Effect of Head Removal on Leaf Senescence of Sunflower

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ABSTRACT

Greenhouse and field studies examined the effect of flower or seedhead removal on leaf senescence and associated changes in sunflower (Helianthus annuus L.) plants. At intervals during seed development, selected leaves (leaves 6 through 8 from the top in the greenhouse and leaf 7 from the top in the field) were harvested and analyzed for chlorophyll, specific leaf weight, N, P, soluble protein, and electrophoretic gel profiles of soluble polypeptides. In both the greenhouse and the field, the leaves of headless plants retained or accumulated more N, P, soluble protein, and dry weight than leaves of plants with heads. Obviously, head removal affected the partitioning of these metabolites during seed development. None of the treatments resulted in the formation of new polypeptides (electrophoretic gel profiles). Comparisons of the rates and extent of loss of chlorophyll, soluble protein, and polypeptide bands (especially ribulose 1,5-bisphosphate carboxylase) from the leaves of headed and deheaded plants showed that head removal delayed the rate of development of leaf senescence for the greenhouse-grown but had much less effect on field-grown plants. These findings illustrate the variability in different parameters commonly associated with the leaf senescence processes of headed and deheaded sunflower plants grown under different environments.

Senescence has been described as the natural deteriorative process leading to death of an organ or organism (12). This process is complex, controversial, and not well understood, especially with respect to the causal factor(s) (26). Additional information concerning the cause, course, and control of senescence is of agronomic importance because of the high positive correlation between leaf area duration and grain yield (9).

There are no comprehensive theories of senescence (13). Most of the published work describes changes that are the reflections of symptoms rather than the cause of senescence. Of the several "theories" of the cause of senescence, two have been most frequently cited. The first, "nutrient drain" or "self-destruction," concerns the translocation or redirection of nutrients from the vegetation to the fruit or seed (15, 22, 23). The second concerns hormones or hormonal balance as a potential trigger of senescence, as described by Leopold and Kriedemann (13) and further promoted by Nooden and co-workers (14, 18, 19). These workers propose that a senescence-inducing compound is synthesized in the seed of soybean plants transported via the xylem to the leaf.

Wittenbach (28, 29) found that leaves of depodded soybean plants (cv Wye) accumulated more dry weight, and retained Chl and protein longer than leaves of podded plants. However, photosynthetic and RuBPCase1 activities (functional activities) declined more rapidly in depodded plants. Because he found that depodding resulted in de novo synthesis of four polypeptides (30), he concluded that depodding altered leaf function (became a sink) rather than delaying or preventing senescence. Crafts-Brandner et al. (7, 8) reported similar results from depodding soybean plants (cv Harosoy), except that RuBPCase was only initially lower in the leaves of depodded plants. By maturity, the depodded plants had accumulated as much dry weight (net photosynthesis) and N in the above ground parts as the podded plants. Apparently the presence of pods only altered the partitioning of plant constituents (7). Based on the seasonal profiles of Chl, and activities of the functional enzymes nitrate reductase, nitrogenase and RuBPCase, the initiation of leaf senescence was similar for podded and depodded plants (7, 8).

Although Moss (17) reported that the prevention of pollination (ear bagging or removal) delayed senescence in maize, other workers (1, 3, 25) found such treatments accelerated leaf and plant senescence. Subsequent work (5, 6, 27) resolved this conflict by showing the response was a reflection of genotype. A detailed comparison of such divergent genotypes (5, 6) permitted the conclusion that the ear per se does not fully dictate the initiation and course of senescence. It appears that metabolic interactions of the whole plant are involved. Although other workers have noted that carbohydrates accumulated in the leaves in response to ear removal, the changes in carbohydrates and nitrogenous compounds were judged to be symptoms (5, 6) rather than the cause of senescence (1, 3).

Seed or fruit removal has been reported to accelerate leaf senescence for pepper (10) and barley (16) and to delay senescence for sunflower (20). These and other divergencies in senescence response to elimination of seed or fruit development emphasize the complexity of the senescence response. Thomas and Stoddart (26) stated that senescence is a genetically controlled and programmed process that can be modified by external factors. Although it can be inferred that hormones or hormonal balance could initiate the senescence process, other initiation processes are possible (26).

This work was undertaken to expand the limited amount of information on the effect of flower or seedhead removal on the senescence patterns and associated changes in metabolites and metabolism of sunflower plants. Auxiliary objectives were to: (a) determine the effects of different environments, and (b) determine if elimination of seed development would be associated with concurrent de novo synthesis of different polypeptides in the leaves.

MATERIALS AND METHODS

Cultural Procedures. Greenhouse. Seeds of sunflower (Helianthus annuus L. hybrid SunGro 372A) were overplanted in 20 L plastic pots on March 5, 1984 and thinned to three plants per pot following germination. The growing media consisted of: (by volume) three parts soil (unsterilized Drummer silty clay loam), one part peat moss, one-half part vermiculite, one-half part sand, and fertilizer (equivalent to 10 g of 0-46-0 phosphate and 5 g of KNO3 per pot). From 15 DAP, pots were watered every 6 d with

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1 Abbreviations: RuBPCase, ribulose 1,5-bisphosphate carboxylase; DAP, days after planting.
500 ml of a nutrient solution of the following composition: 4 mM Ca(NO3)2, 4 mM MgSO4, 2 mM KH2PO4, 2 mM K2SO4, 1 mM NH4NO3, 1 mM CaCl2, 0.70 mM Fe as Chel-330 (0.44 mM), and Chel-138 (0.26 mM) (Ciba-Geigy Corp., Greensboro, NC). 0.14 mM H3BO3, 0.03 mM MnSO4, 9 mM ZnSO4, 1 mM CuSO4, and 0.3 μM Na2MoO4. Pots were watered with tap water as needed between the 6 intervals. Until 38 DAP, natural lighting was supplemented with metal halide lamps (approximately 300 μmol m⁻² s⁻¹ between 400 and 700 nm wavelength) to provide 14 h of illumination. After 38 DAP, all lighting was from natural illumination. Plants were sprayed with Pyrethrin and Diazinon, as needed, for control of whiteflies and aphids.

Treatments consisted of removal of the flower head at the R2 (preflowering) and the R5.8 (postpollination) stages (21). The flower head was left to develop on control plants. For the control and postpollination treatments, hand-cross-pollinations were made to maximize seed set. Each pot contained plants representative of all three treatments. Pots were arranged in a randomized complete block design with three replications.

Field - soil. Plants were planted at the Agronomy-Plant Pathology South Farm, Urbana, IL on June 1, 1984. Plants were thinned to a stand of 44,400 plants ha⁻¹. Soil type was a Flannigan silt loam with high levels of P and K. A spring application of 133 kg N ha⁻¹ was applied to all plots. For weed control, soil was treated preplant with Treflan.

Treatments were arranged in a randomized complete block with three replications. Treatments included plants from which the flower heads were removed at the R5.2 growth stage and control plants with flower heads left intact. An experimental unit consisted of two 6.1 m rows, spaced 0.76 m apart.

Sampling. Leaves were sampled nine times (12, 17, 25, 30, 34, 39, 44, 47, and 53 DAF) for greenhouse grown plants and seven times (8, 17, 25, 30, 37, 44, and 58 DAF) for field grown plants. For each sampling, three leaves (leaf positions 6th–8th from the top) were excised from individual plants in the greenhouse and one leaf (7th from the top) from three individual plants in the field. Samples were taken between 0800 and 0950 h. For each sample, the composited leaves were stacked and folded and disks (0.8 cm diameter) were cut from the midportion of the lamina with a cork borer. Fresh leaf disks were used to extract protein for SDS-PAGE, and to determine Chl concentration and specific leaf weight. The remaining leaf material and 18 leaf disks used for specific leaf weight determination were dried separately to constant weight at 70°C in a forced-draft oven and reweighed. Weight of disks was added back to obtain total leaf weight.

Gel Electrophoresis and Assays. For gel electrophoresis, a modification of the procedure used by Wittenback (28, 29) was used. Leaf disks (36 from each sample) were homogenized for 25 s in 2 ml of 25 mM Tris-HCl buffer containing 1 mM EDTA, 1 mM 2-mercaptoethanol, 1% (w/v, greenhouse), or 2% (w/v, field) Na-ascorbate and 1% (w/v) polyvinylpolypyrrolidone with a Polytron homogenizer. After centrifugation of leaf extracts, aliquots from the supernatant fractions were assayed for protein by the Bio-Rad method (2). Based on the protein determinations, aliquots containing 900 µg soluble protein from the original extract were mixed with 25 mM Tris buffer (pH 7.5) containing 1 mM EDTA, 1% (w/v) SDS, 5% (v/v) glycerol, and 5% (v/v) 2-mercaptoethanol to a final volume of 1 ml. Samples were immersed for 4 min in boiling water bath to dissociate proteins. Aliquots (containing 90 µg soluble protein) of the SDS dissociated extracts were applied to each well. Because the objective of the electrophoretic work was to compare the polypeptide profiles of deheaded with headed plants, equal amounts of extractable soluble protein were applied to each well. The polypeptides were separated on a 9 to 14% polyacrylamide gradient slab gel overlaid with a 6% polyacrylamide stacking gel, using the buffer system described by Chua (4). Electrophoresis was carried out at a constant current of 25 mamp per gel until the bromophenol blue marker in the mol wt standards reached the bottom of the gel (about 4.5 h). A constant temperature of 8°C was maintained during electrophoresis with a circulating water bath. Gels were stained overnight in a solution containing 0.25% (w/v) Coomassie blue, 50% (v/v) methanol, and 7% (v/v) glacial acetic acid and destained in 40% (v/v) methanol and 7% (v/v) glacial acetic acid. Destained gels were stored with distilled H2O in a sealed plastic bag prior to drying and photographing.

Preliminary investigations had shown that satisfactorily electrophoretic protein profiles could not be obtained for sunflower leaves without addition of Na-ascorbate to the homogenization medium (data not shown). Without addition of Na-ascorbate much of the soluble protein (especially the large subunit of RubBPCase) would not enter the polyacrylamide gel. Field-grown leaves required a higher amount of Na-ascorbate than greenhouse-grown leaves. Other tests showed that the free acid form of ascorbate was not effective.

For Chl determination, 18 leaf disks were placed in a glass bottle to which 5.0 ml of 95% (v/v) ethanol was added. Bottles were capped and agitated in the dark at ambient temperature for 20 h. Aliquots were removed from the bottles and diluted with a 4.5 ml of 95% ethanol. Chl concentrations were calculated from absorbance values at 649 and 665 nm according to Holdren (11).

For determination of reduced N and phosphorus concentrations, 100 mg of dry ground (20 mesh) leaf material was digested and analyzed as previously described (5, 24).

Senescence. Senescence of the selected leaves was followed quantitatively by measurements of dry weight, soluble protein, reduced N, P, and Chl (expressed on a leaf area basis) for each treatment. Qualitative changes in soluble proteins during the 25 to 47 DAF period were estimated by gel electrophoretic techniques.

Statistical Analysis. Analysis of variance procedures were used. There were significant differences (P ≤ 0.05) among sampling dates and treatments for all parameters measured for both the greenhouse and field studies. Because the respective error terms for sampling dates and treatments were homogeneous, as indicated by Bartlett's test, the pooled error mean squares from the analyses were used to calculate the LSD (P ≤ 0.05) shown on the figures (applicable between treatments within time and within treatments across time).

RESULTS

Leaf Dry Weight. In response to flower or seedhead removal, dry weight of the selected leaves increased throughout the sampling period for both greenhouse and field grown plants (Fig. 1A and B). In the greenhouse by 53 DAF, the leaf weight of pre- and postflowering deheaded plants was 174 and 246% greater than leaf weight of headed plants, respectively. There was no effect of time of deheading on leaf weight until 44 DAF (Fig. 1A). Between 39 and 53 DAF, plants deheaded after pollination continued to accumulate leaf dry weight at a faster rate than plants that were deheaded prior to pollination; the reason for this difference is not clear. In the field by 58 DAF, the leaf dry weight of deheaded plants was 77% greater than leaf weight of headed plants (Fig. 1B). With the exception of the last sampling of greenhouse plants, leaf weight of all headed plants was relatively constant throughout the experimental period. For all treatments, the leaf weight of field plants were approximately 3-fold greater than greenhouse plants.

Specific Leaf Weight. For both greenhouse- and field-grown plants, specific leaf weights were always greater for deheaded than headed plants (Fig. 1, C and D). A possible explanation for this difference is that in the absence of a reproductive sink, the leaf acts as an alternate sink for deposition of photosynthetic...
The patterns of change in specific leaf weight differed under two environments. For the greenhouse plants, specific leaf weights increased gradually until 47 DAF, regardless of treatment, and until 53 DAF for the postpollination treatment (Fig. 1C). For the field plants, the specific leaf weights increased (8–17 DAF), decreased (17–30 DAF), increased (30–37 DAF), and remained unchanged (37–58 DAF) for both treatments (Fig. 1D). Although the percent increase in specific leaf weight following head removal was greater for greenhouse than field plants, leaf weights for field plants were greater (approximately 35%) than leaf weights for greenhouse plants receiving comparable treatments. Differences in photosynthetic activity or an effect of environment on rates of plant development and seed fill are some of the factors that could affect these patterns.

Because head removal altered leaf weight and not area (data not shown), all other constituents were expressed as concentration per unit area of leaf.

Chlorophyll. For greenhouse-grown plants, flower or head removal resulted in a marked retention of leaf Chl, relative to headed plants (Fig. 2A). There was no consistent effect of the time of head removal on Chl retention. By 53 DAF, the pre- and posttreatments still retained 73 and 77% of the Chl concentration present at 12 DAF, respectively. Leaves of headed plants retained their Chl until 25 DAF and then rapidly lost Chl so that by 53 DAF they were devoid of Chl.

In contrast to greenhouse plants, the leaves of deheaded field plants did not maintain their Chl concentration (Fig. 2, A and B). Between 25 and 44 DAF, the pattern and rate of loss of Chl was similar for headed and deheaded field-grown plants. The rate of Chl loss by the deheaded plants was maintained between 44 and 58 DAF, and at 58 DAF the Chl concentration was similar (1 mg dm$^{-2}$) for both treatments.

Soluble Protein. Headless greenhouse plants maintained and headless field plants retained more of their leaf soluble protein than comparable headed plants during the experimental period (Fig. 2, C and D). Although for all treatments at 12 DAF, the field plants had almost 2-fold more leaf soluble protein than comparable greenhouse plants, all headed plants had lost similar amounts (14–15 mg) of protein per unit leaf area by time of grain maturity. For the headed field plants the loss of protein from the leaves was rapid, occurred early during grain development, and was of short duration (17–37 DAF). For the headed greenhouse plants the loss of protein was gradual and near linear between 12 and 47 DAF. The leaves of the headless field plants lost 9 mg protein dm$^{-2}$ between 12 and 58 DAF, while the loss from the headless greenhouse plants ranged from 1 to 3 mg protein dm$^{-2}$ of leaf depending on time of deheading.

Reduced Nitrogen. Under field as well as greenhouse conditions, the leaves of all headless plants maintained or increased their reduced N concentration (mg dm$^{-2}$ basis) during the experimental period (Fig. 2, C–F). These data indicate that the headless plants remobilized little N to root or stalk when the seed sink was removed. Alternatively, if remobilization of N from the leaf occurred, ultimately it was more than balanced by N newly acquired from the soil or from other plant parts. As with soluble protein, all field-grown plants had more (about 2-fold) total reduced N in their leaves than comparable greenhouse plants. The loss of reduced N per unit leaf area was greater for the headless field plants (10 mg dm$^{-2}$) than for the headed greenhouse plants (7 mg dm$^{-2}$), but the percentage of loss was greater for greenhouse plants.

Gel Electrophoresis. For both greenhouse- and field-grown plants at 25 DAF, PAGE resolved the soluble proteins of the selected leaves into 16 detectable bands regardless of treatment (Fig. 3, A and B). Although the same amount of protein was processed for application to each well, the number of detectable bands and/or the staining intensity of all bands decreased progressively with each successive sampling date. However, the loss in detectable bands and the staining intensity was greater for the headed than the headless plants, especially for the greenhouse
plants. By 47 DAF only two polypeptide bands were detected for the headed greenhouse plants while 13 bands were evident for the headed field plants.

No new or different protein bands were detectable on the gels regardless of treatment or age of plant (Fig. 3, A and B). At 47 DAF, leaves of headless greenhouse plants had as much, and headless field-grown plants had 75% as much soluble protein as at 25 DAF (Fig. 2, C and D). However, for all treatments, much less of the extractable protein was detectable after electrophoretic separation of the extracts from the oldest than from the youngest leaves (Fig. 3, A and B).

The reason for the loss of polypeptide bands and staining intensity of the detectable bands as a function of plant age, even though equal amounts of protein were applied to each well of gel, is not clear. We can only speculate that on each successive sampling the soluble proteins extracted from the plant (see soluble protein and reduced N concentration, Fig. 2, C-F) were present in smaller, yet stainable, fragments due to increased hydrolysis. As a result, more of these small protein fragments could have been lost from the gel during electrophoresis.

Phosphorus. Regardless of environment, head removal ultimately resulted in significant increases in P concentration in the leaves (Fig. 2, G and H). At the final sampling date, the selected leaf of deheaded plants contained 179, 356, and 73% more P than respective headed plants for the pre- and post-treated greenhouse and field grown plants, respectively. For headed plants, except for a marked increase between 44 and 58 DAF of field grown plants, the concentration of P in the selected leaf of greenhouse- and field-grown plants remained relatively constant throughout the experimental period (Fig. 2, G and H).
DISCUSSION

For sunflower, seedhead removal resulted in a marked increase in dry weight (presumably mostly carbohydrates) and phosphorus and a retention of soluble proteins and reduced N (per leaf area basis) for both greenhouse and field grown plants (Figs. 1, 2). These data indicate that in the absence of seeds the leaves served as an alternate sink for photosynthesis and that seedhead removal altered the partitioning of metabolites within the sunflower plant. These findings are consistent with the results obtained in depodding studies with soybean plants (7, 8, 28, 29).

Using the rate and extent of loss of Chl (Fig. 2) and soluble protein (Figs. 2, 3) as criteria of senescence, head removal from greenhouse plants caused a marked delay in the rate of leaf senescence while head removal from field plants had much less effect. Little RuBPCase was detectable on the gel at 47 DAF for the headed plants grown in the greenhouse while strong bands (staining intensity) of RuBPCase were evident for both headed and headless field plants (Fig. 3). For the headed plants, the amount of protein applied to the well represents 1.5- and 3.0-fold more leaf area than for comparable headless plants. If the amounts of protein applied to the well had been representative of equal leaf areas, the effects of deheading under the two environments would have been even more obvious.

These studies, especially under greenhouse conditions, are in agreement with Purohit's (20) observations that head removal delayed the loss of Chl and leaf senescence of field-grown sunflower plants. Under our field conditions and with the cultivar, SunGRO 372A, the loss of Chl was delayed for only 3 weeks following deheading and by the end of the grain-filling period leaves of headed and headless plants had the same Chl concentration (Fig. 2B). However, leaves of headless plants retained more soluble protein per unit leaf area than leaves of headed plants (Fig. 2D). These soluble proteins may represent a retention of anabolic type enzymes. In general, we conclude that removal of heads from sunflower plants delays the development of senescence symptoms and that the rate of development may be altered by environment and probably by cultivar.

The goal of the gel electrophoresis work was to attempt to identify different (newly synthesized) proteins in the leaves of deheaded sunflower plants. No new polypeptide bands were detected (Fig. 3). Consequently, this work is in agreement with similar work with maize (6) and in disagreement with the work with soybean (28, 29). Based on the lack of a major increase in concentration (Fig. 2) or content of soluble protein or reduced N of the deheaded plants, there was little evidence that the leaves were acting as a sink for nitrogenous compounds. In the absence of seed, the N was not remobilized to other plant parts.

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