Plant Physiol. (1980) 66, 164–170
0032-0889/80/66/0164/07/$00.50/0

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[1]

Received for publication November 9, 1979 and in revised form February 7, 1980

ANDREW J. REED[2], FRED E. BELOW, AND RICHARD H. HAGEMAN
Department of Agronomy, University of Illinois, Urbana, Illinois 61801

ABSTRACT

Four maize hybrids, two with high and two with low levels of postanthesis nitrate reductase activity were grown under field conditions. The characteristic enzyme patterns had been established in previous work. Nitrate reductase and proteases were measured in three representative leaves (ear leaf, fourth leaf above and fourth leaf below the ear) at intervals throughout the period of grain development. Concurrent with enzyme sampling, other plants were harvested and subdivided into top, middle and lower leaves, husks, stalks, and ear. Dry weights, nitrate, and reduced N were determined on all plant parts for each sampling. These data established the rate of N accumulation by the grain and depletion from the vegetative material and provide some insight into the relation between newly reduced and remobilized N and accumulation of grain N. Other plants were harvested at maturity for yield and harvest indices for dry weight and N.

Nitrate reductase activity was higher in comparable leaves from the high than from the low nitrate reductase genotypes throughout the grain development period. There was no mathematical correlation between nitrate reductase activity and nitrate content of the leaves or stalks, however the high nitrate reductase genotypes maintained a higher amount of nitrate per plant (largely in the stalk) during the later stages of grain development. From the patterns of plant nitrate content it was deduced that the low nitrate reductase genotypes terminated nitrate absorption sooner than the high nitrate reductase types. Proteolytic activities (casein as substrate at pH 5.5 and 7.5) were higher and increased earlier in the low than in the high nitrate reductase genotypes. The "low nitrate reductase-high protease" genotypes had a higher percentage of grain N, and higher harvest index for N than did the "high nitrate reductase-low protease" genotypes. These results permit the tentative conclusions that: (*a*) redistribution of vegetative N accounted for more of the grain N in the low than in the high nitrate reductase genotypes; and (*b*) leaf protease activities are more closely related to the accumulation of grain N than leaf nitrate reductase activity.

Hay *et al.* (16) observed that by the time of pollination, the corn plant has stored a large quantity of nitrogen, sufficient to "completely supply the maturing grain". The capacity of a maize genotype to accumulate reduced N in the vegetative parts is dependent on nitrate supply, (5, 17) NRA[3] (6, 7, 9, 10) and carbohydrate supply (21), of which the first two requirements are normally limiting. For this reason, significant correlations between NRA and vegetative reduced N have been determined for a number of crop cultivars (7, 9). However, correlation coefficients between NRA and grain reduced N are low (9, 17) which implies that vegetative reduced N accumulation is not always directly related to grain reduced N between genotypes. Hence, although maize plants have the potential to supply the developing grain entirely by redistribution of reduced N accumulated during vegetative growth, other genetic factors must regulate this process.

Dalling *et al.* (7) showed that a significant correlation between NRA and grain N for five wheat cultivars could be obtained if the N translocation efficiency (ratio of grain N to total plant N) was included in the calculations. Therefore, the efficiency of reduced N redistribution is an important regulatory factor in grain N accumulation. In wheat, acid protease activities were correlated with the loss of reduced N from the upper leaves during grain development (8), and Rao and Croy (24) reported that seedling leaf protease activity was higher in cultivars with high grain N compared to low grain N cultivars. Similarly, leaf protease activity and grain N were found to be related in rice cultivars (22). Feller *et al.* (11) showed that in maize leaves, proteolytic activities (casein as substrate at pH 5.4 and 7.5) increased as leaf protein decreased during grain development, and subsequently, significant correlations between proteolytic activity, grain N, and N harvest index were demonstrated (27).

Although proteolytic activity is apparently a key regulator of N reassimilation, the total extent of N reassimilation also depends on the relative distribution of reduced N and protease among the different plant parts. Genotypic differences in the partitioning and redistribution of reduced N from different plant parts has been observed in maize (1, 5, 23), with varying contributions to grain N from upper leaves, lower leaves and stalk. So for maximal redistribution of reduced N, it would be desirable for a maize genotype to accumulate high levels of protein in the tissues that develop high levels of proteolytic activity during grain development.

While some studies (7, 13) indicate that hydrolysis and redistribution of reduced N of the vegetation play a major role in accumulation of reduced N by the grain, it seems possible that nitrate assimilated during the grain filling period could also be transported to the grain. Because current assimilation is dependent upon a supply of nitrate, the relative contributions of the two sources of grain N are controlled both environmentally (8) and genetically (7, 8).

The accumulation of grain protein by a maize genotype depends on the accumulation and partitioning of reduced N accumulated during the vegetative stage of growth, and the relative contributions of nitrate assimilation and N redistribution during grain

[1] This research was supported by the Science and Education Administration of the United States Department of Agriculture under Grant 5901-0410-8-1414-0, and grants from the Frasch 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.

development. To understand better the interrelationships between grain N accumulation and these parameters, this study was designed with the following objectives: (*a*) to examine the relationship between NRA and protease activity in four maize genotypes (two high NRA and two low NRA) during grain development, and determine if these enzyme activities can alter the relative contributions of current nitrate assimilation and N redistribution to grain N accumulation; (*b*) to determine if there are genotypic differences in the distribution of NRA, proteolytic activities and reduced N content throughout the plant, and how this distribution affects the relative contribution of vegetative plant parts to grain N accumulation; and (*c*) to determine how the relationship between NRA, endopeptidase activity, and reduced N partitioning is reflected in the agronomic parameters of grain yield, grain protein, harvest index and N harvest index. We hoped that an understanding of the interrelationships between these parameters would help to establish a physiological basis for selecting maize genotypes with high grain protein.

MATERIALS AND METHODS

Cultural Procedures. Seeds of maize genotypes B37 x B73 (A), B37 x H96 (B), C123 x B14A (C), and Mo17 x H95 (D) were planted May 23, 1978, on Flanigan silt loam. The four maize genotypes were selected on the basis of levels of leaf NRA during the postanthesis period (unpublished); A and B were high and C and D were low NRA genotypes.

The field design was completely randomized with three replications for each genotype. Each plot consisted of three rows, 7.1 m long, spaced 76 cm apart. The center rows were used for determination of final yield, and contained 25 plants (approximately 18,500 plants per acre). The side rows were used for destructive sampling and contained 29 plants. All plots received applications of fertilizer before planting time to provide the equivalent of 200 kg N, 200 kg P, and 280 kg K/ha.

Plant Weight. Total above ground vegetation (two representative plants per plot) was sampled on six dates between anthesis (approximately July 31) and 72 days after anthesis. Judged by black layer formation, genotype D and genotypes A, B, and C matured on October 10 and 16, 71 and 77 days after anthesis, respectively. The plants were weighed and then subdivided into top, middle, and lower leaves, husks, stalks (including leaf sheath), and ears. The composited plant part samples from each harvest were reweighed prior to chopping in a mechanical silage chopper. Each sample was thoroughly mixed, and a 50- to 100-g portion dried at 70 C in a forced air oven for 60 h. The total dry weight of the stover (total above ground vegetation less grain) was calculated from the sum of the parts.

Final harvest for yield and harvest indices for dry weight and N were made at maturity (77 days after anthesis). The plant stover weight was determined directly from five plants in the center row of each plot. The plant material was subdivided, chopped and dried as described. The grain yield was measured by weighing the shelled grain from all plants in the center row of each plot. All dry samples were mechanically ground (20-mesh screen) and stored for assay.

Reduced N Content. The reduced N content of the ground samples of vegetative material (stover) and grain was determined by Nesslerization as previously described (4). The factor 6.25 was used in the conversion of reduced N to protein.

Nitrate and Free Amino Groups. Dry, ground samples of the vegetative material were vacuum-infiltrated (10 mm Hg) in deionized H_2O, and incubated in a shaking water bath for 4 h at 60 C. The water was subsequently assayed for nitrate (20) and free amino groups (28).

Harvest Index. Harvest indices for dry weight and reduced N were calculated as the ratio of grain dry weight of reduced N over total plant plant dry weight or reduced N at maturity.

Chl Concentration. Leaf Chl content was determined as previously described (11).

Enzyme Assays. NR and protease were measured on three leaves of each genotype (ear leaf, and the fourth leaf above and below the earr) at 7- to 10-day intervals between anthesis and grain maturity. All samplings were made at 10.00 h, since diurnal determinations of ear leaf NRA with genotypes A and C showed that maximal enzyme activity, (with insignificant variation) occurred between 10.00 and 14.00 h.

The leaves from two plants per plot were excised, placed in a polyethylene bag on ice, and transported to the laboratory. The leaves were deribbed, weighed, and chopped into 1- × 2-cm sections. The leaf blade sections were mixed thoroughly and portions assayed for NR (assayed immediately) and protease (portions were frozen and assayed within a week).

The *in vivo* NR assay was as previously described (4). Four g leaf sections were vacuum-infiltrated in 80 ml 0.1 M KNO_3, 0.1 M K-phosphate (pH 7.5), and 0.04% Neutronyx 600, and incubated in the dark at 30 C. Aliquots were removed at time intervals for nitrite determination. Activity was expressed as μmol NO_2^- accumulated g^{-1} fresh weight h^{-1} and per plant part. Measurements of NRA were confined to leaf blades because preliminary studies showed that stalks, midribs, leaf sheaths, and husks had low or negligible levels of activity by either *in vivo* or *in vitro* assays.

Protease was assayed with casein as substrate at pH 5.5 and 7.5 by a modification of the method described by Feller *et al.* (11). Frozen leaf sections were ground in extraction medium (1:8 w/v for green leaves, 1:16 for senescent leaves) with a VirTis 45 homogenizer for 1 min at medium and 1 min at high speed. The extraction medium contained 50 mM K-phosphate pH 7.0, 1% (w/v) soluble PVP, and 7 mM mercaptoethanol. The homogenate was filtered through four layers of cheesecloth and then centrifuged at 15,000 rpm for 20 min.

The supernatant was desalted on a Sephadex G-25 column, which had previously been equilibrated with 50 mM K-phosphate, pH 7.0. The K-phosphate proteolytic activity was determined directly on the eluate collected from the column. A 0.5% α-casein solution was prepared in 50 mM acetate [pH 5.5], or Tris-HCl [pH 7.5]) and immediately before use, 7 mM mercaptoethanol was added to the pH 5.5 solution, but not to the pH 7.5 preparation (11). Enzyme extracts (0.05–0.1 ml) were incubated with the substrate solution at 37 C for 3 h. The proteolytic activities were measured by the rate of production of trichloroacetic acid-soluble amino groups from casein and expressed as μmol NH_2 $leaf^{-1}$ h^{-1}. In subsequent studies, attempts to extract proteolytic activities from the stalks were unsuccessful, however both pH 5.5 and 7.5 activities have been found in the leaf sheaths. The activities in the sheath were approximately 10–15% that of the corresponding leaf blade. Patterns of sheath activities over time have not been made.

Statistical Analysis. Analysis of variance procedures were used. The coefficient of variation for each parameter among treatments was calculated. To compare means, the LSD at the 5% level of significance was used.

RESULTS AND DISCUSSION

Nitrate Reductase and Nitrate Content. For all genotypes, leaf NRA declined steadily during grain development at all leaf positions (Fig. 1, A and B, activity expressed as g fresh weight and leaf basis, respectively). As noted in previous experiments, genotypes A and B had significantly higher activities than genotypes C and D, except when activity was expressed on a per leaf basis for the fourth leaf above the ear. The reason for this exception was that the weight of the fourth leaf above the ear was greater for genotypes C and D than for A and B.

Most of the nitrate was in the stalk (includes leaf sheaths) with only 1–3% in the rest of the above ground plant material at 31 days after anthesis and even less at maturity (Table I). Similar

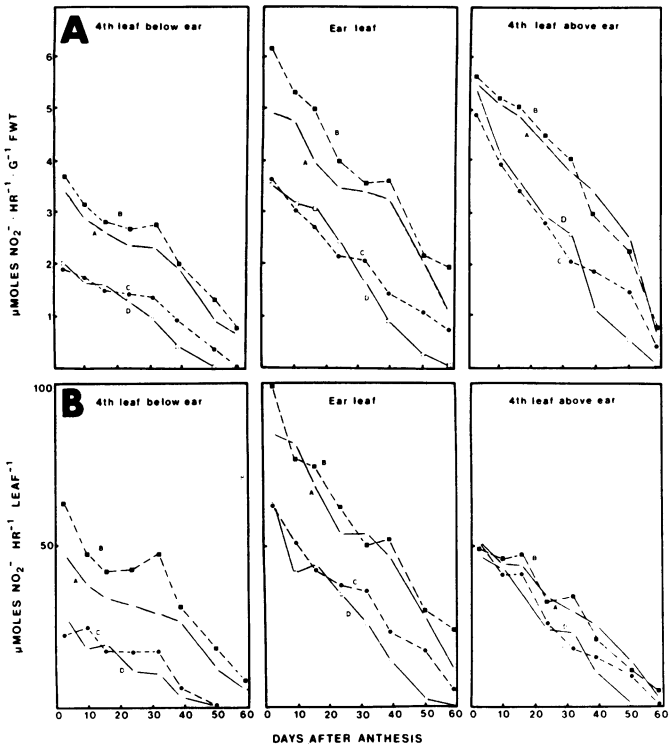

FIG. 1. Changes of *in vivo* NRA (A, expressed on a per g fresh weight and B, per leaf basis) during grain development in the ear leaf, fourth leaf above and fourth leaf below the ear, for four maize genotypes.

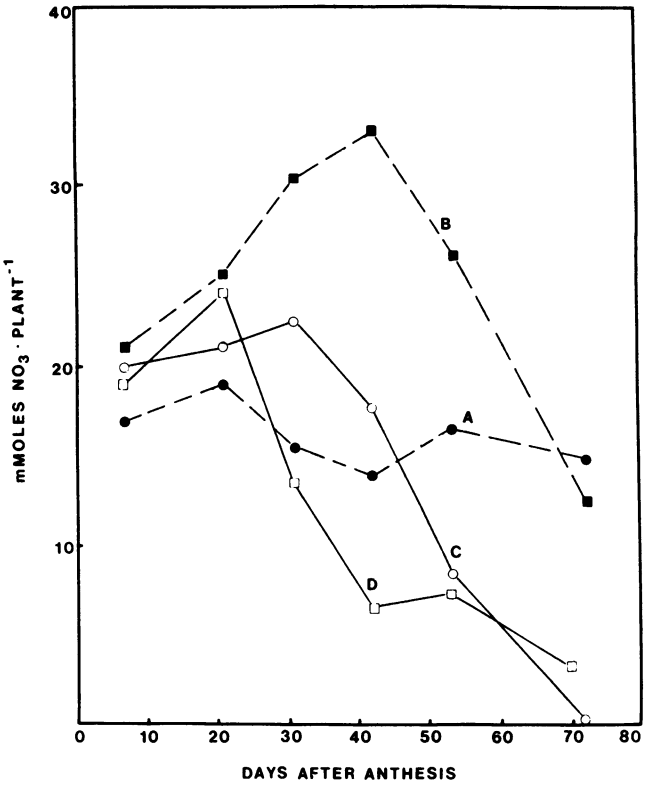

FIG. 2. Changes in the whole plant nitrate content of four maize genotypes during grain development and maturation.

Table I. *Nitrate Content per Plant Part of Leaves (Including Midribs), Ear, Husk, and Stalk (Including Leaf Sheaths) of Four Maize Genotypes*

Genotype	Lower Leaves	Middle Leaves	Upper Leaves	Ear	Husk	Stalk
			31 Days after Anthesis			
			μmol NO_3^-			
A	60	13	5	0	0	15,500
B	461	14	9	0	0	29,800
C	210	64	28	0	0	22,500
D	317	13	8	0	0	13,000
			72 Days after Anthesis			
A	48	0	0	0	0	14,800
B	123	0	0	0	0	12,400
C	9	9	0	0	0	400
D	5	8	0	0	0	3,300

distribution patterns have been reported (5, 9, 12). Most of the leaf nitrate was confined to the midribs as the leaf blades used in the *in vivo* assay accumulated negligible amounts of nitrite, unless the assay medium was fortified with nitrate. With six corn genotypes grown with comparable N fertility, Deckard *et al.* (9) found no more than 0.15 μmol NO_3^- g^{-1} fresh weight in leaf blades after anthesis. Except for genotype A, there was a marked decrease in stalk nitrate between 31 and 72 days after anthesis. Genotype C had the greatest loss of stalk nitrate (22,100 μmol), during grain development. This genotype maintained a higher level of NRA than did the other low NRA genotype which lost 12,700 μmol NO_3^- $plant^{-1}$ during this period (Table I and Fig. 1). Leaf nitrate was also depleted during grain development.

The distribution of NRA from upper to lower leaves (Fig. 1) was the inverse of the leaf nitrate distribution (Table I), especially at 31 days after anthesis. Consistent with previous studies (2) this may indicate that light penetration of the plant canopy was a factor affecting the canopy distribution of NRA. Alternatively the rate of nitrate flux into the cells of the leaf blade may vary with leaf location. The flux rate has been shown to affect induction and activity of NRA more than total leaf nitrate content (26).

During the early stages of grain development there was no relationship between leaf blade NRA levels (Fig. 1) and nitrate content of the various plant parts (Table I) or whole plant (Fig. 2). During the later stages of grain development, the high NRA genotypes maintained a higher level of nitrate, primarily in the stalks than did the low NRA genotypes (Fig. 2). Based on the work of Shaner and Boyer (26) it can be assumed that the flux of nitrate from the soil, stalks and midribs to the cytoplasm of the leaf blade cells in genotypes A and B is higher than in genotypes C and D during this period.

Because the nitrate content of the whole plant (Fig. 2) is a result of nitrate uptake from the soil less assimilation, these data permit some deductions about the rate and duration of uptake. Assuming that *in vivo* NRA (Fig. 1) reflects the *in situ* nitrate reduction (4) and that it is the flux of nitrate into the cytoplasm of the leaf blade cells and not the nitrate content of the plant part that regulates induction and level of NRA (26), it appears that for genotype B, that uptake rate exceeds assimilation rate between 10 and 40 days after anthesis. After 40 days, uptake rate was less than assimilation rate and total plant nitrate decreased. Genotypes C and D have reduced or possibly negligible uptake of nitrate, 30 and 20 days after anthesis, respectively. This is supported by the near depletion of stalk nitrate and loss of NRA by these two genotypes. With genotype A, it appears that nitrate absorption from the soil continues throughout the grain development period. This is supported by the small amount of nitrate (700 μmol) lost from the stalk between 32 and 71 days after anthesis (Table I). The concepts that corn genotypes could vary in duration of nitrate uptake, uptake rates, partitioning, and remobilization are worthy of con-

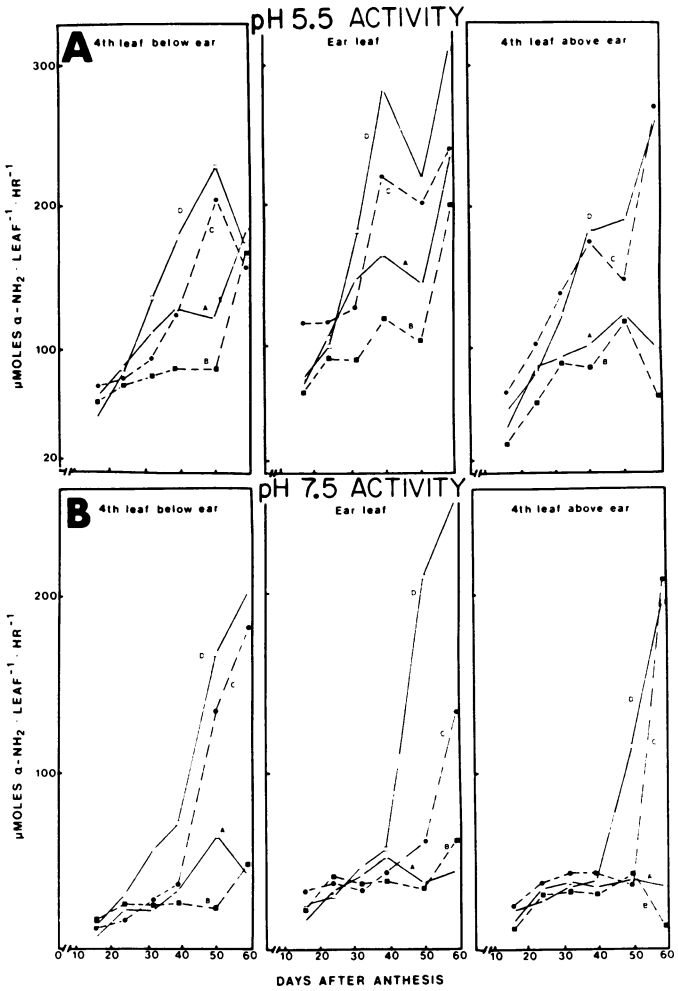

FIG. 3. Postanthesis changes of proteolytic activities in the ear leaf, fourth leaf above and fourth leaf below the ear for four maize genotypes.

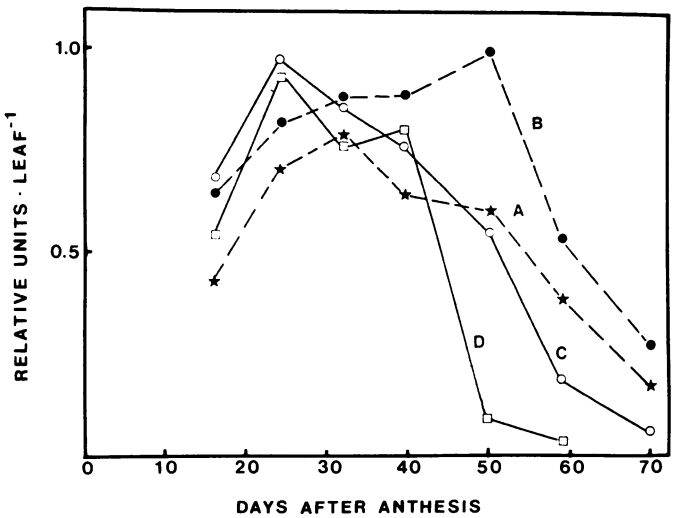

FIG. 4. Relative changes in the Chl content of the ear leaf of four maize genotypes during the grain development and maturation period.

sideration.

Proteolytic Activities. Both the acid (pH 5.5) and neutral (pH 7.5) proteolytic activities (casein as substrate) increased during grain development in the three leaves of all genotypes (Fig. 3, A and B). However, the increase of pH 7.5 activity in the high NRA genotypes was limited. The patterns and levels of activities was a function of genotype. The rate of sustained increase in both activities was initiated earlier and higher levels of activities were achieved by the two low NR genotypes C and D than with the higher NR genotypes A and B. The increase in pH 5.5 activity preceded the increase in pH 7.5 activity in all instances (Fig. 3, A and B). Genotype C that lost more (31-fold) nitrate from the stalk (Table I) and had higher levels of NRA during the latter stages of grain development also had lower levels of protease activities than did genotype D (Fig. 1).

Chl Content. As judged by the loss of Chl (Fig. 4) senescence of the ear leaf of the low NRA genotypes C and D preceded that of the high NRA genotypes A and B. During the later stages of grain development, the loss of Chl from the ear leaves (Fig. 4) occurs in the same order (ranking) of the four genotypes as does the loss in NRA (Fig. 1B), total plant nitrate (Fig. 2) and gain in proteolytic activities (Fig. 3). Previous work with corn indicated that increases in proteolytic enzymes was coincident with loss of Chl and protein from the leaves (11). These observations are also consistent with the tentative deduction that proteolysis may proceed and is associated with the loss of Chl and protein from excised leaves (18).

Losses of Reduced N from the Vegetation. In relation to the total above ground vegetation, the leaves (sheaths excluded) lost the greatest amount of reduced N during grain development (Fig. 5) for all genotypes. The middle leaves, which at anthesis contained the most reduced N, also lost the most N during grain development although roughly proportional amounts were lost from the upper and lower leaves.

The most significant losses of reduced N from the various leaves occurred after 30 days postanthesis (Fig. 5). The loss of reduced N from the middle leaves of genotype C between 10 and 20 days after anthesis was an exception. This early loss of N from genotype C is consistent with the initially high level of pH 5.5 proteolytic activity of the ear (middle group) leaf of genotype C (Fig. 3). In general, the pattern of loss of reduced N from the leaves is coincident with the onset and development of the pH 5.5 proteo-

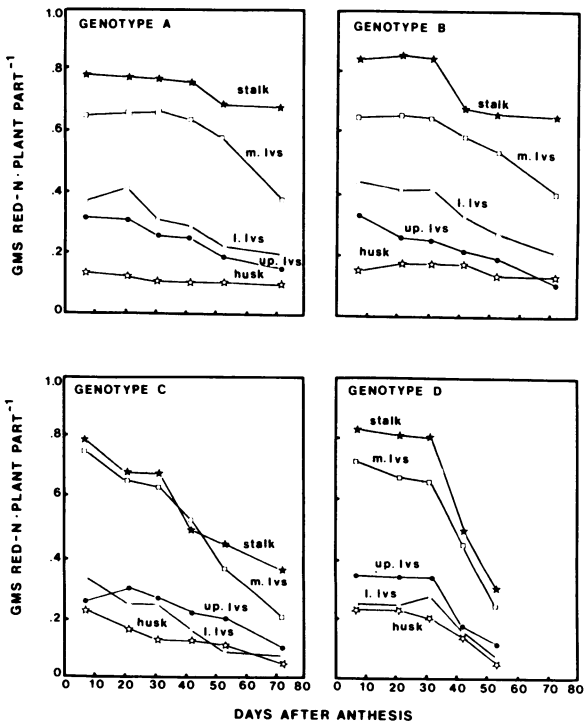

FIG. 5. Changes of reduced N in lower, middle and upper canopy leaves, husk and stalk (includes leaf sheaths) of four maize genotypes during the grain development and maturation period.

lytic activity (Fig. 3) for all genotypes and for the pH 7.5 activity with genotypes C and D. Leaves of genotype A and B that failed to develop appreciable levels of pH 7.5 activity by 60 days postanthesis (Fig. 3) retained more (nearly double) reduced N per leaf at 70 days postanthesis (Fig. 5) than did genotypes C and D that had relatively high levels of pH 7.5 proteolytic activity. The coincidence of these events indicates a causal relationship between the proteolytic activities and the loss of reduced N from the leaves.

The stalks of genotypes C and D lost more than half of their reduced N during the later stages of grain development. In contrast, stalks of genotypes A and B lost no more than one-fourth of their reduced N (Fig. 5). Since these stalks included leaf sheaths, which have been shown to lose N during grain development (14) the amount of reduced N lost specifically by the stalk could not be evaluated. Subsequent studies with other corn genotypes indicated that the loss of reduced N from the leaf sheaths was approximately half that of stalks. Because of the failure to obtain extractable proteolytic activity from the stalk, we are unable to explain the mode of N remobilization of the stalk. The extractable free amino-N compounds constitute a small proportion of the total reduced N of the stalk (Table II), indicating that most of the N in the stalk was protein, in agreement with the results of Hay *et al.* (16).

Redistribution of Reduced N. Several observations are apparent from the decreases of reduced N from the above ground vegetative plant parts (stover) that occurred concurrently with the increases of reduced N in the ears (Fig. 6). (*a*) For all four genotypes, there was little loss of reduced N from the stover until 32 days after anthesis. However within this period the ear accumulated approximately half of its total reduced N at maturity. This indicates that nitrate assimilation is directly or indirectly the source of the early accumulation of reduced N by the ear. Since cob development is complete within 12 days after anthesis (15), the reduced N accumulation by the ear over the first 32 days, must be predominately in the grain. (*b*) Although genotypes A and B had higher levels of NRA than genotypes C and D, no significant differences were observed in the rates of reduced N accumulation in the ears of the four genotypes during the first 32 days of development. Factors other than the level of NRA appear to control the initial rates of accumulation of reduced N by the developing ear. (*c*) Higher rates of accumulation of reduced N by the ears of genotypes C and D, appeared only in the later phases of ear development (Fig. 6), coincident with the appearance of higher proteolytic activities in these genotypes (Fig. 3). (*d*) Decreases in reduced N of the stover between 32 and 72 days after anthesis accounted for only a part of the increases in reduced N of the ears over the same time period except for genotype D. During this period, genotype D had the highest level of proteolytic activity (Fig. 3) and the lowest level of NRA (Fig. 1). Current assimilation of nitrate would seem a logical source for a portion of the reduced N accumulated by the ears of the other three genotypes during the later portion of grain development. Conclusions (*b*) and (*c*) are based on the premise that

Table II. *Changes in the Percentage of Free Amino-N (Leucine Equivalents) to Total Reduced N in the Stalk of Four Maize Genotypes during the Grain Development and Maturation Period*

Time after Anthesis	$\frac{\text{mg Free Amino-N}}{\text{mg Total Reduced N}} \times 100$			
days	*genotype*			
	A	B	C	D
7	18.6	21.5	21.5	23.8
21	19.0	20.6	22.5	14.9
31	14.1	17.7	19.3	11.4
42	25.8	28.3	30.0	26.4
53	18.8	16.6	21.0	20.3
72	20.0	18.2	17.9	

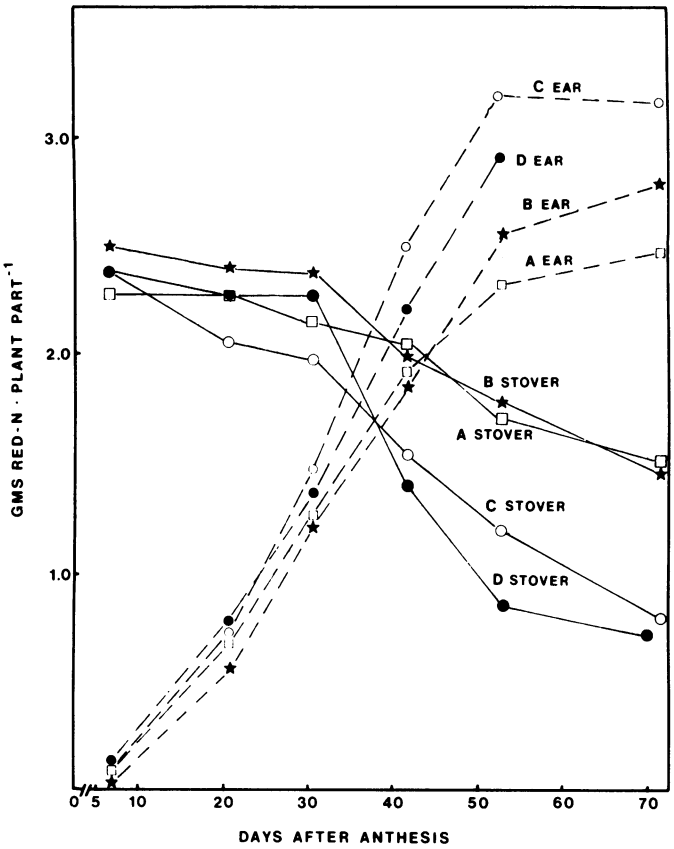

FIG. 6. Changes of reduced N in the stover and ear (includes cob) of four maize genotypes A–D, during the grain development and maturation period.

reduced N is not redistributed from root to ear during grain development, an assumption that is valid under N fertile conditions (13). (*e*) Losses of reduced N from the stover of genotypes C and D were greater than for genotypes A and B. This is consistent with the higher levels of proteolytic activities in genotypes C and D than in A and B and indicate a role for these enzymes in hydrolysis of leaf proteins for translocation to the grain. (*f*) A comparison of the proteolytic activities (Fig. 3) with stover N losses and ear N gains (Fig. 6), indicates that a high level of both pH 5.5 and 7.5 activities are associated with the efficient remobilization of N. We speculated that the failure of genotypes A and B to develop appreciable levels of pH 7.5 proteolytic activity may be causally related to the remobilization efficiency. However, these genotypes also had lower levels of pH 5.5 activity than genotypes C and D. The low levels of pH 7.5 activity of genotypes A and B could be the result of genetic control on enzyme induction (including hormonal effects), environment, or both (*e. g.* temperature, rainfall, and availability of nitrate).

Agronomic Parameters. There was no consistent relationship between the "high NRA, low protease" genotypes A and B or "low NRA, high protease" genotypes D and C and grain yield, grain N (g plant^{-1}), harvest index for dry weight, and dry weight or N content of the whole plant (vegetation and grain) at maturity (Table III). (The last two items can be calculated from Table III data.) Genotypes C and D had higher percentages of grain N, harvest indices for N and retained less N in the stover at maturity than did genotypes A and B. These results indicate that the high proteolytic activity in the leaves and efficient remobilization of N show a more direct relationship to accumulation of grain N than did the level of NRA in the leaves.

The level of NRA in the leaves during the postanthesis period (Fig. 1) did not correlate with the amount of reduced N in the

Table III. *Yields and Nitrogen Content of Grain and Parameters Associated with Translocation of Nitrogen and Dry Weight from Stover to Grain for Four Maize Genotypes*

Harvest was made 72 days after anthesis.

Genotype	Grain (Shelled)			Harvest Index		Loss of Stover N during Grain Development	Grain N Contributed by Stover
	Yield	N		Dry Weight	N		
	g plant^{-1}	*g plant*$^{-1}$	%	*ratio*		*g plant*$^{-1}$	%
A	139.9	1.82	8.14	0.38	0.49	0.37	20
B	163.7	2.21	8.43	0.42	0.56	0.82	37
C	164.4	2.60	9.89	0.46	0.64	1.00	39
D	165.2	2.34	9.22	0.49	0.69	1.34	57
LSD (0.05)	18.4	0.15	0.58	0.05	0.03	0.06	4
CV%	6.2	3.6	3.4	5.8	2.4	3.3	5

whole plant at maturity (3.71, 3.97, 4.05, and 3.39 g N plant^{-1} for A, B, C, and D, respectively, calculated from data of Table III) or at anthesis (Fig. 5).

It was surprising that the later senescence of genotypes A and B (Fig. 4) did not result in superior yields (Table III), inasmuch as the majority of grain dry weight is derived from current photosynthesis (3). Either photosynthate supply did not limit grain yield or photosynthesis in green leaves of genotypes A and B was low during the later stages of grain development.

DISCUSSION

The lack of correlation between NRA in the leaves during grain development and the accumulation of reduced N by the whole plant indicates that the level of the enzyme, as measured, is not a valid index of cumulative accumulation of reduced N among genotypes. The rationale that the level of NRA should be related to accumulation of reduced N by the plant is based on the knowledge that NR is the rate-limiting step between nitrate and amino acids and that NR is substrate-inducible. The level of the enzyme should reflect the rate of supply (flux) of nitrate to the leaf blades (26). The findings that NRA is highly correlated with accumulation of N by wheat plants for a given genotype (4, 10) or for a certain group of wheat genotypes (7) provide support for this view. The current data show that other factors are involved. The measurements of *in vivo* NRA (+ NO_3^-) made only during grain development did not reflect the cumulative accumulation of reduced N. When these same genotypes were grown under growth chamber conditions, genotype C had significantly higher NRA and genotype D had accumulated significantly higher amounts of reduced N over 24 days than the other three genotypes, respectively (25). There appeared to be no correlation between leaf NRA and accumulation of reduced N by the shoot; however, in these studies NRA was not monitored daily as awas done with wheat (4). The reasons for the lack of correlation among these four maize genotypes could be variations in availability of soil nitrate, rate, time, and duration of nitrate uptake, partitioning of nitrate among the plant parts, flux rate of nitrate into the leaf blade cells, availability of reductant, stability of NRA *in situ* and/or sensitivity of the induction site for nitrate.

The parallel ranking of the four genotypes for harvest indices of dry weight and N is of interest; however, the limited data preclude conclusions. The question is raised, "is this parallel ranking due to interaction of genetic traits and a specified environment or will such parallelism prove to be consistent over genotypes and environment?"

The inverse relationship between NRA (Fig. 1) and proteolytic activities (Fig. 3) as well as the delay in development of proteolytic activities in the high NRA genotypes A and B indicated a possible cause and effect relationship between the two processes. Some support for such a view is afforded by the observations with excised oat leaves of Martin and Thimann (19) that certain amino acids enhanced senescence while another acted as an antagonist. Preliminary evaluation of 1979 data obtained with 10 maize genotypes indicated no obligatory relationship between NRA activity and proteolytic activities.

The ever increasing price of fertilizer N indicates the need for development of crop plant genotypes that utilize N most efficiently. Based on harvest index for N, and the ratio of grain yield per g of reduced N accumulated by the whole plant genotype D is a more efficient utilizer of its absorbed N than the other three genotypes (computed from data of Table III). The ratio value and the harvest index for N are not necessarily associated (*e. g.* genotypes B and C have ratio values of 41.5 and 40.5 and harvest indices of 0.56 and 0.64, respectively). The efficient genotypes must be competitive producers of grain dry weight, if these criteria are to be useful in selection of commercial genotypes.

Acknowledgments—The authors wish to express their gratitude to Risé Femmer and Dorothy Carver for their valuable assistance with these experiments, and to Dr. B. Kirkpatrick for his help with the statistical analysis.

LITERATURE CITED

1. BEAUCHAMP EG, LW KANNENBERG, RB HUNTER 1976 Nitrogen accumulation and translocation in corn genotypes following silking. Agron J 68: 418–422
2. BEEVERS L, RH HAGEMAN 1972 The role of light in nitrate metabolism in higher plants. *In* AC Giese, ed, Photophysiology, Vol VII. Academic Press, New York, pp 85–113
3. BIDINGER F, RB MUSGRAVE, RA FISCHER 1977 Contribution of stored preanthesis assimilate to grain yield in wheat and barley. Nature 270: 431–433
4. 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
5. CHEVALIER P, LE SCHRADER 1977 Genotypic differences in nitrate adsorption and partitioning of N among plant parts in maize. Crop Sci 17: 897–901
6. CROY LI, RH HAGEMAN 1970 Relationship of nitrate reductase activity to grain protein production in wheat. Crop Sci 10: 280–285
7. 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
8. DALLING MJ, G BOLAND, JH WILSON 1976 Relation between acid proteinase activity and redistribution of nitrogen during grain development in wheat. Aust J Plant Physiol 3: 721–730
9. 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
10. 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
11. FELLER UK, TT SOONG, RH HAGEMAN 1977 Leaf proteolytic activities and senescence during grain development of field-grown corn (*Zea mays* L.). Plant Physiol 59: 290–294
12. FRIEDRICH JW, LE SCHRADER, EV NORDHEIM 1979 N deprivation in maize during grain filling. I. Accumulation of dry matter, nitrate-N and sulfate-S. Agron J 71: 461–465
13. FRIEDRICH JW, LE SCHRADER 1979 N deprivation in maize during grain filling. II. Remobilization of ^{15}N and ^{35}S and the relationship between N and S

accumulation. Agron J 71: 466–472
14. HANWAY JJ 1962 Corn growth and composition in relation to soil fertility. II. Uptake of N, P and K and their distribution in different plant parts during the growing season. Agron J 54: 217–222
15. HANWAY JJ 1963 Growth stages of corn (*Zea mays* L.). Agron J 55: 487–492
16. 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
17. JOHNSON EB, WJ WHITTINGTON, GC BLACKWOOD 1976 Nitrate reductase as a possible predictive test of crop yield. Nature 262: 133–134
18. MARTIN C, KV THIMANN 1972 The role of protein synthesis in the senescence of leaves. I. Formation of protease. Plant Physiol 49: 64–71
19. MARTIN C, KV THIMANN 1972 The role of protein synthesis in the senescence of leaves. II. The influence of amino acids on senescence. Plant Physiol 50: 432–437
20. MCNAMARA AL, GB MEEKER, PD SHAW, RH HAGEMAN 1971 Use of a dissimilatory nitrate reductase from *Escherichia coli* and formate as a reductive system for nitrate assay. Agric Food Chem 19: 229–231
21. NEYRA CA, RH HAGEMAN 1976 Relationships between carbon dioxide, malate, and nitrate accumulation and reduction in corn (*Zea mays* L.) seedlings. Plant Physiol 58: 726–730
22. PEREZ CM, GB CAGAMPANG, BV ESMANA, RU MONSERRATE, BO JULIANO 1973 Protein metabolism in leaves and developing grains of rices differing in grain protein content. Plant Physiol 51: 537–542
23. POLLMER WG, D EBERHARD, D KLEIN, BS DHILLON 1979 Genetic control of nitrogen uptake and translocation in maize. Crop Sci 19: 82–86
24. RAO SC, LI CROY 1972 Protease and nitrate reductase seasonal patterns and their relation to grain protein production of "high" versus "low" protein wheat varieties. J Agric Food Chem 20: 1138–1141
25. REED AJ, RH HAGEMAN 1979 The relationship between nitrate uptake, flux and nitrate reductase in maize (*Zea mays* L.). I. Genotypic variation. In press
26. SHANER DL, JS BOYER 1976 Nitrate reductase activity in maize (*Zea mays*) leaves. Plant Physiol 58: 499–504
27. SOONG TT 1978 Proteolytic enzyme activities in corn (*Zea mays* L.) leaves during water deficit and senescence. PhD thesis. University of Illinois, Urbana
28. YEMM EW, EC COCKING 1955 The determination of amino acids with ninhydrin. Analyst 80: 209–213