Plant Physiol. (1987) 85, 720–725
0032-0889/87/85/0720/06/$01.00/0

Genotype-Dependent Leaf Senescence in Maize[1]

INHERITANCE AND EFFECTS OF POLLINATION-PREVENTION

Received for publication February 12, 1987 and in revised form July 5, 1987

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ABSTRACT

Objective of research was (a) to evaluate the influence of pollination-prevention on various metabolic parameters of the two maize inbreds B73 and B14A and their F1, and (b) to gain information on the inheritance of leaf senescence, in response to pollination-prevention. The results show that the visual pattern of leaf senescence, in response to prevention of ear pollination, contrasts markedly between the two inbred lines. Relative to control plants, prevention of ear pollination, causes a premature senescence in B73 and B73 × B14A plants, while leaves of unpollinated B14A remain green and similar in appearance to pollinated controls. Furthermore, prevention of ear pollination induces a sizable reduction of dry matter accumulation of all above-ground material and changes in various metabolic parameters. An accumulation of sucrose in the leaves of unpollinated B73 and B73 × B14A plants is correlated with the development of premature senescence. Finally, the genetic analysis supports suggestions that a single dominant gene is responsible for the differences observed, in the visual pattern of leaf senescence, in response to prevention of ear pollination.

Senescence is the deteriorative process that ends the functional life of cells, organs, and organisms. Because the functionality of the leaf apparatus influences directly the extent of dry matter accumulation (9), extensive research has been conducted on leaf senescence of crop plants (for reviews see Thimann [25] and Thomas and Stoddart [28]).

Although causes and mechanisms triggering leaf senescence remain to be clarified, the relationship between reproductive development and senescence has received considerable attention (17, 28). In particular, it has been shown that the removal of the reproductive sink or the prevention of pollination, play an important role in retarding (15, 17) or promoting (14, 18) the senescence of leaves.

In maize there is conflicting evidence on the effects of ear removal or prevention of pollination on the onset of leaf senescence. Moss (20) found that such treatments cause a delay of leaf senescence. Conversely, Allison and Weinmann (2) and Christensen *et al.* (4) reported a premature senescence. More recently, Crafts-Brandner *et al.* (6) found differential expression of leaf senescence in response to ear removal among three maize hybrids. During the grain filling period, the leaves of earless plants of the hybrids P3382 and B73 × Mo17 senesced earlier relative to controls, whereas leaves of earless plants of the hybrid FS 854 remained viable. Furthermore, physiological and biochemical changes, taking place during leaf senescence of maize plants, in response to bagging and ear removal treatments have been described. These studies suggest alteration of several parameters monitoring phytosynthetic activities, Chl content, carbohydrate metabolism, nutrient uptake, and assimilation (2, 4, 6, 7, 20).

The research reported here was designed to (a) evaluate the effects of pollination-prevention on various metabolic parameters, and (b) to gain information on the inheritance of leaf senescence, in response to pollination-prevention.

MATERIALS AND METHODS

Plants. The maize inbreds B14A and B73 used in these experiments, were chosen because of their contrasting differences in senescence patterns in response to ear removal or prevention of pollination: plants of B14A retain green leaves, while leaves from plants of B73 develop a purplish-red pigmentation, followed by a premature death.

Experiment 1. The inbred lines B14A and B73 and their single cross B73 × B14A were grown in an experimental field at Bergamo, during the summer of 1982. The experiment was planted into two-row plots and arranged in a split-plot design in two replicates with genotypes and treatments (pollinated and unpollinated plants) randomly assigned to main plots and subplots, respectively. Individual subplot consisted of a single row, 18 m long and with 76 cm spacing. Subplots were machine planted and thinned to 70 plants per row. Whole plots were 1.20 m apart. The soil fertility was adjusted by adding 280, 120, and 200 kg/ha N, P_2O_5, and K_2O, respectively. Weeds were controlled by herbicides and hand-weeding. Plants were irrigated as needed. All plants of each entry were shoot-bagged at prophyll emergence, including secondary ear shoots if present. At silking, all plants of the pollination treatment, within each entry, were sib-pollinated twice to insure full seed-set. The shoot bags then were removed to be exposed to open pollination. Unpollinated plants remained shoot-bagged throughout the grain-filling period.

Harvests were made at 10-d intervals beginning at silking and continuing through 50 d during the grain filling-period. Harvests dates (sub-subunits) were randomized within the row. At each date, three consecutive well-guarded plants were harvested within each subplot by removing all above-ground material. The samples were taken between 1000 and 1200 h on the harvest days. Each plant was quickly divided into nine parts: stalk internodes below the primary ear; stalk internodes above the primary ear; leaves below the primary ear, including sheaths; leaves above the primary ear including sheaths; tassels; shanks; husks; cobs; and grain (for pollinated plants). After recording the fresh weight, a sample of the various plant parts was dried at 80°C for 36 h to determine the dry weight and the remaining material was frozen in liquid N_2 and lyophilized. The lyophilized samples were ground in a Udy grinder to pass a 20 mesh screen and stored in sealed bottles at 4°C until analyzed.

Total N content was determined by micro-Kjeldhal procedures

[1] Supported by Ministero dell'Agricoltura e delle Foreste, Italy, special grant "Fisiologia produzione delle piante coltivate".

and colorimetric evaluation (11, 23). NPN[2] was evaluated after 3 h precipitation with TCA (15% w/v) of the material extracted from lyophilized tissue with 0.5 M NaCl. The samples were centrifuged at 6000*g* for 20 min and the N content of the supernatants was determined by micro-Kjeldhal analayses as described above. Protein-N was calculated by subtracting NPN from total N. Sucrose content was estimated, after extraction with a methanol-chloroform solution, as described by Van Handel (30). Starch concentration (glucose × 0.9) was analyzed after amyloglucosidase-conversion to glucose and quantified colorimetrically by the method described by Thivend *et al.* (26). Leaf Chl was assayed by the procedure of Feller *et al.* (10).

Data were analyzed by the standard analysis of variance. Fisher's Least Significant Differences were used to compare treatment means within genotypes.

Experiment 2. Plant materials to study the inheritance of the visual pattern of leaf senescence between the line B14A and B73, in response to prevention of ear pollination, consisted of six populations. These included the two inbred lines B14A and B73, their F1 (B73 × B14A), F2 (B73 × B14A)⊗ and two backcross generations ([B73 × B14A] × B73; [B73 × B14A] × B14A). All entries were field-grown at Bergamo during 1982 and 1983. Experimental procedures, treatments (pollinated and unpollinated plants), and cultural practices were similar to those used in experiment 1.

Date of initial emergence of silks was recorded for every plant. Leaf senescence was observed periodically from silking to BLM, which was taken as an indication of physiological maturity (8). BLM stage was ascertained by examining the appearance of the black layer at the bases of 10 kernels removed at 3 d intervals from the central part of the ear of five randomly chosen plants for each genotype and replication of the pollination treatment. At BLM, pollinated and unpollinated plants of each generation were classified, based upon visual observations and comparison with the parental lines, in two classes. When most of the canopy, with the exception of leaves positioned at the lowest nodes, was green and viable in appearance, senescence was excluded. Plants were classified as senescing following the development of a red-purplish pigmentation and the appearance of dry tissue even on younger leaves.

The ratios between green and senescent leaf plants in the various populations were used to develop gene models; goodness-of-fit to these models was tested by chi-square analysis.

RESULTS

Experiment 1. The visual pattern of leaf senescence in response to prevention of ear pollination, contrasted markedly between B14A and B73. Starting 20 d after silking, the uppermost leaves of unpollinated B73 plants developed a visible purplish-red pigmentation of leaf midribs initially and leaf blades ultimately. The symptoms progressed down the plant affecting leaves below as well as above the main ear node and, by 30 d after silking, all leaves and stalk internodes showed a nearly complete red pigmentation. By 45 d after silking, all leaves of unpollinated B73 plants were almost brown. At the same stage of development pollinated plants of B73 were green and viable in appearance. They developed clear leaf symptoms of senescence only approaching the BLM stage, approximately 55 d after silking. It was also observed that for B73 the senescence pattern progressed in basipetal sequence for unpollinated plants, and in acropetal succession for the pollinated plants. The visual pattern of leaf senescence for pollinated and unpollinated B14A plants was indistinguishable. Throughout grain-filling, leaves of unpollinated B14A plants remained green and similar in appearance to pollinated control plants. The pattern of leaf senescence of unpollinated and pollinated F1 plants was found to be virtually identical, respectively, to unpollinated and pollinated B73 plants. However, the development of red color on the leaves of unpollinated plants was clearly detectable 6 to 7 d later than in B73.

Data in Figure 1 document that unpollinated plants had a reduced total dry weight accumulation compared to the controls. It also shows that the differences between the two treatments for each genotype were more pronounced as maturity progressed. The amount of dry matter accumulation in different plant parts (data not shown) were of a similar order of magnitude within each genotype and no distinct treatment effect was observed throughout the sampling dates, with the exception of cobs. For all genotypes, beginning at the second sampling date, dry weights of cobs were significantly higher in pollinated plants in comparison to unpollinated plants.

The differences between the percentage of dry matter as a percentage of fresh weight in different plant parts of pollinated and unpollinated plants are reported in Figure 2. Prevention of pollination induced a higher loss of water from the plant. This was more evident in leaves and stalks, particularly at later maturity stages. In pollinated plants, a higher dry matter percentage was observed in the cobs. No distinct treatment effects were detactable in husks and shanks.

Sucrose fluctuation at various stages of maturity is illustrated in Figure 3. The profiles of sucrose concentration varied for the three genotypes. At all sampling dates for most of the different plant parts no significant changes were found in the level of sucrose in unpollinated plants compared to the controls. Relevant exceptions were sucrose concentrations in the upper and lower leaves of the early senescing unpollinated plants of B73 and B73 × B14A. In fact, it was clearly evident that the level of sucrose in the leaves of unpollinated plants of these two genotypes showed the tendency to rise immediately after prevention of ear pollination, with a sharp rate in the upper and lower leaves of B73, and in lower leaves of B73 × B14A. This tendency was less pronounced for the upper leaves of the hybrid genotype. During grain filling in both genotypes, sucrose content declined to levels more similar to those of pollinated treatments.

The patterns of starch fluctuations in the leaves and stalks are illustrated in Figure 4. The concentration of starch in the stalks increased in all pollinated genotypes up to 20 d after silking. Then it began to decline approaching BLM. The decline was more rapid in B73 than in the other genotypes. For all genotypes, levels of starch in the leaves were more constant than in the stalks throughout the sampling period. For B14A and B73 × B14A plants, prevention of pollination resulted in an increased starch concentration in leaves and stalks, particularly the upper ones. Moreover, unpollinated B14A plants showed a tendency to accumulate starch throughout the sampling period in the stalks. Only for B73, levels of starch in leaves and stalks of

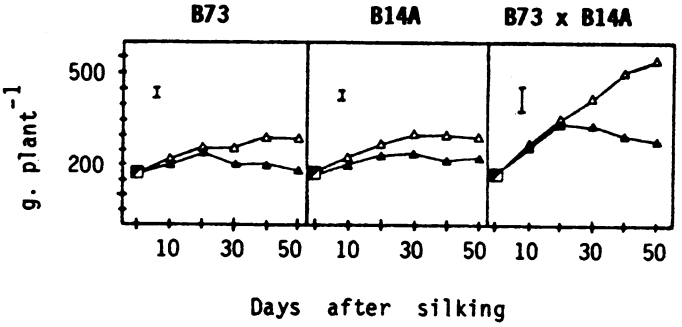

FIG. 1. Effect of prevention of ear pollination on total (above ground) dry weight accumulation, during grain-filling period, of three maize genotypes. (△), Pollinated and (▲), unpollinated plants. In this and following figures vertical bars correspond to LSD values (P = 0.05).

[2] Abbreviations: NPN, nonprotein-N; BLM, black layer maturity.

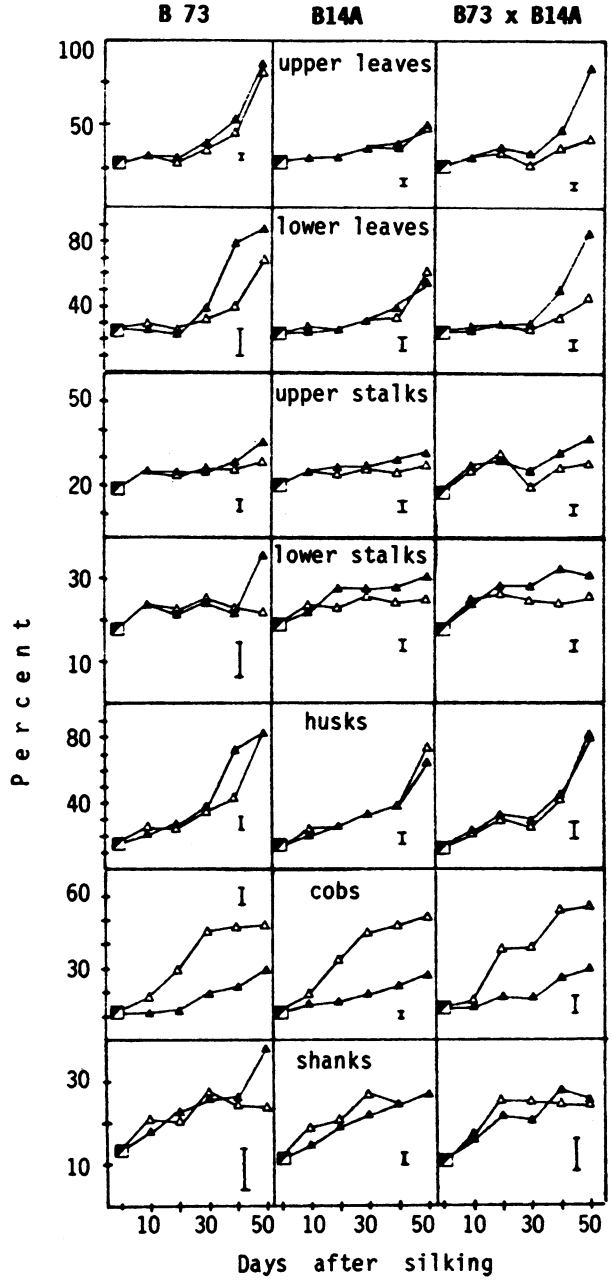

FIG. 2. Effect of prevention of ear pollination on dry matter as a percentage of fresh weight in different plant parts, during grain-filling period, of three maize genotypes. (△), Pollinated and (▲), unpollinated plants.

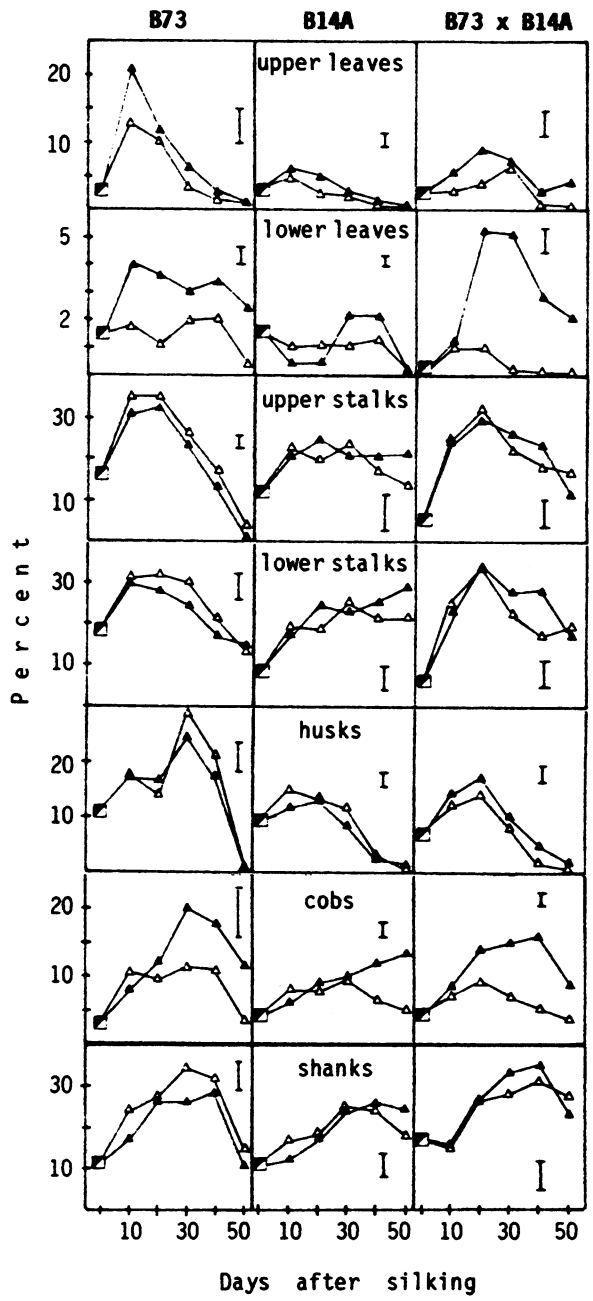

FIG. 3. Effect of prevention of ear pollination on sucrose concentration (percent dry weight) in different plant parts, during grain-filling period, of three maize genotypes. (△), Pollinated and (▲), unpollinated plants.

unpollinated plants were not higher then in the control, though these values did change over time.

Profiles of total N concentration are presented in Figure 5. This parameter as measured in upper and lower leaves and stalks of B73 and B73 × B14A plants remained fairly constant in pollinated plants, while in unpollinated plants it tended to decrease in the leaves and to increase in the stalks. These differences became more pronounced at the latest stages of the sampling period, in particular for stalks. Total N concentrations in the husks, cobs, and shanks tended to decrease in pollinated plants of all genotypes and were, in general, more constant in unpollinated plants, although the trends of total N concentration in the husks and cobs were different than those observed for shanks.

For B73 and B73 × B14A plants prevention of pollination induced an accumulation of nonprotein N in the stalks starting at 20 d after silking (Fig. 6). The tendency was also to some extent evident in unpollinated B14A plants. Patterns of protein-N concentration varied for the three genotypes, with highest levels in the unpollinated plants starting at the third sampling stage. On the other hand the pattern of these metabolic traits in B14A stalks appears to be different than those of B73 and B73 × B14A.

The Chl concentration in the upper and lower leaves declined significantly over the sampling period for all genotypes and treatments (Fig. 7). Prevention of pollination resulted in an accelerated rate of Chl loss in the leaves of B73 and B73 × B14A plants, compared to the control plants. In particular, at 30 and

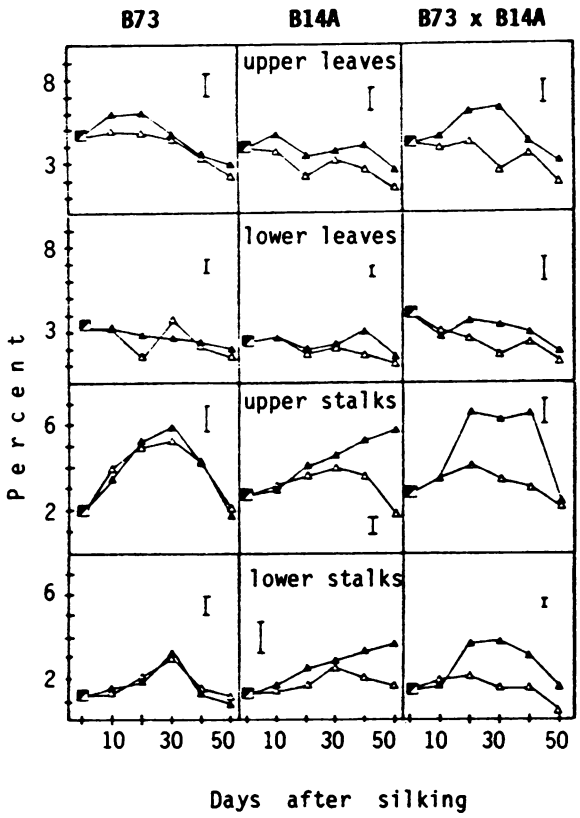

FIG. 4. Effect of prevention of ear pollination on starch concentration (percent dry weight) in leaves and stalks, during grain-filling period, of three maize genotypes. (△), Pollinated and (▲), unpollinated plants.

40 d after silking the upper leaves of these unpollinated genotypes were practically devoid of Chl pigments, while pollinated plants of the same genotypes had a leaf Chl concentration higher than 160 μg/g dry weight of the tissue. The Chl concentration in upper and lower leaves of B14A plants was only moderately affected by prevention of pollination. Chl content of the upper and lower leaves of B14A declined at approximately the same time and at the same rate as it did in the control plants and was, in general, not significantly different at any sampling time.

Experiment 2. The inheritance of leaf senescence, based on visual evaluation, in response to pollination prevention of the ear was studied using unpollinated plants. No differences among replications and years for the nonsegregating populations (parents and F1) were found in this experiment. Therefore, the data obtained were pooled. The results are summarized in Table I.

All unpollinated B73 plants were classified as having senescent leaves, while leaves of B14A plants exhibited green color at the same stage of development. All F1 plants showed a premature leaf senescence similar to B73 plants, indicating that the early senescence trait was dominant to normal senescent type. The F2 data suggest that plants with senescent leaves and green leaves occurred in a 3:1 ratio. Apparently in B73, a single dominant gene governs early leaf senescence, in response to prevention of pollination of the ear.

Data for the backcross to B73 confirm the presence of a single dominant gene for early senescence in unpollinated plants of B73. In the backcross with the parent B14A the number of plants showing early senescent leaves:green leaves were in a 1:1 ratio. The backcross with B73 gave only plants characterized by an early leaf senescence pattern.

DISCUSSION

A growing body of evidence suggests that in plant leaf senescence is typically characterized by an increase in proteolytic

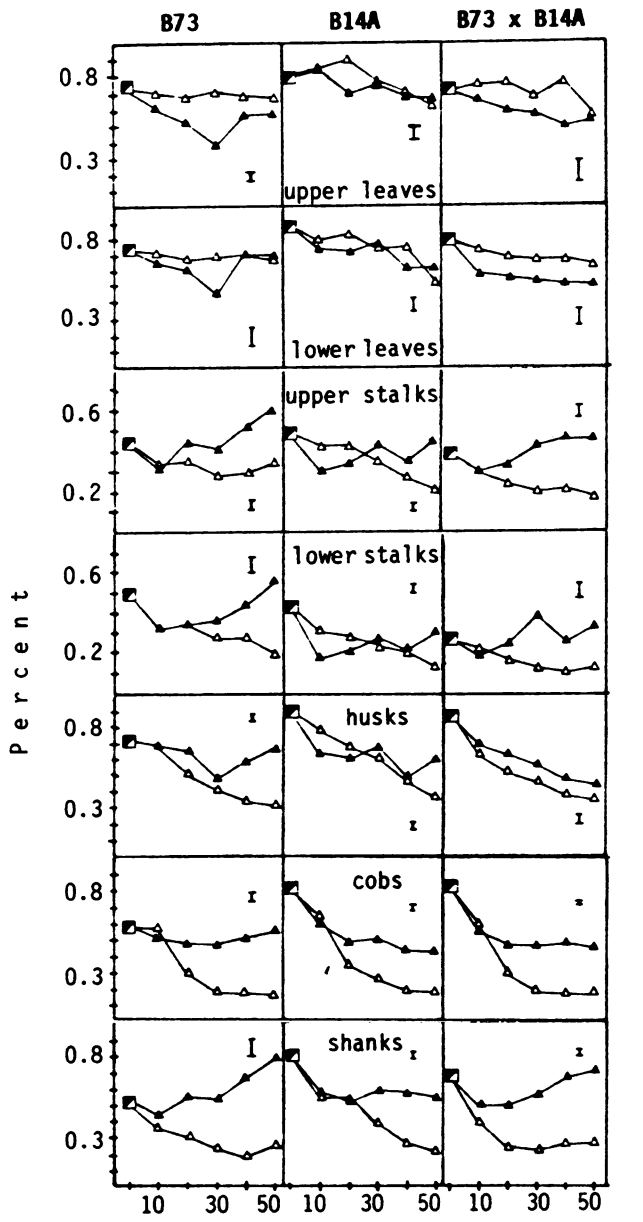

FIG. 5. Effect of prevention of ear pollination on total N concentration (percent dry weight) in different plant parts, during grain-filling period, of three maize genotypes. (△), Pollinated and (▲), unpollinated plants.

activities and by a decline in the levels of Chl, RNA, proteins and nitrogen content, which, in turn, are closely correlated with decline in photosynthetic activity (25, 28). It has also been shown that after the elimination of the reproductive sink, the leaves lose the ability to photosynthesize, while they retain a high level of Chl and protein indicating a separation of functional senescence from death of the leaves (19, 27). From this point of view, genetic variation has been reported to exist both among and within crop species (6, 17, 19, 27).

The results presented in this paper report the finding of a differential expression of leaf senescence in maize, in response to prevention of pollination, as judged by loss of green color from the leaf. In fact, relative to control plants, prevention of pollination of B73 and B73 × B14A plants caused the development of a red pigmentation in leaves and stalks and premature

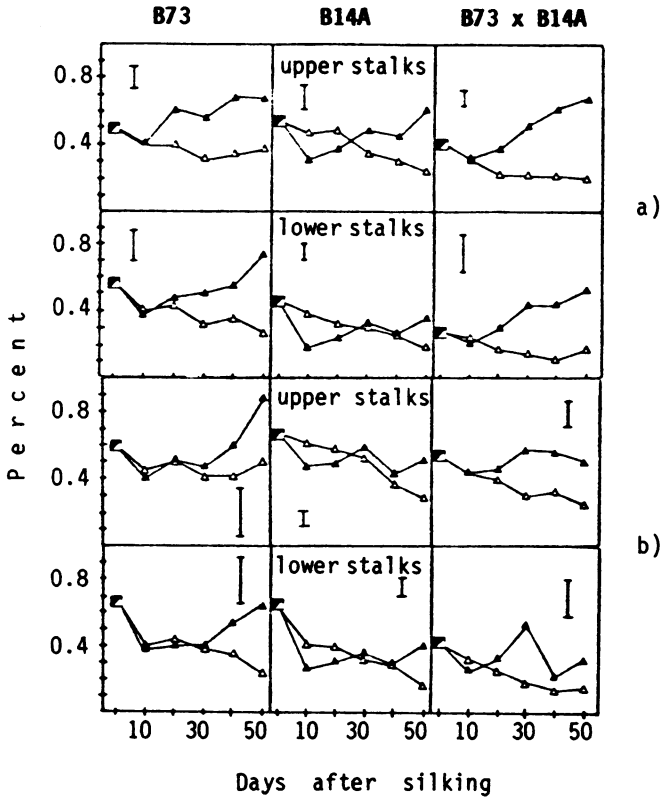

FIG. 6. Effect of prevention of ear pollination on nonprotein-N (a) and protein-N (b) concentrations (percent dry weight) in stalks, during grain-filling period, of three maize genotypes. (△), Pollinated and (▲), unpollinated plants.

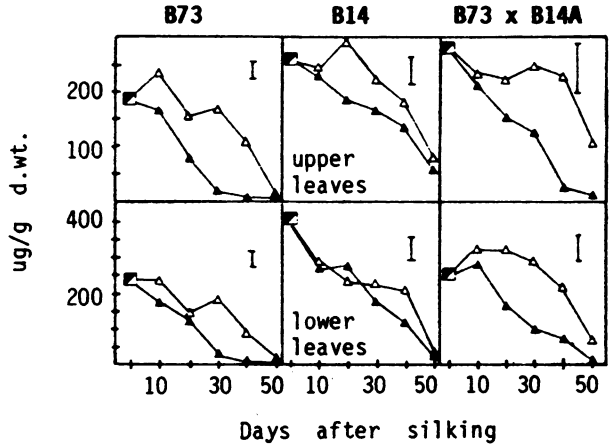

FIG. 7. Effect of prevention of ear pollination on Chl concentration (μg/g dry weight) in leaves, during grain-filling period, of three maize genotypes. (△), Pollinated and (▲), unpollinated plants.

senescence and death of the leaves. Conversely, leaves of unpollinated B14A plants remain green and retain a relatively higher level of Chl manifesting the normal senescence pattern showed by the controls. These results are in agreement with previous observations made by other workers in maize (2, 4, 6, 7, 20) and with investigations revealing that differences in the visual pattern of leaf senescence, in response to ear removal, are genetically dependent (6). In addition, the genetic analysis herein reported support suggestions that a single dominant gene, or a gene complex, is responsible for the differences observed in the visual pattern of leaf senescence, in response to prevention of ear pollination. The existence of major genes controlling the expression of leaf senescence has been already reported for several crop species. Abu-Shakra *et al.* (1) found that delayed senescent lines of soybeans depend on the concomitant presence of the recessive alleles *dt1* and *e1* although in different genetic situations *dt1* and *e1* must be reinforced by the alleles *dt2* and *e2* (24). Thomas and Stoddart (27) found that a genotype of *Festuca pratensis* L., that does not exibit leaf yellowing is due to a single recessive gene mutation. In maize, Gentinetta *et al.* (13) reported that a major dominant gene determined the stay-green habit of the inbred line Lo876o2.

Table I. *Segregation Ratios of Plants with Leaf Symptoms of Senescence and with Green Leaves, in Response to Ear Prevention Pollination, in the Experimental Population Involving B73 and B14A Lines*

Population	Classification No. of plants with senescent leaves:green leaves	Ratio	χ^2	P
Parent				
B73	197:0	1:0		
B14A	0:219	0:1		
F1 cross:				
B73 × B14A	198:0	1:0		
F2 generation:				
(B73 × B14A ⊗)	134:43	3:1	0.05	0.90–0.75
Backcrosses				
(B73 × B14A) × B73	167:0	1:0		
(B73 × B14A) × B14A	90:81	1:1	0.47	0.50–0.25

A series of observations reported in this investigation concerns the changes of several metabolic parameters, following prevention of ear pollination. The available literature in maize indicates that ear removal or prevention of ear pollination is characterized by a decline of photosynthetic activity and nitrate and phosphate uptake and N accumulation (4, 6, 20), a loss of reduced N and Chl (7) as well as by an increased starch and sugar content in leaves and stalks (2, 4, 6, 7). In agreement with these findings the data presented confirms that prevention of pollination leads to a reduction of dry matter accumulation of all above-ground materials as already known from studies of several crop species (16, 17, 19, 20, 22). Apparently, the accumulation of carbohydrates in photosynthesizing tissues may negatively affect photosynthetic activity (5, 19, 21, 29) suggesting a control of photosynthesis by level of soluble sugars (12, 22).

In the experiment described here, sucrose accumulates in leaves of B73 and B73 × B14A plants, but not in unpollinated B14A plants. This excess of soluble carbohydrates in the leaves may be related to the development of red pigmentation and premature senescence of the leaf apparatus for unpollinated B73 and B73 × B14A plants (see also similar results by Allison and Weinmann [2]). Unpublished data of the authors indicating that reduction of light intensity retard significantly the appearance of visible symptoms of leaf senescence in unpollinated plants of B73 support this interpretation.

In conclusion, while the data presented may add circumstantial evidence in favor of a product inhibition mechanism by soluble carbohydrates on leaf photosynthesis, it is attractive to postulate that an abnormal level of sucrose in the leaves of unpollinated B73 and B73 × B14A plants is related to an accelerated senescence process. This is particularly so considering that a single gene seems to trigger in B73 and B73 × B14A the senescence reactions. This mutant approach may contribute to assess the physiological role of those phythormons which changes their concentrations according to aging (3, 17, 28).

Acknowledgments—We express our thanks to Drs. F. Salamini and W. D. Park for their critical reading of the manuscript.

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