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Effect of Pod Removal on Leaf Photosynthesis and Soluble Protein Composition of Field-Grown Soybeans[1]

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ABSTRACT

Well nodulated, field-grown soybeans (*Glycine max* [L.] Merr. var Williams) were depodded just prior to seed development and near mid pod-fill. Both treatments caused a considerable increase in leaf dry weight, suggesting continued photosynthate production following pod removal. Moreover, depodding had a marked effect on leaf soluble protein without affecting total proteolytic activity. Early depodding caused a 50% increase in leaf protein, and both early and late depodding caused the retention of protein for several weeks following the decline in control leaves. But despite this retention of protein, leaves of depodded plants showed no difference in the onset of the irreversible decline in photosynthesis. Therefore, although depodding delayed the loss of leaf chlorophyll and protein, it did not delay the onset of functional leaf senescence and in fact, actually appeared to enhance the rate of decline in photosynthesis. There was a good correlation between the irreversible decline in ribulose bisphosphate carboxylase (activity and amount) and that of photosynthesis. In contrast, the correlation did not seem as good between stomatal closure and the onset of the irreversible decline in photosynthesis. The reason total soluble protein remained high following depodding while carboxylase, which normally comprised 40% of the soluble protein, declined was because several polypeptides increased in amounts sufficient to offset the loss of carboxylase. This change in leaf protein composition indicates a change in leaf function; this is discussed in terms of other recent findings.

Senescence of soybean leaves is normally characterized by a decline in photosynthesis and the loss of leaf protein and Chl (12), leading to death of the leaf. However, recently it was shown that following pod removal, the leaves lose the ability for photosynthesis but retain high levels of Chl and protein (7, 10), indicating a separation of functional senescence from death of the leaf. Therefore, removing the pods from soybeans apparently does not delay functional senescence of the leaves as has been claimed (5). Instead, depodding causes a change in the soluble protein pattern of the leaf suggesting a change in leaf function (10). The leaf appears to change from a photosynthesizing source organ to a sink organ.

In the previous growth room study (10), Wye soybeans, a determinate variety, were grown with applied N which resulted in poor nodulation. Under these conditions, the plants had essentially only one sink, the pods, following flowering. Removing this sink caused a rapid decline of photosynthesis and a change in the soluble-protein composition of the leaf. The decline in photosynthesis was closely associated with stomatal closure, implying a possible cause and effect relation as has been suggested by Thimann and Satler (8).

The present study examines the effect of depodding on leaf senescence in field-grown plants. It expands the previous study to include an indeterminate variety, Williams, grown under conditions favoring nodule production and activity, thereby providing alternative sinks for photosynthate normally used by developing pods. In addition, this work more closely examines the effect of pod removal on stomatal resistance, Rubisco[2] activity, and photosynthesis to critically evaluate cause and effect relations.

MATERIALS AND METHODS

Plant Material. Soybeans (*Glycine max* [L.] Merr. var Williams) were planted in a Metapeake silt loam soil on June 11, 1981. Seed was treated with Nitragen *Rhizobium* inoculum prior to planting. The row spacing was 76 cm, and the stand was about 300,000 plants/ha. Irrigation was used as necessary to prevent water stress. Flowering occurred between July 24 and August 13.

Leaf and pod samples were collected from node 12 (plants had a total of about18 nodes), as the trifoliate at this node was at the outer canopy and remained unshaded during plant growth. The same node was used throughout plant development. Early pod removal was started 1 week after flowering occurred at node 12 (August 6), and late pod removal was started 3 weeks later. Newly initiated pods were removed from depodded plants at weekly intervals. All measurements and samples were taken between 1 and 3 PM. Tissue samples were immediately frozen in liquid N_2 and transported to the lab on dry ice. They were then stored in liquid N_2 until assayed.

Measurements. Leaf Chl, protein, and specific weight determinations were made as previously described (10). Proteolytic activity was assayed using tobacco Rubisco as the substrate according to the procedure outlined earlier (9). Photosynthesis and leaf conductance measurements were made when the PAR was 1500 $\mu E \cdot m^{-2}\ s^{-1}$ or greater, using a $^{14}CO_2$ pulsing unit for photosynthesis and a Lambda LI 1600 steady state porometer for leaf conductance as described previously (10). Rubisco activity and amount were determined using NaH $^{14}CO_3$ incorporation and immunoelectrophoresis as outlined earlier (9). The changes in soluble protein were followed using SDS-polyacrylamide gel electrophoresis. Sample preparation and running conditions were the same as described in the previous paper (10).

All experimental values are based on at least three replications, and although results are only presented for the 1981 season, similar results were obtained from a study in 1980.

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[2] Abbreviation: Rubisco, ribulose biphosphate carboxylase.

RESULTS AND DISCUSSION

Both early and late pod removal resulted in a rapid increase in specific leaf weight (Fig. 1A). From previous work (1, 10), this increase in specific weight is largely due to the accumulation of starch in the leaves of depodded plants. The increase in leaf weight following early pod removal occurred over the same period as seed growth (Fig. 1B) and accounted for at least 50% of the normal pod dry matter at the node. If the increase in dry matter of the petiole and stem at this node is included, it would equal about 80% of the pod dry matter. This is close to the value reported earlier by Ciha and Brun (1) who compared the total shoot weight for podded and depodded plants, and implies there is not a rapid and complete feedback inhibition of photosynthesis following pod removal.

Although both early and late pod removal delayed the loss of leaf Chl (Fig. 2A), the effect was much less pronounced than that demonstrated in the growth room study (10). In contrast, the effect on leaf soluble protein content was more pronounced in the field (Fig. 2B). Early depodding caused the level of protein to continue to increase on an area basis to a final content approximately 50% greater than in the leaves of control, podded plants, while late depodding resulted in retention of the high level of protein attained in control leaves. This buildup and retention of protein following depodding was not due to any major change in proteolytic activity (Fig. 2C). There was no observed effect of depodding on proteolytic activity until 5 weeks after early and 2 weeks after late pod removal. Inasmuch as these changes followed the observed change in protein, it is doubtful whether they had any significant effect on the initial buildup or retention of protein, although they may have had a role in retention of the high levels of protein during the latter 2 weeks.

The dramatic effect of depodding on leaf soluble protein content certainly implies that the leaf is still functioning at a very late stage, but is it still functioning normally? Because the most

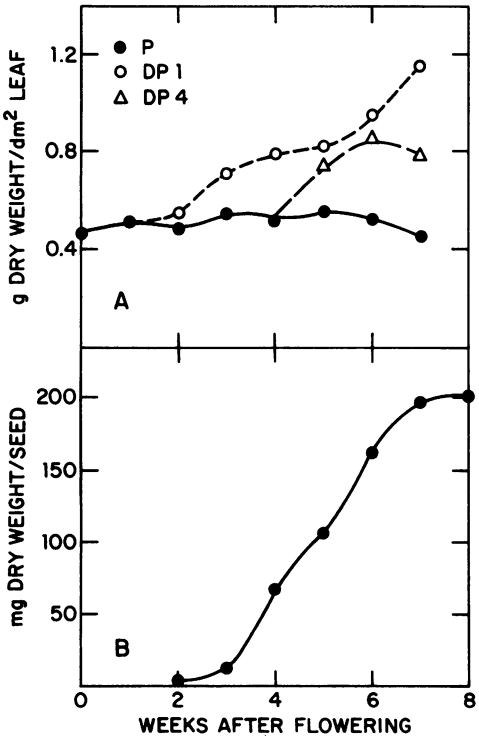

FIG. 1. A, Changes in specific weight of leaves from podded (P, ●) plants and plants continuously depodded beginning 1 week (DP1, O) or 4 weeks (DP4, Δ) after flowering. B, Seed development at node 12 in control, podded plants.

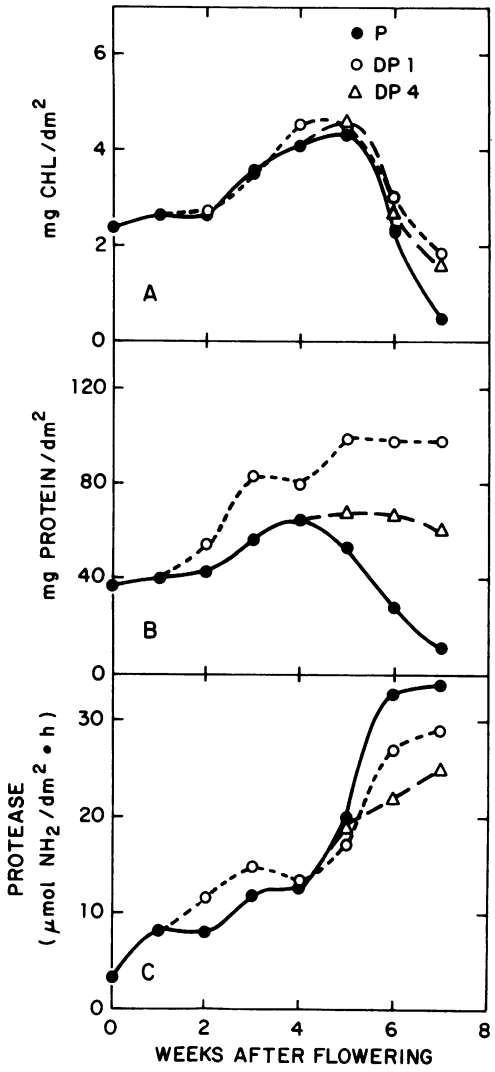

FIG. 2. Changes in Chl (A), protein (B), and proteolytic (C) activity of leaves from control, podded (P, ●) plants, and plants continuously depodded beginning 1 week (DP1, O) or 4 weeks (DP4, Δ) after flowering. The SD for CHl ranged from ± 0.13 to 0.24 mg/dm²; for protein, from ± 5.7 to 9.6 mg/dm²; and for protease, from ±1.8 to 4.9 μmol NH_2/dm²·h.

important and primary function of the leaf is photosynthesis, the effect of depodding on this function was observed (Fig. 3A). Early pod removal caused nearly a 40% decline in the rate of photosynthesis. Although there was also a decline (for unknown reasons) in photosynthesis of leaves from podded plants at this time, this rate recovered the following week, whereas that of depodded leaves recovered at a far slower rate and never did fully recover. This initial effect of depodding on photosynthesis was coupled with the effect of depodding on leaf conductance (Fig. 3B), indicating the initial decline in photosynthesis resulted from stomatal closure. However, the stomatal influence was reversible, and in fact, leaf conductance recovered to about 40% of the value for leaves from podded plants, whereas the photosynthetic rate recovered to within 20% of the control rate. Thus, although stomatal conductance is effected by depodding and can have a significant effect on photosynthesis, it appears that stomatal aperture is not the principal controlling agent in leaf senescence as has been suggested (8).

The onset of the irreversible decline in photosynthesis occurred at the same time in leaves of podded and depodded plants,

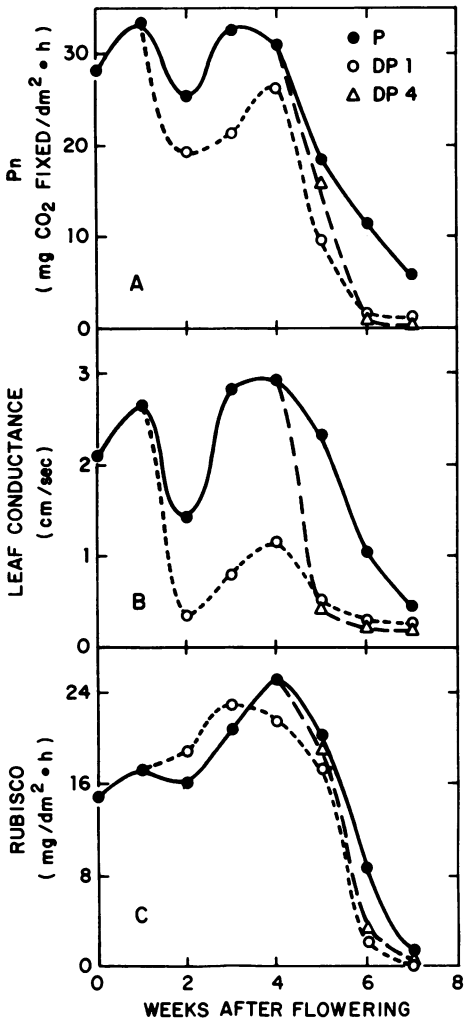

FIG. 3. Changes in photosynthesis (A), leaf conductance (B), and Rubisco protein (C) of leaves from control, podded (P, ●) plants, and plants depodded beginning 1 week (DP1, O) or 4 weeks (DP4, Δ) after flowering. The SD for photosynthesis ranged from ±0.3 to 6.1 mg/dm^2·h; for leaf conductance, from ±0.1 to 0.5 cm/s; and for Rubisco, from ± 0.1 to 4.1 mg/dm^2·h.

suggesting the initiation of functional senescence is not coupled with pod development. The decline in photosynthesis for leaves of podded plants was correlated with an irreversible decline in leaf conductance, Rubisco, soluble protein, and after 1 week's lag, Chl content (Figs. 2 and 3). In addition, there was an associated acceleration of the increase in proteolytic activity (Fig. 2C). All these changes were also evident in leaves of depodded plants with the exception of the decline in soluble protein.

Although depodding did not effect the onset of senescence, it did appear to influence the rate, resulting in an increased rate of decline in photosynthesis, Rubisco, and leaf conductance (after late depodding). This indicates a slightly faster breakdown of carboxylase and yet there was actually less total proteolytic activity in the depodded than podded leaves. Hence, compartmentation is apparently more important than total proteolytic activity in protein degradation of soybean leaves as has been previously shown for wheat and barley leaves (4, 6, 13).

The decline in leaf photosynthesis for both podded and depodded plants was correlated with the decline in amount of Rubisco (Fig. 3C). Rubisco activity also was followed, but the results are not presented since they were essentially identical with the quantitation results (Fig. 3C). The specific activity of carboxylase averaged 2.63 mg CO_2 fixed/h·mg Rubisco (994 nmol/min). In both this study and the earlier growth room study (10) with the determinate Wye cultivar, there was a close correlation between the decline of Rubisco (activity and amount) and photosynthesis in control podded and late depodded leaves, although they were not closely coupled following early depodding. Based on the results of this study, it seems likely that the initial decline in photosynthesis observed following early depodding in the growth room was induced by stomatal closure which was reversible, but because the irreversible decline in photosynthesis began 1 week later there was no chance to observe the recovery in either conductance or photosynthesis.

From these results, it would appear the decline in photosynthesis of soybean leaves is closely correlated with the decline in Rubisco as was previously shown for wheat (9). There are, however, certainly exceptions to this statement; for instance, there are soybean genotypes (R. L. Bernard, University of Illinois) in which the mature but not yet senescing leaves lose their Chl (y_3 gene) and photosynthesis begins to decline irreversibly prior to the decline in Rubisco (Wittenbach, unpublished results). Also, in a previous study (12) on leaf senescence in field-grown Kent soybeans, we failed to observe a close correlation between the decline in Rubisco and photosynthesis at a node equivalent to that in the present study. However, that variety exhibited a marked increase in specific leaf weight during the first 3 to 4 weeks after flowering (in 2 years of field studies with Williams, we have not observed a similar response). This increase in specific leaf weight was reflected by an increase in leaf and palisade thickness (12). Associated with the increase in palisade thickness was a large increase in protein and Rubisco on an area basis, and this synthesis may have continued into the period when photosynthesis had begun to decline. Also, it has recently been shown (2) that senescence does not proceed uniformly through the leaf but occurs first in the upper palisade layer and then proceeds to the second palisade layer and spongy mesophyll layer. Thus, depeding on the distribution of Rubisco and the period during which its synthesis continues, it may be possible for photosynthesis to decline while the level of Rubisco appears to remain constant, as synthesis and breakdown could be occurring simultaneously in different cell layers. This would seem to be a possible, though untested, explanation for our earlier results.

The field grown plants had several sinks at the early depodding stage because of indeterminate plant growth and the presence of nodules. Still, early pod removal resulted in accumulation of the same proteins (27, 29, and 80 kD) observed in the leaves of growth-room grown plants (Fig. 4). Although accumulation occurred at a slower rate in the field, an increase in these proteins was evident after 1 to 2 weeks and was very pronounced by 3 weeks after depodding. By 6 weeks after depodding, these three polypeptides represented the major protein bands. Although many other polypeptides were maintained, there were several that declined following depodding, most notable of which are large and small subunits of carboxylase (53 and 13 kD). Following late depodding, these same changes in polypeptide composition were observed. Thus, depodding soybeans maintains or increases the level of soluble protein but causes major changes in the composition of this protein, thereby indicating a change in leaf function.

The function of the polypeptides that accumulate following depodding is unknown. However, the 27 and 29 kD polypeptides appear to represent a single protein, and this protein has been localized in the vacuoles of the paraveinal mesophyll and associated bundle sheath cells (3). Inasmuch as it is a glycoprotein and appears to be synthesized in a manner similar to that of seed storage proteins, it seems reasonable that it may be a storage form for N in the leaf. It is normally present in leaves of soybean plants (but not seeds) and appears to accumulate just prior to

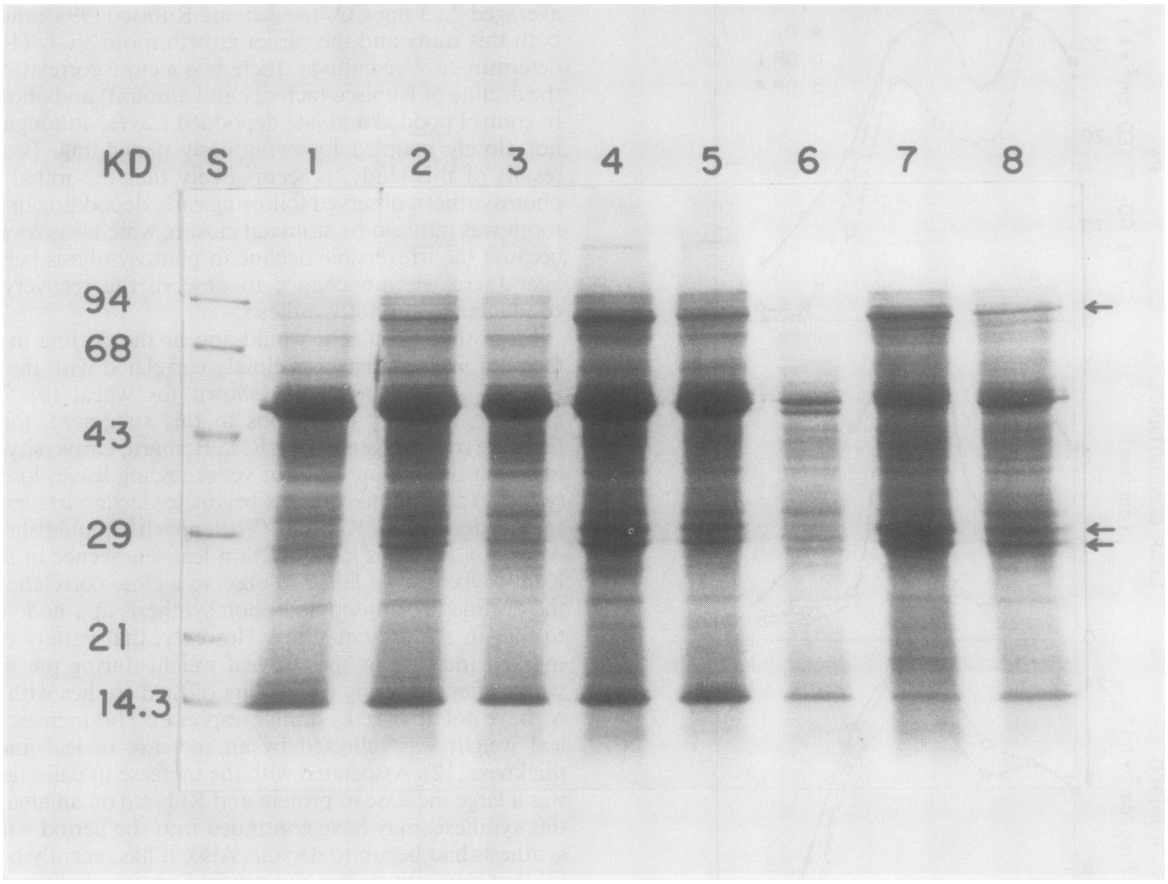

FIG. 4. SDS-gel electrophoretogram of soluble proteins from leaves of control, podded plants (lanes 1, 3, and 6) and plants continuously depodded beginning 1 week (lanes 2, 4, and 7) or 4 weeks (lanes 5 and 8) after flowering. Leaves were taken 3 weeks (lanes 1 and 2), 5 weeks (lanes 3, 4, and 5) or 7 weeks (lanes 6, 7, and 8) after flowering. Each lane represents the same leaf area. Lane S is the weight standards: 94, 68, 43, 29, 21, and 14.3 kD (top to bottom).

seed growth. Hence, it may function to insure an adequate supply of N to the seeds. If this is true, then, following depodding, an accumulation of both N (protein) and carbon (starch) occurs (10). This suggests there is not a rapid feedback control of photosynthesis following sink removal, which may be advantageous to the plant as it can then accumulate reserves for later remobilization and use by newly formed pods initiated following depodding.

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