fig. 4). All NRA were similar to the values obtained with samples from plants of comparable age grown on nutrient solution (Fig. 2). The average concentration of shoot reduced N of the vermiculite-grown hybrid C was lower at 2.5 and higher at 7.5 and 15 mM nitrate than for hybrid B, at all sampling dates. With plants of comparable age similar patterns of reduced N had been found with nutrient solution-grown plants (Fig. 3). However, the Vermiculite-grown plants of both hybrids had lower concentrations of reduced N than did the 25-day-old plants grown on nutrient solutions. This difference is attributed to growth dilution of N as the vermiculite-grown plants were more vigorous and larger and/or that the vermiculite medium supplied less nitrate (19).

Data of Figure 4, recomputed as $\mu\text{mol N/shoot}$, were used to calculate the correlation values between estimated input of reduced N (NRA activity) and actual shoot reduced N, as was done previously with wheat (8). The average NRA (recomputed as $\mu\text{mol shoot}^{-1} \text{ leaf canopy h}^{-1}$ from data of Fig. 4) was obtained by summing the three values of the first (12-day) and second (17-day) harvest and dividing by 2. A second set of three values was obtained for the second and third harvest. Estimated input for the time intervals between harvest were calculated by multiplying average NRA by the total photoperiod (h) between the harvest periods by a diurnal factor (0.97). The factor was estimated from data previously published (19). It was assumed that nitrate was not reduced in leaf blades during dark (3). These input values were correlated with the increase in shoot reduced N between harvest periods (e.g. reduced N at 17 days - reduced N at 12

days). For hybrid C, the correlation values (r) were +0.987, +0.990, and +0.913* for the first (12- to 17-day), second (17- to 24-day), and both time periods, respectively; for hybrid B, the corresponding values were +0.997*, +0.972 and +0.934*. For both hybrids over both time periods (12 comparisons), r was +0.921** and the equation was y (reduced N) = $1.44x + 10.13$. The positive intercept value could indicate that approximately one-third of the shoot reduced N was derived presumably from the roots of maize plants at this stage of development when grown on 2.5 to 15 mM nitrate. The slope value (1.44) indicates that the *in vivo* assay is underestimating the *in situ* reduction. Lack of available substrate and/or reductant are the logical factors causing this underestimation, unless NR is inactivated *in vivo* and activated by extraction as the *in vitro* assays show that in the leaf sections, NR is not limiting. These correlations between *in vivo* NRA and reduced N are higher than comparable comparisons obtained with the *in vitro* assay with a single wheat genotype grown under field conditions (8).

We conclude that the *in vivo* measurement of leaf NRA provides a reasonable estimate of the ability of these maize hybrids to accumulate reduced N in the shoot during vegetative development under growth chamber conditions. These two hybrids had similar growth characteristics at all levels of nitrate and comparable rates of nitrate uptake and flux, except when grown at 2.5 mM nitrate.

The work presented here constitutes a part of an ongoing program designed to identify biochemical and physiological traits that can be used to select maize genotypes that are highly productive under field conditions. The study presented here and two others (19, 20) indicate some of the problems encountered and provide some leads for future investigation. A typical problem concerns how to determine which of the parameters, nitrate uptake, flux or reduction, is best suited for the identification of a maize genotype that accumulates the greatest amount of reduced N/plant by the termination of vegetative development (anthesis). High reduced N in the plant at anthesis is assumed to be a desirable trait because it provides adequate N for development of the photosynthetic apparatus and kernel initials (sink). Recurrent divergent selection techniques would also identify genotypes with low or intermediate levels of reduced N. A comparison of the correlation values (Table III) indicates that nitrate uptake and flux provide a better estimate of reduced N accumulation than does NRA. The higher correlation between flux \times NRA and reduced N indicate that both flux and NRA affect the rate of nitrate reduction. This is supported by the observations that hybrid B accumulated more reduced N per plant at 2.5 mM nitrate and less at 7.5 mM nitrate than did hybrid C. The higher level of reduced N per plant of hybrid C at 7.5 mM nitrate is consistent with previous results (19). For all nitrate treatments, hybrid B had higher flux rates and lower NRA than hybrid C, but exceeded hybrid C in accumulation of reduced N only at 2.5 mM nitrate. For the best estimates of reduced N accumulation, all three parameters should be measured; however, this is time-consuming and not readily adapted to the genetic screening of hundreds of individual plants under growth chamber conditions and nearly impossible with field-grown plants. Although a direct assay for total plant reduced N would be the best measurement, such assays are more laborious than for NRA and require the destruction of the plant. NRA assays are relatively simple and rapid, can be nondestructive to the plant, and are adaptable to genetic screening. The main faults are: (a) nitrate flux also affects accumulation of reduced N, and NRA and flux are not closely correlated among genotypes; and (b) ranking, with respect to NRA level, may change with plant development (19). This altered ranking may be due to changing rates of uptake and flux. For example, under growth chamber conditions, hybrid C consistently had higher levels of leaf NRA than did hybrid B, regardless of nitrate supply (also see ref. 19). In contrast, under field conditions during the

postanthesis period, the reverse was true (20). Is this shift in ranking, with respect to NRA, with plant development due to the inability of the roots of hybrid C to supply nitrate to the plant during the postanthesis period? With respect to the ultimate selection criterion used by the plant breeder (grain yield), under field conditions in 1978, the yields of these two hybrids were nearly identical, although hybrid B had accumulated more reduced N by anthesis than did hybrid C. Over a 3-year period (1975, 1976, and 1978), the average yield of hybrid B exceeded that of hybrid C by 12.6 quintals/hectare.

A typical lead was the observation that hybrid B is apparently more efficient than hybrid C in absorbing nitrate when nitrate supply was low (2.5 mM). If one assumes that soil nitrate levels decrease and are low during the postanthesis period, this higher efficiency of uptake of hybrid B may explain how hybrid B maintained a higher level of plant nitrate and a higher level of NRA than hybrid C during grain development (20). Alternatively, it is possible that patterns of root senescence of the two hybrids differed. The possibility of genetic differences in nitrate uptake efficiency needs to be investigated with these and other hybrids.

Acknowledgments—The authors wish to express their appreciation to Fredrick Below for statistical analysis, helpful discussions, and proofreading and to Jane Below for draftsmanship.

LITERATURE CITED

- BEEVERS L, LE SCHRADER, D FLESHER, RH HAGEMAN 1975 The role of light and nitrate in the induction of nitrate reductase in radish cotyledons and maize seedlings. *Plant Physiol* 40: 691–698
- BRUNETTI N, RH HAGEMAN 1976 Comparison of *in vivo* and *in vitro* assays of nitrate reductase in wheat (*Triticum aestivum* L.) seedlings. *Plant Physiol* 58: 583–587
- CANVIN DT, CA ATKINS 1974 Nitrate, nitrite, and ammonia assimilation by leaves: effect of light, carbon dioxide, and oxygen. *Planta* 116: 207–224
- CHEVALIER P, LE SCHRADER 1977 Genetic differences in nitrate absorption and partitioning of N among plant parts in maize. *Crop Sci* 17: 897–901
- COUNCIL FOR AGRICULTURAL SCIENCE AND TECHNOLOGY 1973 Report (November), Energy in Agriculture. Ames, Iowa
- DALLING MJ, GM HALLORAN, JH WILSON 1975 The relation between nitrate reductase activity and grain nitrogen productivity in wheat. *Aust J Agric Res* 26: 1–10
- DECKARD EL, RJ LAMBERT, RH HAGEMAN 1973 Nitrate reductase activity in corn leaves as related to yields of grain and grain protein. *Crop Sci* 13: 343–350
- EILRICH GL, RH HAGEMAN 1973 Nitrate reductase activity and its relationship to accumulation of vegetative and grain nitrogen in wheat (*Triticum aestivum* L.). *Crop Sci* 13: 59–66
- EVANS LT 1975 Crops and world food supply, crop evolution, and the origins of crop physiology. In LT Evans, ed, *Crop Physiology*. Cambridge University Press, Cambridge, pp 1–22
- HAGEMAN RH 1979 Integration of nitrogen assimilation in relation to yield. In EJ Hewitt, CV Cutting, eds, *Nitrogen Assimilation of Plants*. Academic Press, New York, pp 591–611
- HAGEMAN RH, D FLESHER 1960 Nitrate reductase activity in corn seedlings as affected by light and nitrate content of nutrient media. *Plant Physiol* 35: 700–708
- HAY RE, EB EARLEY, EE DeTURK 1953 Concentration and translocation of nitrogen compounds in the corn plant (*Zea mays*) during grain development. *Plant Physiol* 28: 606–621
- HOENER IR, EE DeTURK 1938 The absorption and utilization of nitrate nitrogen during vegetative growth by Illinois high-protein and Illinois low-protein corn. *J Am Soc Agron* 30: 232–243
- NEYRA CA, RH HAGEMAN 1975 Nitrate uptake and induction of nitrate reductase in excised corn roots. *Plant Physiol* 56: 692–695
- OLDAY FS, AV BARKER, DN MAYNARD 1976 A physiological basis for different patterns of nitrate accumulation in cucumber and pea. *J Am Soc Hort Sci* 101: 219–221
- POLISETTY R 1977 Characteristics of nitrate uptake from nutrient solution and root nitrate reductase activity among corn genotypes. PhD thesis. University of Illinois, Urbana-Champaign
- RADIN JW 1977 Contribution of the root system to nitrate assimilation in whole cotton plants. *Aust J Plant Physiol* 4: 811–819
- RADIN JW, LL PARKER, CR SELL 1978 Partitioning of sugar between growth and nitrate reduction in cotton roots. *Plant Physiol* 62: 550–553
- REED AJ, RH HAGEMAN 1980 The relationship between nitrate uptake, nitrate flux and nitrate reductase in four maize (*Zea mays* L.) genotypes. I. Genotypic variation. *Plant Physiol* 66: 1179–1183
- REED AJ, FE BELOW, RH HAGEMAN 1980 Grain protein accumulation and the relationship between leaf nitrate reductase and protease activities during grain development in maize (*Zea mays* L.). I. Variation between genotypes. *Plant Physiol* 66: 164–170
- SCHRADER LE, D DAMSKA, PE JUNG JR, LA PETERSON 1972 Uptake and assimilation of ammonium-N and nitrate-N and their influence on the growth of corn (*Zea mays* L.). *Agron J* 64: 690–695
- SHANER DL, JS BOYER 1976 Nitrate reductase activity in maize (*Zea mays* L.) leaves. I. Regulation by nitrate flux. *Plant Physiol* 58: 499–504
- TURNER TW 1922 Studies of the mechanism of the physiological effects of certain mineral salts in altering the ratio of top growth to root growth in seed plants. *Am J Bot* 9: 415–445
- WARNCKE DD, SA BARBER 1974 Nitrate uptake effectiveness of four plant species. *J Environ Qual* 3(1): 28–30
- WALLACE W, JS PATE 1965 Nitrate reductase in the field pea (*Pisum arvense* L.). *Ann Bot* 29: 655–671
- WALLACE W, JS PATE 1967 Nitrate assimilation in higher plants with special reference to the cocklebur (*Xanthium pensylvanicum* Wallr.). *Ann Bot* 31: 213–228