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The Effects of Ear Removal on Senescence and Metabolism of Maize[1]

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ABSTRACT

Ears were removed from field grown maize (*Zea mays* L.) to determine the effects on senescence and metabolism and to clarify conflicting literature reports pertaining to these effects. Ears were removed at three days after anthesis and comparisons were made of changes in metabolism between eared and earless plants until grain of the eared plants matured as judged by black layer formation.

The initial visual symptom following ear removal was the development of reddish colored leaves. As judged by leaf yellowing, the removal of ears not only initiated an earlier onset but enhanced the rate of senescence. With this exception, the visual patterns of senescence were similar for earless and eared plants. Other characteristics associated with ear removal were: (a) marked decrease in dry weight and reduced N accumulation by the whole plant; (b) progressive, parallel decreases in leaf reduced N, nitrate reductase activity, and chlorophyll; (c) increases in carbohydrate content of both the leaf and stalk and of reduced N in the stalk. These changes indicate that ear removal reduced photosynthesis and nitrate reduction by approximately equal proportions and that the stalk serves as an alternate sink for both carbohydrate and nitrogen.

The remobilization of nitrogen from the leaf was not dependent on the presence of an ear. A logical reason for the more rapid loss of nitrogen from the leaf of the earless plants appears to be the cessation of nitrate uptake and/or flux of nitrate to the leaves.

From these results and from related experiments we tentatively conclude that the loss of nitrogen from the leaf is a major cause of death of the intact maize plant.

Recent reviews (11, 17, 18, 28, 30) indicate that senescence of plants is complex and not well understood. Of the several theories of senescence that have been proposed, competition for nutrients has received the most attention. The theory had as its inception the observation that leaf longevity of numerous plant species may be extended if flowering or pollination is prevented or if fruits or seeds are removed (30). However, in some cultivars of some species (*Zea*, *Hordeum*, *Capsicum*) ear or fruit removal induces rather than delays early senescence of the leaves (1, 8, 12).

In attempting to resolve these differences among species, Thomas and Stoddart (30) suggested that removal of sinks (seed) with relatively high N:C ratio from plants (*e.g.* leguminous species) causes a greater reduction in sink demand for N and consequently a reduced rate of loss of N from the leaves than does removal of seeds from other species. They also state, 'Removal or enervation of sink organs with a high demand for current photosynthate and a relatively low requirement for mobile minerals leads to accumulation of carbohydrates in the source leaf, a depressed rate of photosynthesis and the induction of senescence.' These statements infer that removal of sinks decreases the remobilization of N from the leaves for leguminous species. Conversely, depletion of N from leaves of plants with seeds would be extensive. This view is consistent with the self-destruct concept of Sinclair and de Wit (26).

However, Mondal *et al.* (14) have found that, relative to the seed bearing controls, carbohydrate as well as protein accumulated in the leaves of 'desinked' soybean plants and that photosynthesis was only partially inhibited during the normal seed filling period. Leaves of the desinked plants remained green and did not exhibit the normal senescing pattern (leaf yellowing) shown by the controls. Lawn and Brun (10) found that, relative to the control, partial depodding increased acetylene reduction of soybean nodules. At maturity the vegetation of the partially depodded plant had a greater N content than the control although the seed N contents were equal. Wilson *et al.* (31) compared male sterile soybean plants having limited pod set with a normal counterpart. They found that the partially podded plants had delayed senescence, greater nodular acetylene reduction, and greater N content of vegetation and seeds. Collectively, these studies indicate that any variable that enhances N_2 fixation by the nodules and maintains a greater N content in the vegetation was associated with delayed senescence. These studies provide little evidence for the view that build up of carbohydrates in the leaves was associated with plant senescence as proposed for corn (1, 30).

Sesay and Shibles (21) found that foliar applications of mineral elements to soybean plants did not delay the onset or alter the course of senescence. However, the concentration of leaf lamina N was only slightly higher immediately after spraying and the rates of loss of N from the leaves during senescence were similar and as extensive for both sprayed and unsprayed plants.

Thomas and Stoddart (30) infer and Thimann (29) states that, while the remobilization of N and C compounds may play a role in leaf senescence, they are not causal. Nooden (17) ascribes to the theory that a hormone exported from the seed *per se* is the cause of senescence. Although ABA has been suggested as the senescence hormone, the experimental evidence for its direct involvement in senescence is not convincing (3, 20). Studies with excised leaves (or leaf sections) have shown treatments that alter stomatal aperture also alter leaf senescence under conditions of light or dark (29). Thimann (29) concluded that stomatal aperture controls or operates in parallel with leaf senescence.

In 1962, Moss (15) reported that maize plants senesced later when the ears were bagged to prevent fertilization of the kernel. After bagging, the plants developed purple stems and leaf margins characteristic of barren plants. However, they still retained dark green leaves at the time the eared plants had matured. When pollination was prevented, photosynthesis was initially depressed

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(up to 65% within 11 days for some cultivars) but was higher than for the eared plant at the end of grain filling. Sugar concentration (Brix) in the stalk was higher (62 to 112%) in the barren than in the eared plant. No other parameters were measured. In contrast, Allison and Weinmann (1) state that bagging, or ear removal, caused development of red pigmentation and premature senescence of leaves of maize. Similar observations were noted with unpollinated maize plants by Thiagarajah *et al.* (27). In leaves of barren plants, Allison and Weinmann (1) found that total nonstructural carbohydrate content was up to 75% higher than in comparable control leaves. They imply that the high concentration of carbohydrates is associated with premature senescence. Genetic differences among maize cultivars or environmental effects may be the cause of these divergent results.

The objectives of this work were to reinvestigate the conflicting reports of Moss (15) and Allison and Weinmann (1) that ear removal from maize plants hastens leaf and plant senescence and to determine the effect of ear removal on metabolic parameters.

MATERIALS AND METHODS

Cultural Procedures. Plants were field grown on Flanagan silt loam with high levels of P and K and a spring application of 190 kg N/ha. *Zea mays* (L.) kernels, hybrid B73 × Mo17, were overplanted on May 3, 1980, and thinned to a final stand of 39,500 plants/ha. The field design was a randomized complete block with three replications. Each block was divided into 15 plots (0.7 by 3.0 m) containing eight plants. Treatments consisted of removal of ear shoots from half of the plants in each plot at three DAA[2] and no ear shoot removal. Treatments were assigned at random to individual plants in each plot. Second ear shoots that were initiated after removal of the primary ear shoot were also excised as soon as they formed. Each plot was randomly assigned a sampling date.

Sampling. Whole plants were harvested at 4 dates (3, 28, 42 and 57 DAA). An individual leaf (first leaf below the ear leaf) was harvested on 11 dates (3, 5, 9, 13, 18, 21, 31, 34, 41, 46 and 57 DAA) between anthesis (July 19) and black layer grain maturity (Sept. 14). At each sampling date (either for whole plants or individual leaf) three uniform plants in each treatment were sampled and the samples composited for each plot-treatment combination.

For whole plant sampling, plants were taken at 1500 h, subdivided and composited into leaf and stalk (including sheaths) samples. The sub-samples were weighed before passage through a silage chopper. A weighed portion (100 g) of the chopped stalk or leaf sample was dried for 60 h at 70 C in a forced-draft oven, reweighed, and then ground through a 20-mesh screen of a Wiley mill. The ground material was used for chemical assays.

An individual leaf was selected for detailed study because previous work had shown that such a leaf provides a reasonable estimate of changes in the critical central canopy (19). The composited three-leaf samples were placed in a polyethylene bag and placed on ice for transport to the laboratory. The leaves were deribbed and the laminae and midribs were weighed before chopping into 1 × 2-cm sections. Portions of the chopped laminae were used for enzyme and Chl assays. Other portions (4 g) of both laminae and midribs were used to determine moisture content. The dried samples were then ground through a 20-mesh screen of a micro Wiley mill and used for chemical assays. Midribs were used for nitrate assay because the amounts of nitrate in the laminae are negligible and highly variable during the reproductive phase. The midribs, especially the thickened cells above and below the vascular system and the structural (support) elements, apparently serve as a storage site for nitrate as midrib nitrate is depleted more slowly than laminae nitrate when nitrate supply is terminated. The intersecting veinal network of the parallel veined leaf would permit redistribution of this stored nitrate. We have been unable to detect NRA in midribs (unpublished data).

Nitrate Reductase Activity. Leaf *in vivo* nitrate reductase activity (NRA) was determined as described(19).

Chlorophyll. Leaf Chl was determined as described (7).

Proteases. Proteases were assayed by the method described by Reed *et al.* (19), except for the following modifications: (a) the homogenization medium contained 0.1% (v/v) Triton X-100 because its inclusion approximately doubled extractable activity, and (b) activity was measured at pH 5.8 and 8.0 using 50 mM citrate-phosphate and 50 mM phosphate buffers, respectively.

Reduced-N. A 100-mg sample of dry, ground tissue was weighed into a Folin-Wu digestion tube to which 4 ml of concentrated H_2SO_4 were then added. Tissue was digested at 240°C on an aluminum block. At intervals after appropriate cooling several drops of 30% H_2O_2 were added and the digestion continued until the solution became clear. After digests had been diluted and mixed, an aliquot was assayed for NH_4^+ (2).

Total N. Total N (includes reduced N plus nitrate) was determined by the procedure of Nelson and Sommers (16). In the samples that contained appreciable levels of nitrate, reduced N was estimated by subtracting nitrate-N from total N, because nitrate-N is partially reduced during the digestive phase of the reduced-N assay.

Nitrate N. One hundred mg dry, ground material (leaves, stalks or midribs) was weighed into a bottle to which 25 ml deionized H_2O was added. Bottles were capped and incubated in a shaking water bath for 90 min at 60°C. After filtering, the solution was assayed for NO_3^- (13).

Carbohydrate. A solution of 50 mg dried tissue in 35 ml distilled H_2O was autoclaved (20 min, 1.05 kg/cm^2). Extracts were filtered, and total nonstructural carbohydrate contents were determined (5).

Free Amino-N. Extracts analyzed for carbohydrate were also analyzed for free amino-N (32).

Senescence. Plant senescence was followed visually by the sequential yellowing and death of the leaves. Senescence of the first leaf below the ear leaf was followed quantitatively by measurements of reduced N and Chl content. Grain maturity was judged by black layer formation.

RESULTS

Total Plant. The results obtained were similar to those obtained by Allison and Weinmann (1). The initial visual symptom after ear removal was the development of reddish pigmentation (presumed to be anthocyanins and normally associated with sugar accumulation) in the uppermost leaves. The red coloration moved progressively down the plant and ultimately affected leaves below as well as above the ear. As judged by leaf yellowing, the removal of the ears not only initiated an earlier onset but enhanced the rate of senescence. Leaf yellowing proceeded sequentially and concurrently from the top and bottom leaves to the mid-canopy leaves as previously described (7). Except for an earlier (18 to 20 days) onset and completion of the senescence process, the visible pattern of leaf yellowing and death were similar for eared and earless plants. A preliminary survey showed that ear removal induced the red pigmentation of the leaves and the visual symptoms of senescence of five other maize genotypes. In some instances ear removal as late as 40 DAA also resulted in reddish colored leaves and enhanced senescence.

Plants with ears nearly doubled (1.8-fold) in total above ground dry weight between 3 and 28 DAA and acquired 90% of their final weight (Table I). Plants without ears had a 19% increase in dry weight over this same period and were heavier than when harvested at grain maturity. These data indicate that ear removal

[2] Abbreviations: DAA, days after anthesis; NRA, nitrate reductase activity; CER, carbon dioxide exchange rate.

Table I. *Total Dry Weight and Reduced N Content of Eared and Earless Maize Plants at Intervals during Ear Development*

Total dry weight was determined from above-ground plant parts and ear when present.

Time after Anthesis	Ear	Dry Weight	Reduced N
days		*grams·plant*$^{-1}$	
3	+	236	3.5
28	+	436	5.9
	−	282	3.4
42	+	468	6.3
	−	293	3.8
57	+	484	7.0
	−	272	4.1
LSD (0.05)[a]	treatments	56	0.8
	days	25	0.5

[a] Means of three replicates are compared using LSD at the 5% significance level. LSD treatments compares earless *versus* eared for the same date. LSD days compares differences due to time for a given treatment.

depressed photosynthesis. Thiagarajah *et al.* (27) found that unpollinated maize plants lost photosynthetic activity during the grain filling period more rapidly than pollinated controls. In other crops, seed removal was associated with a depression of CER rates (9, 14).

Plants with ears increased their total reduced N some 1.7-fold between 3 and 28 DAA and had acquired 84% of their final reduced N content (Table I). Similar to the increases in dry weight, reduced N content of eared plants continued to accumulate slowly over the last two sampling periods. Plants without ears showed no change in reduced N content between 3 and 28 DAA. However, their reduced N content was approximately 20% higher by maturity. Based on plant contents of reduced N (Table I), the earless plant reduced 0.6 g NO_3^--N and the eared plant 3.5 g NO_3^--N between 3 and 57 DAA. Thus, removal of the ear depressed nitrate reduction as well as photosynthesis.

Carbohydrates. Ear removal had no immediate effect on total nonstructural carbohydrate content of the laminae of the first leaf below the ear leaf (Fig. 1). The carbohydrate content of the leaf of the earless plant increased (1.8-fold) by 21 DAA. Subsequently, the carbohydrate content decreased, increased again between 31 and 41 DAA, and then declined with the final stages of senescence. Laminae carbohydrate content of the eared plant decreased (25%) below the initial level by 9 DAA and never rose above the initial level until 45 DAA. The leaf carbohydrate content then continued to increase as the grain matured.

Carbohydrate content of all leaves (composite sample including midrib) of the earless plant increased (15%) between 3 and 28 DAA, remained relatively unchanged until 42 DAA, and then declined to 40% of the initial level by maturity (Table II). For the eared plants, leaf carbohydrate content remained relatively constant through the first 42 DAA and then declined to 65% of the initial level by maturity.

Stalk carbohydrate content of earless plants increased (1.7- to 1.8-fold) overall, and all of this increase occurred between 3 and 28 DAA (Table II). The stalk served as a substitute sink (6). This alternate storage site may be the cause for the lag period in accumulation of carbohydrate by the leaf (Fig. 1). Stalk carbohydrate of the eared plants increased slowly with each successive sampling date and exceeded the initial level by 1.3 fold at maturity.

Reduced N and Amino N. Regardless of treatment, leaf blade reduced N remained relatively constant between 3 and 18 DAA, and then declined. The rate of decrease, however, was more rapid for the earless than for the eared plant (Fig. 1). For eared plants, leaf amino N decreased progressively between 3 and 47 DAA (Fig. 1). For the earless plant, leaf amino N content decreased between 3 and 31 DAA and then increased rapidly over the next 10 days before declining. For both treatments, total N and reduced N contents of all leaves (composite sample including midribs) also decreased from anthesis to maturity (Table II). The rate of decrease was greatest during the period of early ear development. Except for the final sampling, the leaves of the earless plant also had lower amounts of reduced N than leaves from the eared plants. Between 3 and 57 DAA, the eared and earless plants lost 1.2 and 1.1 g plant^{-1} reduced N from the leaves. The stalk served as an alternate sink for the remobilization of the leaf nitrogen (Table II).

Ear removal had little effect on the amino N content of all leaves (Table II). The difference in the amino N patterns of the first leaf blade below the ear leaf (Fig. 1) and for all leaves (Table II) is attributed to the varying stages of senescence of the leaves comprising the composite leaf sample.

Stalks of earless plants had a progressive increase in both reduced N [total N − (NO_3^--N)] and amino N throughout the sampling period (Table II). By maturity the reduced N content of the earless stalk had doubled and the amino N content had increased 5-fold. In contrast for plants with ears, the amount of reduced N remained about 30% below the initial level throughout grain development. Amino N content of the eared stalk remained relatively unchanged.

Nitrate and NRA. Ear removal had little effect on the changes in nitrate content of the midrib of the individual leaf (Fig. 1) except that the rate of depletion was more rapid between 9 and 18 DAA for the earless plant. These data show that rate of transport of nitrate from soil and stalk to this leaf was inadequate to prevent depletion of midrib nitrate during the critical phases of grain development. For both treatments, midrib nitrate content increased between 41 and 46 DAA. Whether this increase, especially for the earless plant, reflects continued nitrate uptake or transport from the stalk reserve is not clear. Irrespective of treatment, nitrate content of all leaves (including midribs) decreased rapidly between 3 and 28 DAA and then decreased gradually thereafter (Table II). Stalk nitrate content for both eared and earless plants increased between 3 and 28 DAA (Table II). For the eared plant stalk nitrate content decreased between 28 and 42 DAA and then remained essentially unchanged between 42 and 57 DAA. These changes in storage nitrate throughout the grain filling period infer a shifting balance between the rate of nitrate uptake and the rate of nitrate assimilation by the whole plant. For the earless plant, stalk nitrate remained constant from 28 DAA to maturity, which indicates that ear removal affected in some way nitrate uptake and/or transport to the leaves.

For the earless plants, there was a progressive decrease in leaf blade NRA from 13 DAA to maturity (Fig. 1). For the eared plants, NRA also decreased during grain filling, however peaks of activity were noted at 18, 31 and 46 DAA (Fig. 1). Based on the work of Shaner and Boyer (25) these peaks of activity may be attributed to increases in nitrate flux into the leaf. In each case the peaks of activity occurred subsequent to rainfall and the increases at 18 and 46 DAA are concurrent with increases in midrib nitrate in the same leaf (Fig. 1) and with increases in stalk nitrate (Table II). The lack of increase in leaf NRA of the earless plant in response to increased midrib nitrate at 46 DAA is attributed to leaf dehydration (moisture, Fig. 1) and the advanced state of senescence of the leaf (Chl, Fig. 1).

The average level of leaf NRA of the eared plant was 1.8-fold higher than the average level of leaf NRA of the earless plant. This difference is consistent with the higher amount (1.7-fold) of reduced N of the total plant of eared *versus* earless plants (Table I). The patterns of leaf NRA and reduced N are similar (Fig. 1).

The reason for the more rapid and uninterrupted decline in leaf NRA of the earless plants does not appear to be due to differences in nitrate content of the midrib (Fig. 1) or leaves and stalks (Table II) between earless and eared plants. For reasons cited (25) the

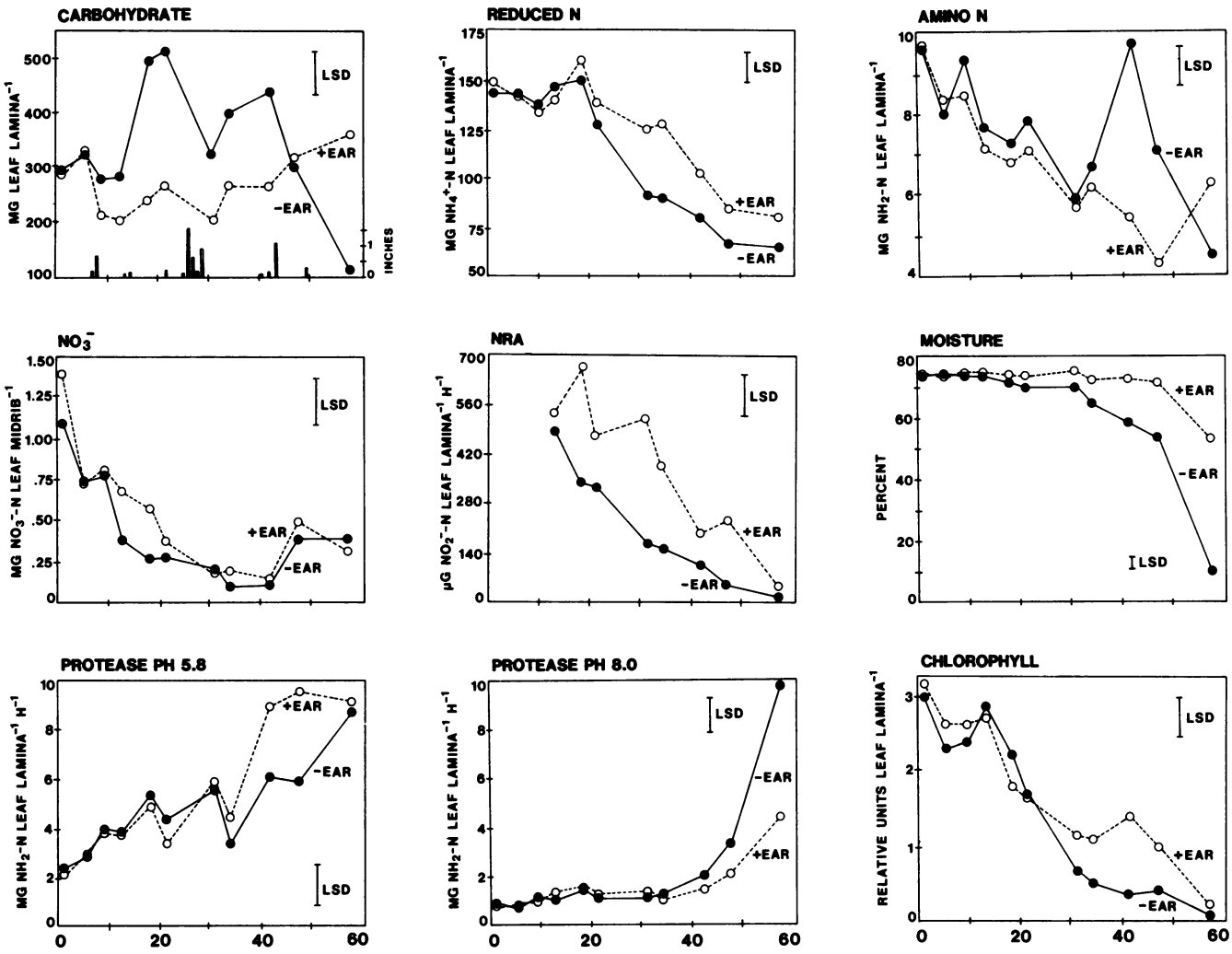

FIG. 1. Changes in several metabolic parameters of the first leaf below the ear leaf of maize plants with and without ears. Ears were removed at 3 days after anthesis and measurements stopped when kernels of eared plants were mature as judged by black layer formation. Means are compared using LSD at the 5% significance level. Initial NRA samples were inadvertently lost. Amount and time of occurrence of rainfall is shown on X axis of carbohydrate plot.

lack of corresponding peaks in leaf NRA of the earless plants indicates that nitrate is not fluxing into the leaf laminae.

Protease Activity. The effects of ear removal on leaf blade proteolytic activities measured at pH 5.8 and 8.0 (casein as substrate) are shown in Figure 1. The pH 5.8 activity increased erratically but progressively from anthesis to maturity for both treatments, although activity was higher in the eared plants at 41 and 46 DAA. The leaf pH 8.0 proteolytic activity remained relatively constant from 3 to 34 DAA for both eared and earless plants. Thereafter the activity of the earless plants increased at a faster rate than for the eared plants. The more rapid rate of increase of the pH 8.0 activity of the earless plant is attributed to the earlier senescence of these plants as judged by leaf moisture and leaf Chl (Fig. 1). The increase in alkaline protease activity with final stages of leaf senescence has been reported (7, 19).

Ear removal did not affect the time of initiation of increased proteolytic activities (Fig. 1). Subsequently the rate of change of these parameters varied with treatment and time. The presence of the ear hastened the increase in pH 5.8 proteolytic activity and slowed the rate of development of the pH 8.0 activity.

While the increase in leaf proteolytic activities and loss of reduced N from the same leaf were concurrent, there was no statistical correlation between these two parameters. The lack of correlation may be more apparent than real. Although there was a gradual increase in pH 5.8 activity between 3 and 18 DAA there was no corresponding loss of reduced N from the leaf. The actual export of reduced N from protein turnover could be increasing if the loss of reduced N were balanced by protein synthesis from newly reduced nitrate. Alternatively, the level of activity present at anthesis was more than adequate for remobilization of leaf protein.

The marked increases in leaf amino N that occurred between 31 and 41 DAA for the earless plant and between 46 and 57 DAA for the eared plant (Fig. 1) were concurrent with increases in pH 8.0 proteolytic activity and high levels of pH 5.8 activity (Fig. 1). Based on the level of NRA and Chl (Fig. 1) of the leaf from the earless plant, we conclude that the marked increase in amino N in the same leaf is due to *in situ* hydrolysis of protein and not from nitrate reduction. For the eared plant the late increase in leaf amino N could have been derived from either protein hydrolysis or nitrate reduction.

The steady depletion of leaf amino N, regardless of the presence

Table II. *Dry Weight, Nonstructural Carbohydrate, Total N, Amino N and Nitrate N of Leaves and Stalks of Maize Plants with and without Ears at Intervals during Ear Development*

Plant Part	Time after Anthesis	Ear	Dry Wt	Carbohydrate	Total N	Amino N	NO_3^--N
	days				*grams·part*$^{-1}$		
Leaves (composite of all leaves)	3	+	67	8.3	2.0	0.11	0.033
	28	+	64	8.0	1.4	0.08	0.010
		−	65	9.6	1.2	0.11	0.011
	42	+	60	7.9	1.2	0.07	0.009
		−	62	9.8	1.0	0.11	0.009
	57	+	54	5.4	0.8	0.08	0.006
		−	51	3.3	0.9	0.09	0.005
	LSD (0.05)[a]	treatments	NS	NS	NS	NS	NS
		days	6	1.0	0.2	NS	0.003
Stalk (including sheaths)	3	+	158	51	1.7	0.17	0.36
	28	+	164	55	1.4	0.20	0.47
		−	216	90	2.7	0.58	0.47
	42	+	166	63	1.2	0.20	0.29
		−	231	91	3.2	0.71	0.45
	57	+	163	65	1.5	0.28	0.36
		−	221	87	3.7	0.90	0.43
	LSD (0.05)	treatments	19	18	0.3	0.09	0.10
		days	20	10	0.3	0.11	0.11

[a] Statistics as in Table I.

of the ear during the first half of the grain filling period, would indicate that the capacity for transport of reduced N from the leaf exceeded the rate of supply from either nitrate reduction or proteolysis. As previously noted, in the absence of an ear the stalk serves as a sink for the reduced N remobilized from the leaf.

DISCUSSION

Senescence. Relative to the eared plant, ear removal caused an earlier initiation and hastened the course of senescence as indicated by leaf yellowing and death and reduced N and Chl content of the selected leaf. Within 10 to 14 days after the removal of ears the uppermost leaves developed a visable red pigmentation which probably was associated with an accumulation of carbohydrates. In this work, red leaf coloration was not used as a criterion of senescence. However, if sugar accumulation in the leaves is associated with enhanced senescence as proposed (1, 30), the red pigmentation could signal the onset of plant senescence.

Other characteristics associated with ear removal were: (a) marked decreases in dry matter and reduced N accumulation by the whole plant (Table I); (b) increase in carbohydrate content, and a more rapid decrease in reduced N content of the leaves (Fig. 1 and Table II); and (c) an increase in both carbohydrate and reduced N content in the stalk (Table II). The ability of the maize stalk to serve as an alternate sink may increase the time required to build up high concentrations of carbohydrates in the leaves and may hasten the loss of reduced N from the leaves.

Possible reasons for the more rapid depletion of nitrogen from the leaf of the earless maize plant (Fig. 1) are: (a) the cessation of nitrate uptake and/or flux to the leaves; and (b) the stalk served as an alternate sink, as the data shows that the remobilization of N from the leaf was not dependent on the presence of an ear (Fig. 1, Table II).

Soybean plants with seeds which senesced earlier than desinked (4, 14) or male sterile plants (31) had limited increases in starch or carbohydrate concentrations but major decreases in reduced N or protein concentrations of the leaves during the final stages of senescence. Earless maize plants that senesced earlier than eared plants had an appreciable increase in carbohydrate content ([1], see also Fig. 1 and Table II) and an extensive depletion of reduced N content of the leaves during the grain filling period (Fig. 1, Table II). With respect to the 'competition for nutrients' theory of senescence, the loss of nitrogen from the leaves would be the common characteristic for early senescence of both species. However, if depletion of leaf nitrogen is to be universally associated with earlier leaf senescence, it has to be assumed that the bagged or barren maize plants described by Moss (15) did not lose appreciable amounts of leaf nitrogen. Unfortunately, he did not measure leaf nitrogen or carbohydrate. Alternatively, it can be assumed that the cultivars used by Moss (15) were efficient in partitioning carbohydrate to the stalk or root and that leaf carbohydrate never reached the critical threshold level proposed by Allison and Weinmann (1).

Setter *et al.* (22–24) reported that within 5 h after removal of pods, soybean plants showed a significant reduction in CER and stomatal conductivity, and a 10-fold increase in free ABA concentration in the leaves. The increase in ABA resulted from decreased transport rather than increased synthesis. They suggest that ABA buildup in the leaf may be responsible for partial stomatal closure. These results indicate that the accumulation of ABA in the leaf or partial stomatal closure do not lead to senescence of soybean plants, because depodding delays senescence and death (4, 14).

Relative to controls, barren (unpollinated) maize plants showed a marked increase in stomatal resistance and a more rapid decrease in CER during the grain filling period (27). Because Moss (15) also noted that ear removal depressed photosynthetic activity, it seems likely that ear removal also affected stomatal aperture. Because increased stomatal resistance was associated with enhanced senescence in maize (27) and delayed senescence in soybean (14, 23) it seems that with intact plants stomatal closure is not universally associated with senescence.

Data of Table I show that plants with ears removed accumulated only 0.6 g of total N plant^{-1} during the grain filling period (3.5 g plant^{-1} for control). Because the earless plants had adequate levels of carbohydrate in the above ground vegetation, the reason for the cessation of N accumulation is not readily apparent. One possible explanation is that ear removal may have caused stomatal closure which in turn reduced the flux of nitrate in the above ground vegetation.

Other experiments also indicate that nitrogen supply affects the

course of senescence. Visual observation of nitrate grown (nodulation inhibited) and urea-grown (nodulated) soybeans indicated that senescence was delayed in the urea-grown plants (private communication, J. E. Harper, United States Department of Agriculture, Science and Education Administration, Agricultural Research, Urbana, Illinois). Application of NH_4NO_3 (equivalent to 80 kg ha^{-1}) to pot grown maize plants just prior to the time when ears were bagged to prevent fertilization also delayed senescence relative to control (eared) plants. Leaves of the slower senescing plants also retained a higher concentration of reduced N than did the control plants during the latter stages of grain filling and maturation (private communication, S. N. Kelly, Agronomy Department, University of Illinois, Urbana, Illinois). Thomas and Stoddart (30) state that foliar application of N will reverse the senescence process in *Nicotiana*. Currently we are attempting to identify corn-belt genotypes of comparable maturity class that show divergent senescence patterns in response to ear removal. Until such genotypes are identified we can only tentatively conclude that the loss of nitrogen from the leaf is a major cause of death of the intact maize plant.

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