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Effect of Pod Removal on Leaf Senescence in Soybeans[1]

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ABSTRACT

Depodding soybean (*Glycine max* [L] Merr. cv Wye) plants results in an apparent inhibition of senescence as indicated by leaf chlorophyll and soluble protein retention. However, leaf photosynthesis and ribulose bisphosphate carboxylase (Rubisco) levels begin to decline earlier in depodded than in control, podded plants. The initial decline in photosynthesis is correlated with a decrease in leaf transpiration, while the latter decline is associated with the loss of Rubisco. Total soluble protein remains high in depodded plants because several polypeptides, three in particular, increase in amounts sufficient to offset the loss of Rubisco. Thus, depodding appears to change the function of the leaf rather than simply delaying or preventing the decline in leaf function. Changes in specific leaf weight and starch content following depodding suggest that the leaf may be changing to a storage organ.

Senescence of soybean leaves is characterized by a decline in photosynthesis and the loss of leaf protein and Chl (11, 22). Clearly, the most dramatic visual symptom is leaf yellowing, and because of this, it is widely used as an index of plant and leaf senescence. From our field studies with winter wheat (21) and soybeans (22), loss of Chl appears to be a good initial index of leaf senescence. Yet caution must be taken in using this as the only indicator of senescence since Chl loss is not always an inevitable event in senescence (18, 19).

Over 50 years ago, Molisch (10) recorded the observation that plants delayed in the reproductive stages showed delayed senescence. Leopold *et al.* (7), following up on this report, were able to demonstrate a marked delay in soybean leaf and plant senescence following the continuous removal of either the flowers or pods. In recent years, this response in soybeans has been investigated further in an attempt to understand the 'senescence signal.' Lindoo and Noodén (9) were able to duplicate the pod removal effect by only removing the seeds from the pods, indicating that the senescence signal was associated with the developing seeds. The same laboratory (13) later provided evidence which separated seed dry matter accumulation from the senescence response, thereby contradicting the theory that seeds caused senescence by diverting or withdrawing needed nutrients from the leaves.

Although these studies on pod and seed removal imply a maintenance of normal leaf function, this has never been conclusively demonstrated. In fact, Mondal *et al.* (11) found that depodding soybeans partially inhibited and caused photosynthesis to decline earlier than in control, podded plants. This suggests that the normal photosynthetic capacity of the leaf may not be maintained following depodding. Therefore, the purpose of this study was to evaluate critically the effect of pod removal on photosynthesis, inasmuch as this represents the most important single function of the mature leaf. If the photosynthetic capacity is maintained, then this system may indeed provide insight as to how plant productivity can be increased.

MATERIALS AND METHODS

Plant Material. Soybean (*Glycine max* [L] Merr. cv Wye) plants were grown in 15-cm diameter pots containing Metro Mix 350 (Grace Horticultural and Agricultural Product Co., Cambridge, MA). The plants were thinned to two plants per pot following germination. Growth chamber conditions were as follows: 11-h day with a light intensity of 600 μE m^{-2} s^{-1} at the plant level, 24°C day, 18°C night, and 75% RH. Leaf samples were taken between the 5th and 6th h of the photoperiod from the leaf at the 7th node (plants had a total of 10 nodes). Tissue samples were either assayed immediately or frozen and stored in liquid N_2 until assayed.

Enzyme Assays. Rubisco[2] activity was determined by following $^{14}CO_2$ incorporation into acid-stable products as described previously (20). Leaf samples (five 1.5-cm diameter leaf disks) were extracted in 5 ml of 25 mM Hepes (pH 7.5) containing 4 mM DTT, 1 mM Na_2-EDTA, and 1% (w/v) PVP using a Polytron homogenizer. The extracts were centrifuged for 15 min at 30,000*g*, and the supernatant fractions were used to assay for Rubisco activity. Aliquots from the same fractions were used to quantitate the level of Rubisco, using the procedure of rocket immunoelectrophoresis outlined earlier (22). Proteolytic activity was determined using tobacco (*Nicotiana tabacum* L.) Rubisco as substrate by the procedure described previously (21).

Photosynthesis and Leaf Transpiration Measurements. Measurements of apparent photosynthesis were made using a $^{14}CO_2$ pulsing apparatus similar to that of Naylor and Teare (12). The C loss from photorespiration was minimized by limiting the pulse time to 15 s. Transpiration measurements were obtained using a LI-1600 steady-state porometer. Leaf transpiration rates were calculated assuming that transpiration of the individual surfaces acted in parallel with respect to total leaf transpiration.

Chl, Protein, and Starch Determinations. Chlorophyll content was determined using an 80% acetone extract following the method of Arnon (1). Protein analysis of samples was made following centrifugation using the Bio-Rad procedure (2). Starch content was determined using the methods of Outlaw and Manchester (14) as modified for use with soybean leaf tissue (22). Starch levels are reported on the basis of anhydroglucose equivalents. No correction has been made for starch recovery, although it is assumed to be about 80% based on a corn starch standard.

Gel Electrophoresis. After centrifugation of leaf extracts, a 0.5-ml aliquot was taken from the supernatant fractions and added to 0.5 ml 50 mM Tris (pH 7.3) containing 2% (w/v) SDS, 10% (v/v)

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[2] Abbreviation: Rubisco, ribulose bisphosphate carboxylase.

glycerol, 10% (v/v) 2-mercaptoethanol, and 0.01% phenol red. The proteins were completely dissociated by immersing the samples for 2 min in boiling water. Electrophoresis was carried out at a constant current of 40 mamp for 5 h on 9 to 18% polyacrylamide slab gels overlaid with a 5% polyacrylamide stacking gel. Gels were stained overnight in a solution containing 0.1% (w/v) Coomassie Blue, 40% (v/v) methanol, and 10% (v/v) glacial acetic acid and were destained in 30% (v/v) methanol and 10% (v/v) acetic acid. Photographs were taken after the gels were dried.

RESULTS AND DISCUSSION

When soybean plants were continually depodded beginning either 1 or 4 weeks after flowering, the loss of leaf Chl was markedly delayed (Fig. 1A). Moreover, the soluble protein content did not decline and, in fact, increased following late depodding (Fig. 1B). These results confirm and extend the observations of others (7, 9, 11) and are consistent with the proposal that depodding delays leaf senescence.

The initial increase and retention of protein following early pod removal was not associated with a reduction in proteolytic activity (Fig. 1C). There was no significant difference in activity of podded and depodded leaves during the first 3 weeks following early depodding. However, between 4 and 5 weeks after flowering, proteolytic activity began to increase at a faster rate in the leaves of podded than depodded plants. Thus, after this time, the lower activity in leaves of depodded plants could have influenced the level of protein following early and late pod removal. However, when compared to the marked difference in protein content

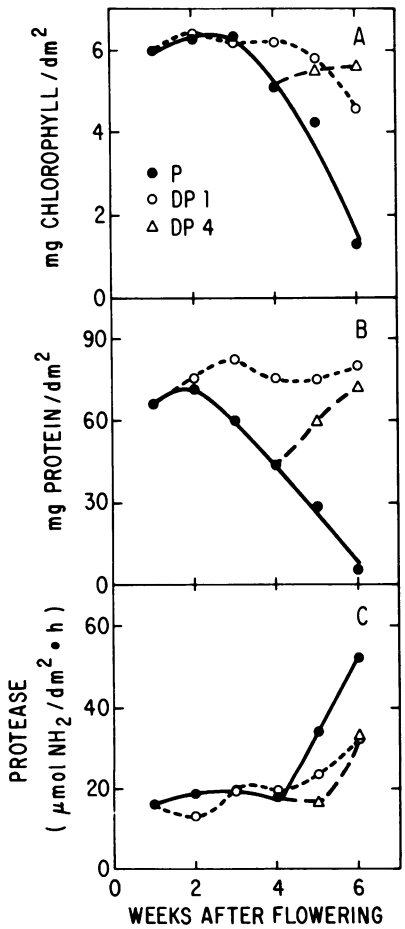

FIG. 1. Changes in (A) Chl, (B) protein, and (C) proteolytic activity of leaves from control, podded (●) plants and plants continuously depodded beginning 1 week (○) or 4 weeks (△) after flowering.

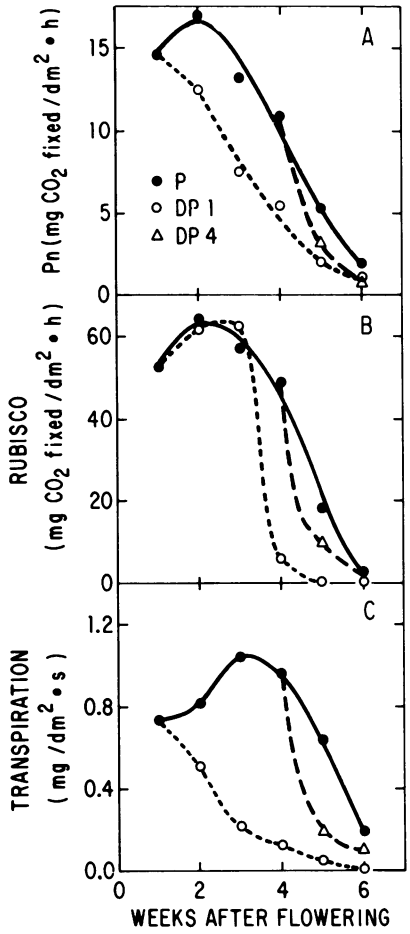

FIG. 2. Changes in (A) photosynthesis (Pn), (B) Rubisco activity and (C) transpiration of leaves from control, podded (P, ●) plants and plants continuously depodded beginning 1 week (DP1, ○) or 4 weeks (DP4, △) after flowering.

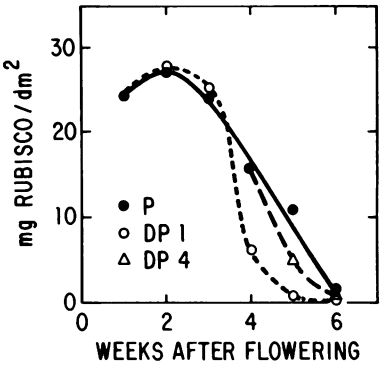

FIG. 3. Rubisco protein in leaves from podded (P, ●) plants and plants depodded beginning 1 week (DP1, ○) or 4 weeks (DP4, △) after flowering.

resulting from pod removal, the difference in proteolytic activity appears small, although this may imply that compartmentation of proteases is more important than total leaf activity, as has been demonstrated for barley and wheat during senescence (4, 8, 23).

In spite of the apparent delay in senescence resulting from depodding, photosynthesis began to decline within 1 week following early pod removal, preceding the onset of the normal decline by 2 weeks (Fig. 2A). It then continued to decline nearly linearly during the remainder of the experiment. Even late pod removal, which occurred midway during the normal decline in photosynthesis, appeared to enhance the rate of decline in photosynthesis.

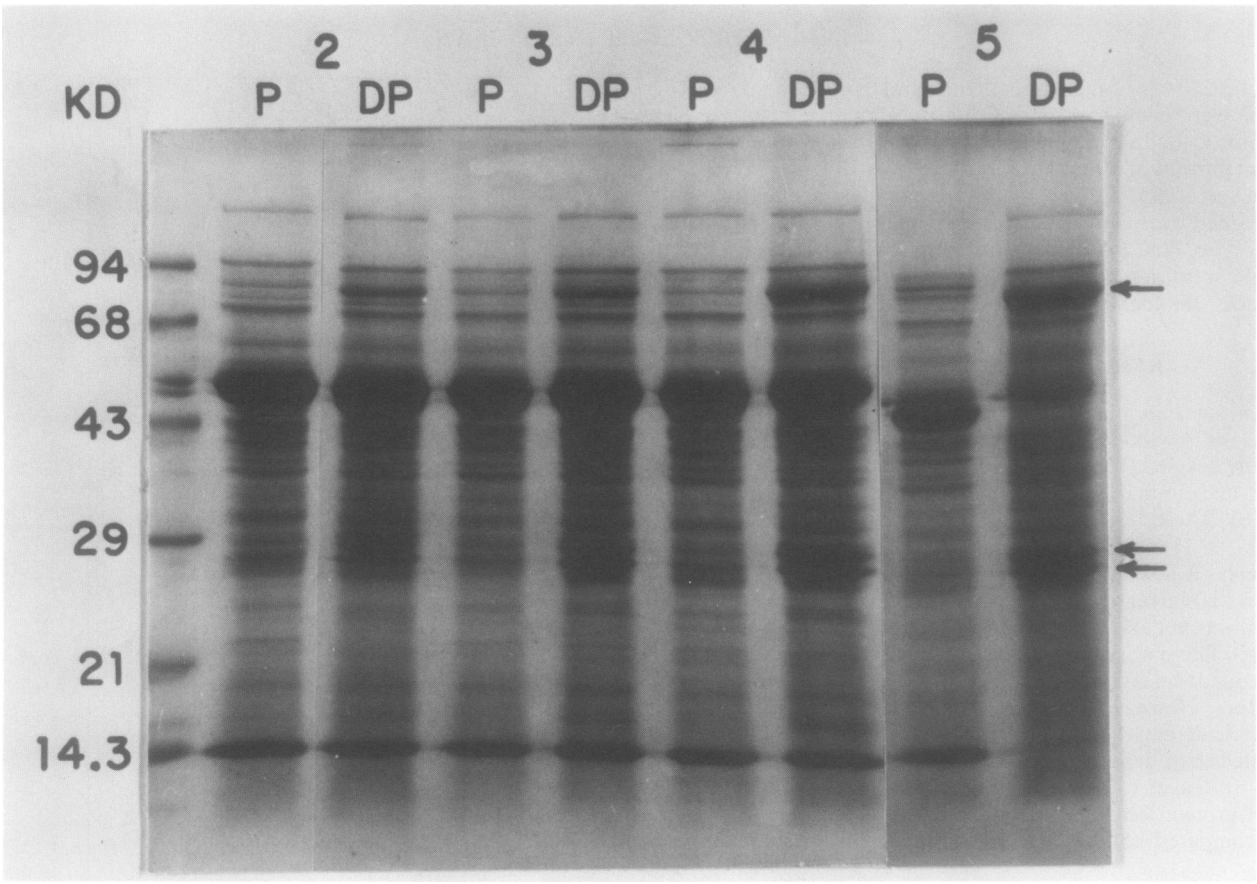

FIG. 4. SDS gel electrophoretogram of soluble proteins from leaves of control, podded (P) plants, and plants continuously depodded (DP) beginning 1 week after flowering. Samples were taken 2, 3, 4, and 5 weeks after flowering. Arrows denote the three major polypeptides that increase following pod removal.

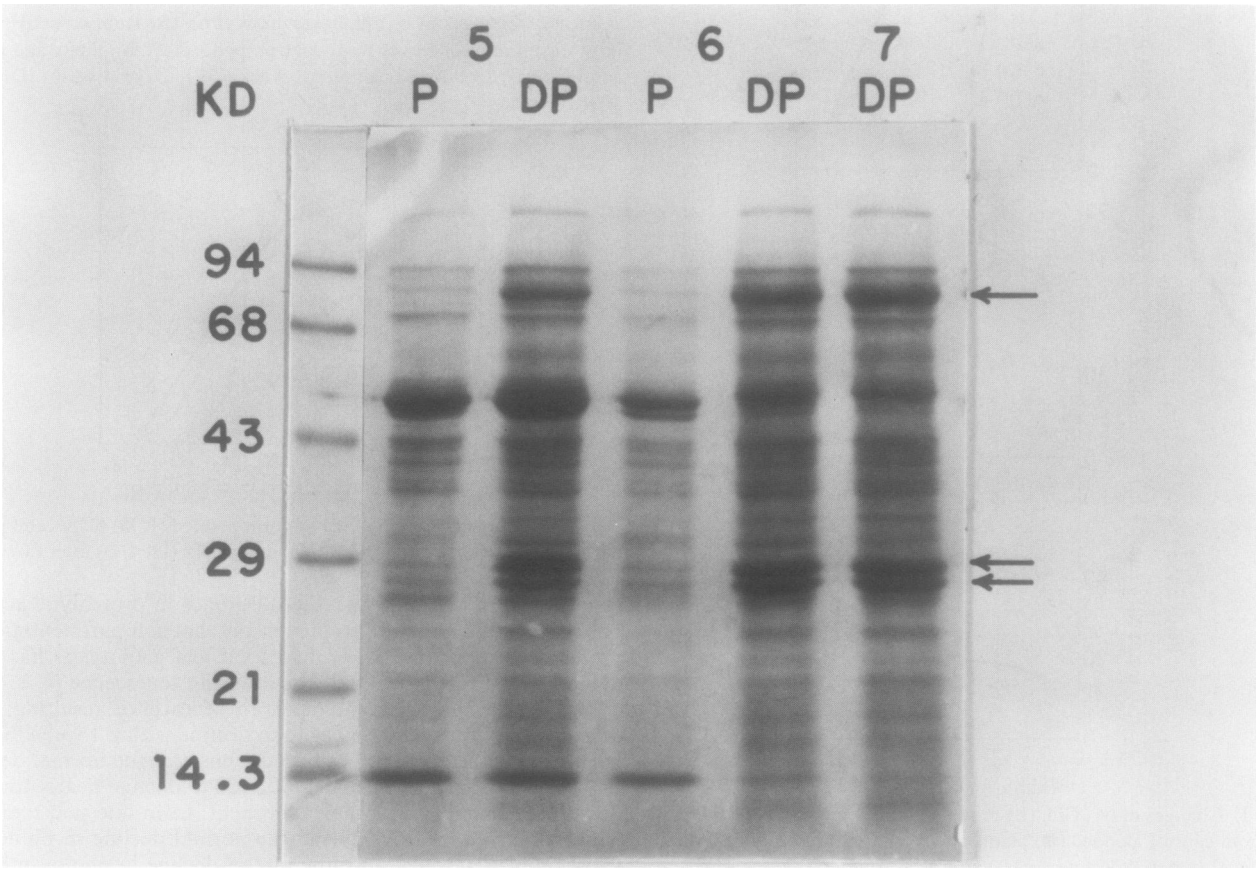

FIG. 5. SDS gel electrophoretogram of soluble proteins from leaves of podded (P) plants and plants continuously depodded (DP) beginning 4 weeks after flowering. Samples were taken 5, 6, and 7 weeks after flowering. Arrows denote the three major polypeptides that increase following pod removal.

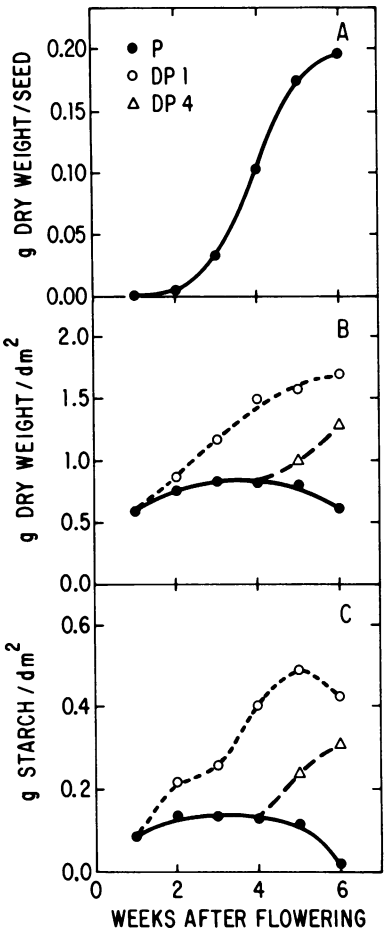

FIG. 6. Seed development in control, podded plants (A), and changes in specific leaf weight (B) and starch content (C) of leaves from podded (P, ●) plants and plants depodded beginning 1 week (DP1, ○) or 4 weeks (DP4, △) after flowering.

The initial decline in photosynthesis following early pod removal was not caused by a decline in Rubisco activity (Fig. 2B) or amount (Fig. 3). There was no apparent change in Rubisco until between 2 and 3 weeks after depodding when a very rapid decline in activity and amount was observed. The initial decline in photosynthesis was correlated with a decline in leaf transpiration (Fig. 2C), indicating stomatal closure. Other investigators (5, 15) have also observed this early increase in stomatal resistance following depodding. Setter *et al.* (15) concluded that the early decline in photosynthesis following depodding was induced by stomatal closure. My results support this interpretation for the initial decline in photosynthesis; however, the later decline must also be influenced by the loss of Rubisco. In a subsequent report, Setter *et al.* (16) showed a rapid rise in leaf ABA content following pod removal, which they suggested may be responsible for inducing the stomatal closure. If this is true, then ABA may also directly or indirectly influence the decline in Rubisco, even though it lags considerably behind the stomatal closure response. This concept is even more intriguing when one observes the effect of late depodding (Fig. 2). As seen here, there is a good correlation between the enhanced decline in Rubisco and stomatal closure; moreover, photosynthesis appears to mimic both responses. This close correlation between stomatal closure and functional senescence following late depodding resembles the observations of Thimann and Satler (17) on senescence of detached oat leaf segments.

The preceding results pose an interesting question. How does the leaf maintain its level of soluble protein while losing Rubisco, which normally comprises nearly 50% of this level? To answer this question, I observed the changes in the major soluble proteins following pod removal using gel electrophoresis (Figs. 4 and 5). Within 1 week after early (Fig. 4) or late (Fig. 5) depodding, there was already evidence of an increase in the amount of at least three polypeptides (denoted by arrows), two near 30 kD and one near 80 kD. These three polypeptides continue to increase in amount, becoming the dominating ones by 3 to 4 weeks after early depodding and 2 to 3 weeks after late depodding. The large and small subunits of Rubisco (~53 and 13 kD) showed little change in amount during the first 2 weeks following early pod removal, but between 2 and 3 weeks, a dramatic loss of both subunits was observed (Fig. 4). These qualitative changes concur with the quantitative Rubisco activity and immunoassay results (Figs. 2B and 3). The marked increase in the three designated polypeptides plus the retention and/or continued synthesis of other minor bands explains how the leaf maintains its level of soluble protein while losing Rubisco.

At present, we do not know the identity of these three major polypeptides. The two smaller polypeptides (~27 and 29 kD) could conceivably be breakdown products of the large subunit of Rubisco. However, three pieces of evidence suggest that this is unlikely. First, neither of these proteins reacts with antibody to intact Rubisco in spite of their relatively large size. Second, protein levels following late pod removal strongly suggest a marked increase in protein synthesis. Finally, these polypeptides show a relatively large increase in amount prior to any detectable loss in activity or amount of Rubisco following early pod removal.

These changes in soluble protein following pod removal confirm a change in leaf function. Although death of the leaf is delayed, the leaf is definitely losing its ability to function as a source leaf. Therefore, if one defines senescence (6) as the deteriorative changes which terminate the functional (photosynthetic) life of an organ (leaf), then depodding does not delay, but in fact, enhances senescence. However, if one defines senescence as the deteriorative changes which result in the death of the organ (24), then pod removal does delay senescence. Regardless of the definition used, it is obvious that there are distinct differences between leaves from podded and depodded plants; hence, caution must be used when comparing these leaves.

Both early and late pod removal cause a large increase in specific leaf weight (Fig. 6B). This increase in dry weight is due largely to an accumulation of starch (Fig. 6C). Interestingly, this increase in dry weight nearly paralleled the increase in seed weight at the same node of a normal podded plant (Fig. 6A). Ciha and Brun (3) examined this increase in dry weight in greater detail and found that starch accumulated first in the stem and petiole and then in the leaf of depodded plants. Furthermore, the shoots of depodded plants attained a maximum dry weight equivalent to nearly 90% of that for shoots plus pods of control plants. Thus, depodding apparently causes a build-up of photosynthate along the entire translocation pathway in soybeans. It seems the whole plant becomes a storage reserve, perhaps for utilization by new growth from buds which are continually being initiated at each node.

In summary, these results combined with those of other workers indicate a change in leaf function following pod removal. The leaf apparently changes from a photosynthesizing, source organ to a sink organ. This change is accompanied by a change in the soluble protein complement of the leaf. In particular, three polypeptides increase greatly in amount and apparently at the expense of Rubisco. At present, we do not know the role of these polypeptides or what stimulus signals their accumulation. However, stomatal closure is an early response to pod removal and, because ABA has been implicated in this response, it is natural to question whether ABA may be a signal for this change in proteins.

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