

Effect of Ear Removal on CO₂ Exchange and Activities of Ribulose Bisphosphate Carboxylase/Oxygenase and Phosphoenolpyruvate Carboxylase of Maize Hybrids and Inbred Lines¹

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ABSTRACT

The effects of ear removal on gas exchange traits, chlorophyll, and leaf N profiles, and activities of ribulose 1,5-bisphosphate carboxylase/oxygenase and phosphoenolpyruvate carboxylase were examined using four maize hybrids (B73 × Mo17, B73 × LH38, FS854, and CB59G × LH38) and four inbred lines (B73, Mo17, LH38, and CB59G) as experimental material. A diverse genotypic response to ear removal was observed which was generally typified by (a) greatly accelerated loss of chlorophyll, leaf N, enzyme activities, and CO₂ exchange relative to controls for B73, B73 × Mo17, and B73 × LH38, (b) intermediate rate of decline for leaf constituents for FS854, LH38, and Mo17, or (c) loss of leaf constituents at similar or slower rates than for control plants for CB59G and CB59G × LH38. For all genotypes which had accelerated senescence relative to controls, loss of CO₂ exchange activity was correlated with increased internal CO₂ concentrations. Thus, it was concluded that metabolic factors and not stomatal effects were responsible for loss of CO₂ exchange activity. Loss of chlorophyll, leaf N, and enzyme activities correlated well with loss of CO₂ exchange activity only for some of the genotypes. Accelerated leaf senescence in response to ear removal for the inbred line B73 and the hybrids B73 × Mo17 and B73 × LH38, as well as the apparent delayed leaf senescence for the inbred line CB59G and the hybrid CB59G × LH38 show that the contrasting responses to ear removal, rapid *versus* delayed senescence, can be transmitted as dominant traits to F₁ hybrids. The intermediate response by some genotypes, and the dominance of contrasting senescence traits, suggested a relatively complex inheritance for expression of the ear removal response.

Brandner *et al.* (5, 6), ear removal led to a marked decrease in dry weight accumulation by above-ground plant parts, which indicated that net photosynthesis was diminished for all hybrids. Loss of leaf constituents such as N, P, Chl and nitrate reductase activity was slightly greater than controls for one hybrid (FS854) but greatly accelerated for the other two hybrids (B73 × Mo17 and P3382).

The objectives of this study were (a) to determine effect of ear removal on CO₂ exchange and associated carboxylase activities, and internal CO₂ concentrations in order to determine the relative importance of metabolic *versus* stomatal effects in the ear removal response; and (b) to determine how ear removal affects inbred parents of hybrids which differ markedly in response to ear removal.

MATERIALS AND METHODS

Cultural Procedures, Treatments, and Measurements. Four single-cross hybrids (FS854, CB59G × LH38, B73 × LH38, and B73 × Mo17) and four inbred lines (LH38, CB59G, Mo17, and B73) of maize (*Zea mays* L.) were grown in the field in 1985 using standard cultural practices previously described (4). Experimental design was previously described (4), and inbreds and hybrids were analyzed as separate experiments. Two adjacent interior rows of each six-row plot were kept continuously free of ear shoots. For all hybrids, anthesis occurred on approximately July 9 and physiological maturity (black layer formation) occurred on approximately September 13 except for CB59G × LH38, which reached physiological maturity on approximately September 8. Anthesis occurred on approximately July 6 for Mo17 and LH38 and on approximately July 10 for B73 and CB59G. Physiological maturity occurred before September 13 for all inbreds.

Leaves (first or second leaf above the ear) from two plants per plot were measured for gas exchange parameters, as previously described (4), on clear days between 1100 and 1400 h. For other leaf measurements, three leaves (leaf above the ear) per plot were sampled between 1000 and 1200 h, transported to the laboratory on ice, stripped of midribs, and used for Chl, total N, Rubisco,² and PEPCase assays as previously described (4).

RESULTS

Data pertaining to the effect of ear removal on leaf senescence for the hybrids and inbreds used in this study will be presented

Extended leaf area duration (or delayed leaf senescence) has been shown to be positively related to yield in several crop species (7, 8). Present understanding of the complex process of leaf senescence, particularly for intact plants, is not well developed (15). It has recently been demonstrated that removal of ears from maize plants leads to either accelerated or comparable leaf senescence rates relative to control plants with ears (5, 6). This work reconciled contrasting literature reports pertaining to the effects of ear removal or prevention of pollination on maize leaf senescence (1, 3, 11, 14). For all three hybrids used by Crafts-

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² Abbreviations: Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase; PEPCase, phosphoenolpyruvate carboxylase.

as values relative to control plants with ears. Absolute values for the various measurements are reported in a companion paper (4).

For the hybrids, leaves of earless B73 \times Mo17 and B73 \times LH38 had developed red midveins and some red coloration of lamina, as previously described (5), by August 3, whereas earless FS854 and CB59G \times LH38 plants appeared similar to controls at this time. By August 22, earless B73 \times Mo17 and B73 \times LH38 plants had 90 and 65% brown leaves, respectively, whereas earless FS854 plants were beginning to develop red midveins and lamina. Earless CB59G \times LH38 plants looked similar to controls at this date. By September 2, earless B73 \times Mo17 and B73 \times LH38 plants were completely senesced (lost all green color) and earless FS854 plants had red leaves throughout the canopy, but leaves of earless CB59G \times LH38 plants were similar in appearance to controls. All control plants retained green leaves throughout the canopy at this date. By September 13 earless FS854 plants had very few green leaves but earless CB59G \times LH38 still appeared similar to controls. Control B73 \times Mo17 plants were completely senesced at this date but the other three hybrids retained green leaves.

Earless B73 visually appeared the same and senesced on a time course similar to B73 \times Mo17, while CB59G visually responded to ear removal similar to CB59G \times LH38. Earless Mo17 plants were visually affected by August 22, but the effect was different than B73 in that there was not red coloration of midveins and lamina; the lamina simply turned yellow relative to controls. By

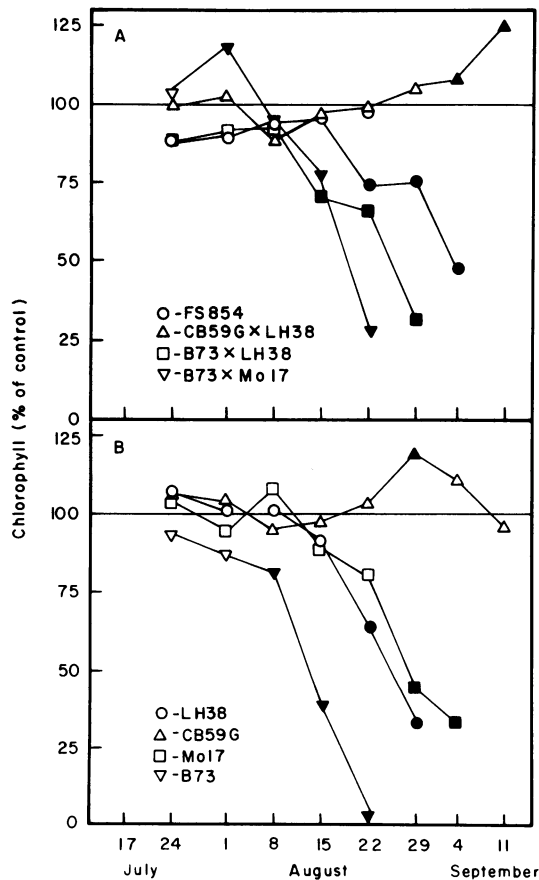


FIG. 1. Effect of ear removal on Chl content of maize hybrids (A) and inbreds (B) during the grain-filling period. Chl was measured on the leaf above the ear. Data represents relative values compared to control plants with ears. Data points which differed significantly from control values are indicated by closed symbols; open symbols indicate that data points were not significantly different from controls.

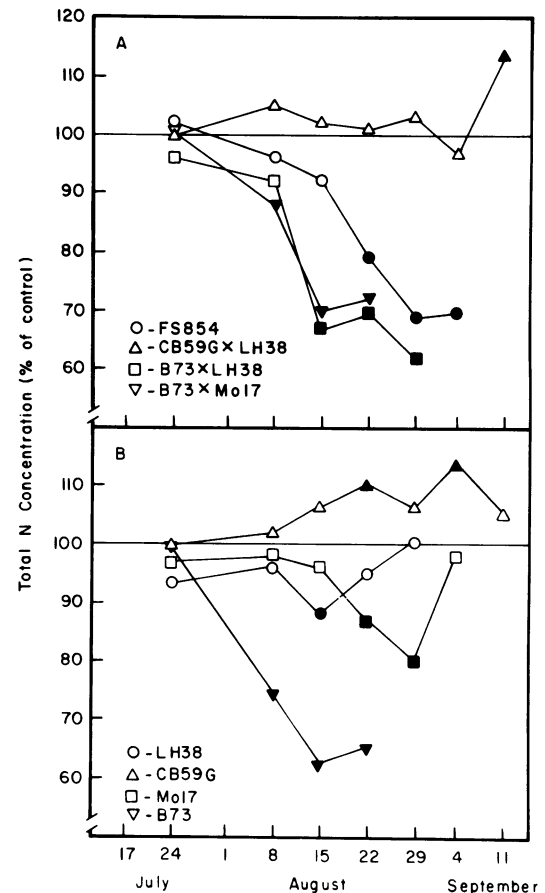


FIG. 2. Effect of ear removal on total N concentration of maize hybrids (A) and inbreds (B) during the grain-filling period. Sample collection and statistical analysis were as described in Figure 1.

September 2 both earless and control Mo17 plants had a mixture of pale yellow and completely brown leaves. Earless LH38 developed red coloration of lamina but not midveins by August 10, and by August 22 approximately 40% of the leaves were brown. After August 29, however, LH38 became infested with corn borers which prevented further evaluation of this inbred.

Visual appearance of the effect of ear removal on leaf senescence was reflected by leaf Chl profiles (Fig. 1, A and B). As previously reported (3, 5, 6), earless B73 \times Mo17 lost Chl at a very fast rate relative to controls, whereas the effect of ear removal on Chl in FS854 was much less pronounced (Fig. 1A). Relative to controls, earless B73 \times LH38 lost leaf Chl at a rate intermediate to FS854 and B73 \times Mo17 but earless CB59G \times LH38 tended to have higher Chl levels than controls over the sampling period. For the inbreds, earless B73 and CB59G had Chl profiles similar to B73 \times Mo17 and CB59G \times LH38, respectively (Fig. 1B). Earless Mo17 and LH38 lost Chl at a greater rate than respective controls, but the effect of ear removal on these inbreds was much less marked than for B73.

Compared to controls, ear removal led to significant genotypic differences for leaf N concentration profiles (Fig. 2, A and B). Relative to controls, leaf N remobilization was greatly accelerated for B73 \times Mo17 and B73 \times LH38, but for CB59G \times LH38 leaf N profiles essentially overlapped for earless and control plants (Fig. 2A). Earless FS854 remobilized leaf N at a greater rate than control plants but the effect of ear removal was much less than for B73 \times Mo17 and B73 \times LH38. For the inbreds (Fig. 2B), leaf N concentration profiles for earless B73 and CB59G were similar to B73 \times Mo17 and CB59G \times LH38, respectively. The effect of ear removal on leaf N concentration profiles of LH38

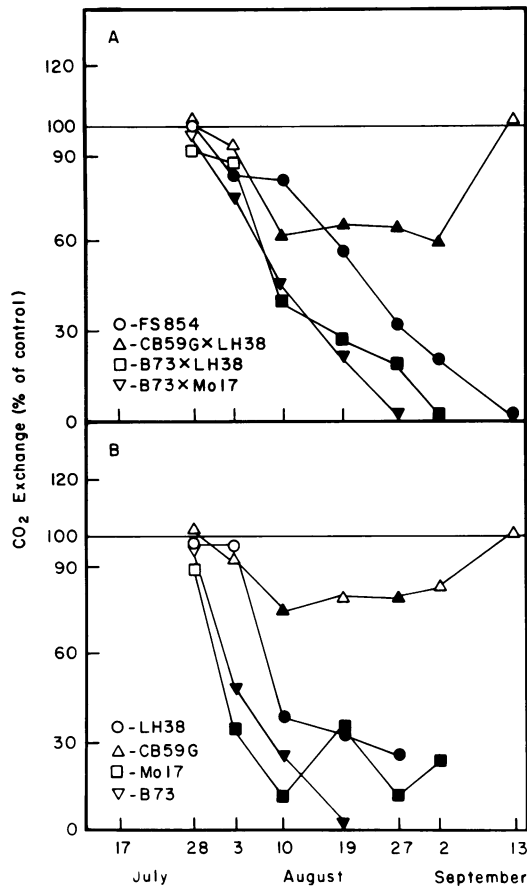


FIG. 3. Effect of ear removal on CO_2 exchange rates of maize hybrids (A) and inbreds (B) during the grain-filling period. Sample collection and statistical analysis were as described in Figure 1.

and Mo17 were intermediate to the effect on B73 and CB59G. Previous work demonstrated that most of the leaf N remobilized from earless plants was found in the stalk (3, 5).

For all genotypes, ear removal led to a decrease in CO_2 exchange rate relative to controls (Fig. 3, A and B). For the hybrids (Fig. 3A) CO_2 exchange rate decline rapidly compared to controls for B73 \times Mo17 and B73 \times LH38. Complete loss of CO_2 exchange capacity occurred approximately 1 week earlier for earless B73 \times Mo17 than earless B73 \times LH38. Earless FS854 completely lost CO_2 exchange capacity much sooner than controls, but the relative effect was not pronounced as for B73 \times Mo17 and B73 \times LH38. By August 10, earless CB59G \times LH38 retained 60% of the CO_2 exchange rate of the control plants, and this relative level of CO_2 exchange was maintained for the rest of the sampling period. The inbreds B73 and CB59G responded to ear removal similar to B73 \times Mo17 and CB59G \times LH38, respectively (Fig. 3B), whereas the effect of ear removal on CO_2 exchange activity of LH38 was intermediate to B73 and CB59G. Earless Mo17, however, initially lost relative CO_2 exchange activity even faster than B73, but after August 10 maintained a low CO_2 exchange rate for the duration of the sampling period.

For all earless genotypes, internal CO_2 concentration remained within 45% above or 30% below control values which ranged from 120 $\mu\text{l/L}$ on July 17 to 195 $\mu\text{l/L}$ on September 2 (Fig. 4, A and B). Internal CO_2 concentrations for earless B73 \times Mo17 was higher than controls by August 3 and remained higher than controls until the leaves senesced (Fig. 4A). Internal CO_2 concentrations for the other three earless hybrids initially dropped slightly below control values before gradually increasing to levels above controls, first for B73 \times LH38 followed by FS854 and

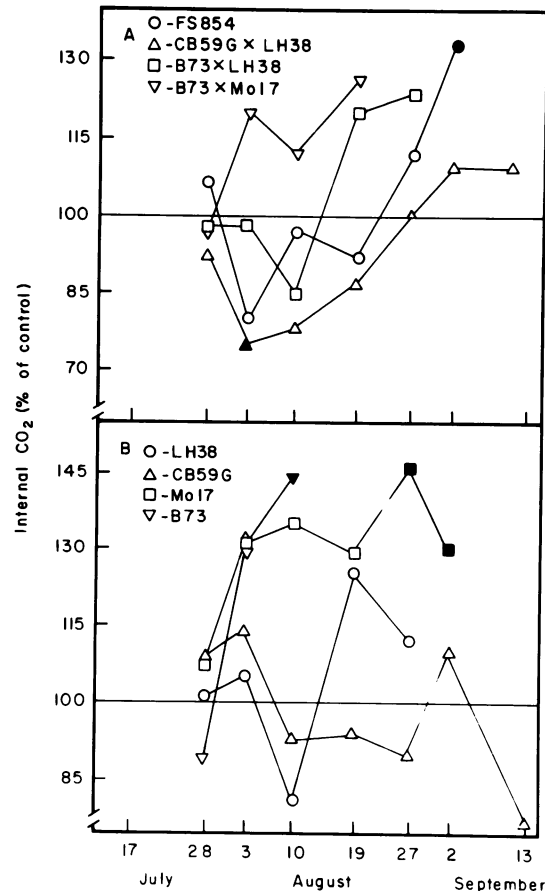


FIG. 4. Effect of ear removal on internal CO_2 concentrations of maize hybrids (A) and inbreds (B) during the grain-filling period. Sample collection and statistical analysis were as described in Figure 1.

finally CB59G \times LH38. The earless inbreds B73 and CB59G had internal CO_2 concentration profiles similar to B73 \times Mo17 and CB59G \times LH38, respectively (Fig. 4B). Earless LH38 had a short-term decline in internal CO_2 concentration before increasing to levels above control plants and earless Mo17 immediately developed and maintained much higher internal CO_2 concentrations than controls.

Genotypic responses for Rubisco and PEPCase activities of earless plants were comparable to relative changes for several of the other physiological parameters measured (Figs. 5 and 6). In general, ear removal tended to affect PEPCase activities to a greater degree than Rubisco activities. Relative to controls, earless B73 \times Mo17 lost Rubisco and PEPCase activities at a greatly accelerated rate whereas earless CB59G \times LH38 developed higher levels of both enzyme activities (Figs. 5A and 6A). Both earless B73 \times LH38 and FS854 lost enzyme activities at greater rates than controls, but the effect of ear removal was greater for B73 \times LH38 than for FS854. For the inbreds, earless B73 and CB59G had Rubisco and PEPCase profiles similar to B73 \times Mo17 and CB59G \times LH38, respectively (Figs. 5B and 6B). Earless Mo17 and LH38 lost relative enzyme activities at an intermediate rate compared to B73 and CB59G.

DISCUSSION

The results of this study extend previous work which demonstrated that maize genotypes differ markedly in response to ear removal (3, 5, 6). Declines in net photosynthesis, which were inferred from previous whole plant dry weight data of Crafts-Brandner *et al.* (5), were verified in this study by CO_2 exchange

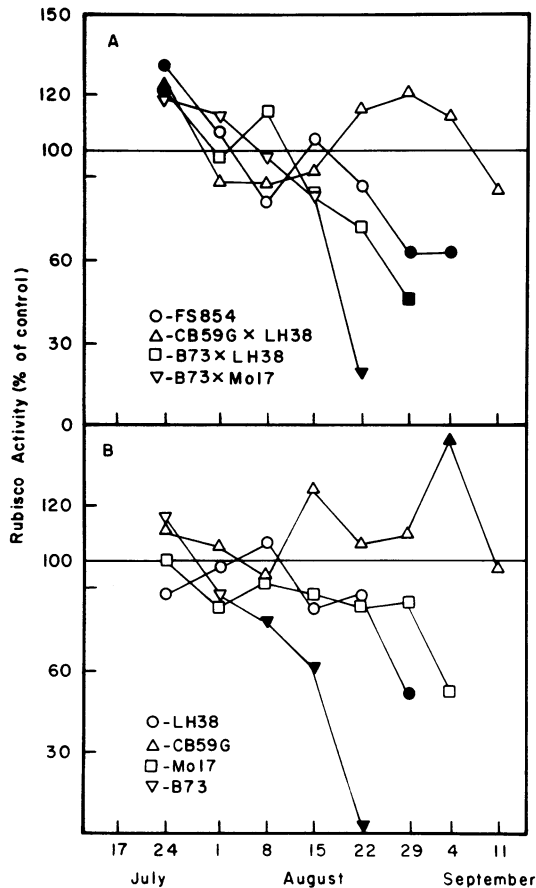


FIG. 5. Effect of ear removal on Rubisco activity of maize hybrids (A) and inbreds (B) during the grain-filling period. Sample collection and statistical analysis were as described in Figure 1.

rate determinations (Fig. 3). Furthermore, the internal CO_2 concentration and Rubisco/PEPCase profiles (Figs. 4–6) indicated that metabolic factors, and not stomatal effects, were responsible for the observed declines in CO_2 exchange rates. The internal CO_2 values, which for controls were consistent with values previously reported for maize (17), increased for earless genotypes that senesced at an accelerated rate relative to controls which indicated that internal CO_2 was apparently not limiting CO_2 exchange for these genotypes.

For most of the genotypes, changes in CO_2 exchange which were induced by ear removal were, in general, followed by comparable changes in Chl, leaf N, Rubisco, and PEPCase activities (Figs. 1, 2, 5, 6). For the inbreds LH38 and Mo17, however, loss of Chl, leaf N, and enzyme activities occurred but not to an extent consistent with loss of CO_2 exchange activity. Thus, no single parameter was outstanding with regard to explaining changes in CO_2 exchange induced by ear removal. It was notable, however, that PEPCase activities appeared to be affected by ear removal to a greater extent than Rubisco activities (Figs. 5 and 6). For example, for B73 \times Mo17, B73 \times LH38, and B73 PEPCase activities declined more rapidly relative to controls than Rubisco activities. In addition, for CB59G \times LH38 and CB59G, PEPCase activities increased to a greater extent, relative to controls, than Rubisco activities. Previous work (4) indicated that PEPCase activity changes were much more closely related to CO_2 fixation changes than were changes in Rubisco activity during leaf senescence of control plants with ears. These findings, in addition to recent work which indicates that PEPCase levels are more sensitive to N nutritional status than Rubisco (13) and that PEPCase activity is light modulated (9, 10) and

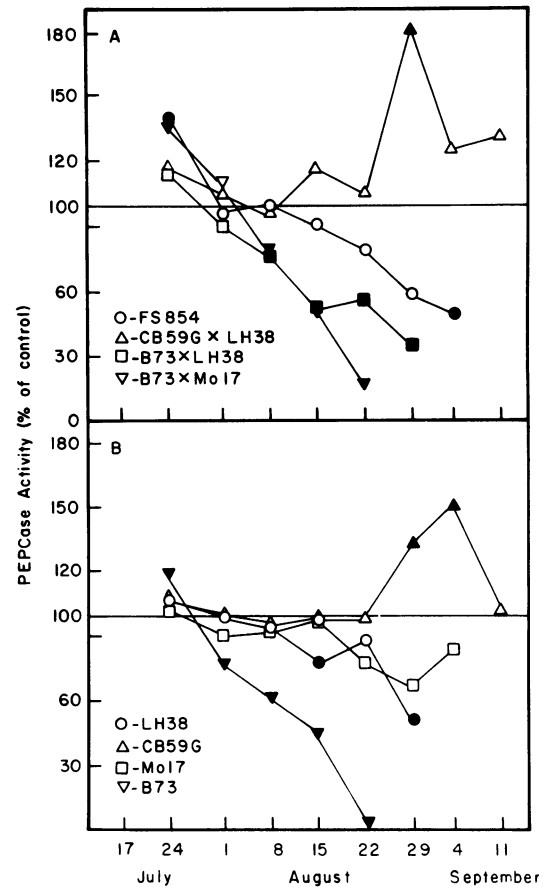


FIG. 6. Effect of ear removal on PEPCase activity of maize hybrids (A) and inbreds (B) during the grain-filling period. Sample collection and statistical analysis were as described in Figure 1.

regulated by levels of cellular metabolites (12) suggest a possible regulatory role for this enzyme in photosynthesis *per se* and as a major target in degradation of the photosynthetic apparatus.

Willman *et al.* (16) visually evaluated the effect of ear removal on 161 hybrids and found that 14% did not undergo accelerated senescence relative to controls. In addition, the authors conducted a genetic study which suggested that relatively few loci were involved in causing the accelerated senescence as observed for B73 \times Mo17. Data for the inbreds and hybrids obtained in the present study support the results of Willman *et al.* (16) in that the rapid senescence characteristic of inbreds is prominently expressed in F₁ hybrids. Our observations, however, suggested that rapid senescence or delayed senescence may be under separate controls that may have more complex inheritance than suggested by Willman *et al.* (16). The inbred CB59G did not undergo accelerated senescence in the absence of ears and when crossed to LH38, the hybrid responded to ear removal the same as CB59G. In fact, senescence may have actually been delayed to some extent by ear removal for these genotypes. On the other hand, B73 senesced rapidly after ear removal as did B73 \times Mo17 and to a slightly lesser extent B73 \times LH38. These results demonstrated that the contrasting characteristics of both inbreds were dominant in their respective hybrids. The present study also demonstrated that the effect of ear removal is not simply an all or none phenomenon. FS854, characterized as a nonsenescent genotype (5, 6, 16), clearly responded to ear removal intermediately to CB59G \times LH38 and B73 \times Mo17. Both Mo17 and LH38 also were negatively affected by ear removal but not nearly to the extent of B73. Since both the rapid senescence of B73 and the delayed senescence of CB59G were dominant in respective

F₁ hybrids, additional genetic analyses are needed to understand the genetic control mechanisms.

It was clear that in the extreme case of the inbreds B73 and CB59G and the hybrids B73 × Mo17 and CB59G × LH38, ear removal led to marked biochemical differences, probably at the level of gene expression, associated with the leaf senescence process. In one case (B73 type), relative to controls, enzyme activities associated with photosynthesis declined, Chl was degraded and leaf N was rapidly remobilized. Since Rubisco, PEP-Case, and PPK account for approximately half of the maize leaf soluble protein (2, 13) it is likely that these proteins to a large extent accounted for the remobilized leaf N (Fig. 2) (6). In the case of earless CB59G type plants, however, enzyme activities actually increased, and Chl and leaf N also tended to increase relative to controls. The paramount question is: Why does one corn plant rapidly senesce in the absence of developing grain whereas another corn plant is not affected or perhaps has a slightly delayed senescence process? The present data are not sufficient to answer this question. We now know, however, that stomatal effects can apparently be discounted as having any influence on the ear removal effect. In addition, we also now know that this markedly divergent response to ear removal can be easily transferred from inbred parents to single cross-hybrids. By using the genotypes B73 × Mo17 and CB59G × LH38, which represent the extremes of the ear removal response and which have markedly different senescence patterns under normal (plants with ears) conditions (4), the rate of leaf senescence can be manipulated both within and between genotypes. This system should provide a useful tool to study biochemical events associated with the initiation and progression of the leaf senescence process using maize plants grown under standard cultural conditions in the field and experiencing senescence as a natural phase of their life cycle.

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