

Effects of Sink Removal on Photosynthesis and Senescence in Leaves of Soybean (*Glycine max* L.) Plants¹

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ABSTRACT

Photosynthetic rate, ribulose 1,5-bisphosphate carboxylase activity, specific leaf weight, and leaf concentrations of carbohydrates, proteins, chlorophyll, and inorganic phosphate were determined periodically from midbloom until maturity in leaves of soybean plants (*Glycine max* L., var. Hodgson) from which reproductive and vegetative sinks had been removed 32 hours before measurement, or continuously since midbloom.

Leaf photosynthesis, measured in the top of the canopy, was partially inhibited by both sink removal treatments. This inhibition was of constant magnitude from midbloom until maturity.

Leaf photosynthesis in the top of the canopy declined from midbloom until maturity in the control as well as in the desinked plants. The decline in photosynthesis was gradual at first, but later became more abrupt. The photosynthetic decline was equally evident in the yellowing leaves of control plants and in the dark green leaves of the continuously desinked plants.

Neither the inhibition of photosynthesis by sink removal nor the decline in photosynthetic rate with time was clearly related to any of the measured traits.

Inhibition of photosynthesis by the removal of a sink has been demonstrated in several species (12, 16, 18) including soybean. Thorne and Koller (23) found an increase in photosynthesis in soybeans when sink demand was increased by shading all but a single source leaf.

The removal of floral buds and young pods has been shown to delay or prevent soybean leaf senescence, as judged by their loss of green color (10, 15). Such yellowing of leaves has been attributed to adverse water relations during flowering and fruiting (2), a depletion of available nutrients (20), or to hormonal signals associated with the reproductive sink (15).

Effects of developing sinks on photosynthesis and senescence were studied separately and at single stages of development. The objectives were to examine the effects of sink removal on soybean leaf photosynthesis, on senescence, and on a number of leaf traits commonly associated with these processes.

MATERIALS AND METHODS

Soybeans (*Glycine max* L., var. Hodgson) were seeded in the field in 8-m rows spaced 60 cm apart on May 17, 1976, and thinned to a uniform plant spacing of 8 cm on June 1, 1976.

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Three treatments replicated four times were imposed on July 12 when plants were flowering at approximately 50% of the nodes (midbloom). A randomized complete block design was used. Treatments were: (a) control; (b) recently desinked, in which all flowers, floral buds, pods (if any), and shoot apices were removed from the main stem and branches at 0800 hr; and (c) continuously desinked, in which the plants were desinked as above on July 12 and then every other day, as necessary. Within replications, treated rows were bordered on both sides with nontreated guard rows. The plots were thoroughly watered 2 days prior to each sampling date.

Fully expanded leaves in the top of the canopy (fifth node from the top) were sampled for photosynthesis and chemical composition every 8 to 10 days from midbloom until late in the pod-filling period when photosynthetic activity had declined to nearly zero. Sampling was done between 1500 and 1700 hr, 32 hr after the desinking treatments.

For each treatment within each replicate, two plants were used for measuring photosynthesis and one for the determination of chl content, RuBP Case⁴ activity (EC 4.1.139), Pi, protein, and carbohydrate, as described below.

Photosynthesis. Leaf photosynthetic rates were measured in the field using an IR gas analyzer (Beckman 215). The intact terminal leaflet of the sampled leaf was inserted into a Plexiglass assimilation chamber (3.2 × 3.2 × 1.5 cm) mounted in the jaws of a modified vise-grip. The assimilation chamber consisted of two halves covering the upper and lower leaf surfaces, respectively. Three cool white fluorescent tubes (F4T5CW) were mounted about 1 cm from each assimilation chamber half, providing supplemental photosynthetic photon flux density of 385 $\mu\text{E sec}^{-1}\text{m}^{-2}$ to each leaf surface as measured with Lambda Instruments' LI-190S quantum sensor. In addition, the upper surface received about 18% of full sunlight transmitted through the fluorescent tubes and the assimilation chamber. With an air flow rate of 0.5 liters min^{-1} , the boundary layer resistance to CO₂ diffusion of this assimilation chamber was previously determined to be 1.6 sec cm^{-1} .

Carbon dioxide (320 $\mu\text{l/l}$) was metered at 0.5 liters min^{-1} and passed through the assimilation chamber and back to the IR analyzer housed in a nearby instrument trailer. The photosynthetic rate (mg of CO₂ fixed $\text{dm}^{-2}\text{hr}^{-1}$) was calculated as the product of the flow rate and the CO₂ depletion of the air stream passing through the chamber divided by the area of the leaflet surface within the chamber.

After leaf photosynthetic rates were determined, leaf blades were collected, placed on ice, and brought to the laboratory where fresh weights and areas of the leaflets were quickly determined. Leaf areas were measured on a Hayashi-Denko type AAM-5 leaf area meter. Dry weights were determined after drying at 65 C for

⁴ Abbreviations: RuBP: ribulose 1,5-bisphosphate; RuBP Case: RuBP carboxylase.

48 hr. Dried tissues were then ground with a mortar and pestle and stored for subsequent analyses.

Carbohydrates. Approximately 100 mg of dried leaf tissue were extracted with 80% (v/v) aqueous ethanol following the method of Clegg (4). The soluble carbohydrate contents of the extracts were determined by the method of Dubois *et al.* (5). Starch left in the residue was extracted according to the procedure described by Clegg (4) and analyzed by the method of Dubois *et al.* (5).

Inorganic Phosphate. Inorganic phosphates of the leaf samples (100 mg of dry sample) were extracted following the method of Hall and Hodges (9) and assayed by the Fiske-Subbarow (6) method.

Protein. A 100-mg sample of dried leaf tissue was first extracted with methanol. The protein in the methanol-insoluble residue was then extracted following the procedure of Thorne and Koller (23), and assayed by the method of Lowry *et al.* (17).

Chlorophyll. To extract Chl, two 1.2-cm fresh leaf discs were ground in methanol with a Polytron homogenizer. The mixture was incubated at room temperature for 30 min in the dark, centrifuged, and the Chl *a* and *b* contents determined by the method of Arnon (1).

RuBP Case. A crude extract was prepared and assayed for RuBP Case activity using the procedure of Johnson *et al.* (11) with slight modification. The reaction mixture contained 50 μ mol of HEPES (pH 8.1), 3 μ mol of reduced glutathione, 0.1 μ mol of disodium EDTA, 5 μ mol of $MgCl_2$, 2.5 μ mol (0.4 μ Ci μ mol⁻¹) of $NaH^{14}CO_3$, and 0.1 μ mol of RuBP in a total volume of 1 ml. The reaction, run at room temperature (25 ± 1 C), was initiated by adding 0.1 ml of the enzyme extract and was stopped by adding 0.1 ml of 6 N acetic acid after 3 min. RuBP Case activity was determined by measuring the incorporation of $^{14}CO_2$ into acid-stable products.

Statistical Analysis. The data for the respective treatments and sampling dates were tested for homogeneity of error. Those data that had homogeneous error values were subjected to two-way analysis of variance to test for interactions between treatments and sampling dates. Means were compared using a Tukey's test at the 5% level.

RESULTS

The desinked plants showed a significantly (and fairly constant) reduced photosynthetic rate, compared to the control plants, at all sampling dates except the first and the last (Fig. 1). The photosynthetic rate of continuously desinked plants differed very little from that of the recently desinked plants. Photosynthetic rates of both the control and the treated plants remained relatively unchanged until 38 days after midbloom and then declined rapidly to low levels during the last sampling dates. The continuously desinked plants had dark green leaves throughout the sampling period, yet their photosynthetic rate started declining at approximately the same time and at the same rate as it did in the control plants.

When photosynthetic rates were measured at various times between 4 and 32 hr after desinking, treatment effects were evident at 8 hr, but more pronounced at 24 and 32 hr (data not shown).

Leaf Chl contents/unit area of the continuously desinked plants did not change significantly with time between 38 and 60 days after midbloom (Table I), while in control and recently desinked plants it dropped significantly during this time.

Starch concentrations in sampled leaves of continuously desinked plants were significantly higher than in those of the recently desinked plants which in turn were higher than those of control plants after day 22 (Fig. 2). After day 30, the starch concentration of continuously desinked plants remained constant, whereas the starch concentrations in leaves of recently desinked and control plants decreased about equally. At the final harvest on day 60, leaf starch concentrations of all treatments were significantly different from each other.

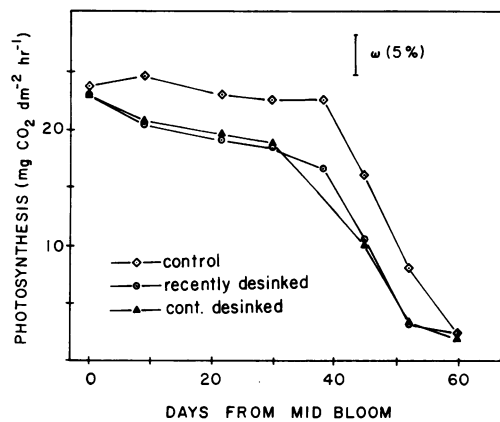


FIG. 1. Leaf photosynthetic rates of control, recently desinked, and continuously desinked soybean plants. All means differing by a value greater than ω are significantly different at the 5% level (Tukey's test).

Table I. Concentrations of chlorophylls *a* and *b* in sampled leaves of control, recently desinked and continuously desinked soybean plants

The leaf sample taken was fifth from the top.

Treatments	Days after Mid-Bloom					
	38		45		60	
	a	b	a	b	a	b
Control	3.77 ^c	1.45 ^c	2.27 ^d	1.01 ^d	1.60 ^e	0.61 ^e
Recently desinked	3.79 ^c	1.43 ^c	2.31 ^d	1.05 ^d	1.57 ^e	0.63 ^e
Continuously desinked	3.85 ^c	1.44 ^c	3.75 ^c	1.42 ^c	3.80 ^c	1.41 ^c

¹ Means of chlorophylls A or B followed by different letters are significantly different at the 1% level (Student-Newman-Keuls' test).

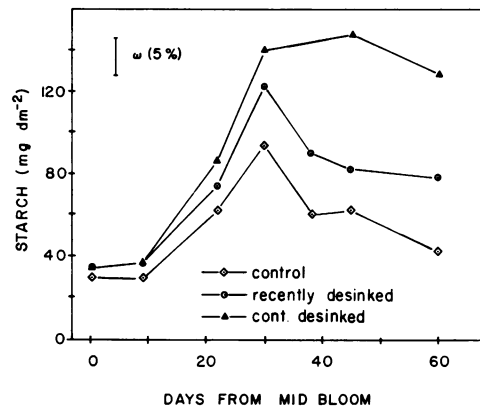


FIG. 2. Starch concentrations in leaves of control, recently desinked, and continuously desinked soybean plants. See Figure 1 for statistics.

At the last two sampling dates, the amounts of soluble carbohydrates were significantly greater in the sampled leaves of continuously desinked plants than in those of control and recently desinked plants (Fig. 3). There was, however, no significant change in the level of soluble carbohydrates in control and recently desinked plants throughout the sampling period.

At the first two sampling dates, there was no difference in the levels of protein between the treatments (Fig. 4). On day 22, leaf protein concentrations in control and recently desinked plants dropped significantly (about 13% [w/w]) and then remained constant through the final harvest. Protein concentrations in the continuously desinked plants, on the other hand, did not change at all until after the 30th day when they increased significantly.

The RuBP Case activities of leaves from the treated plants did not differ significantly except on the last sampling date when the activity in leaves of the continuously desinked plants was about

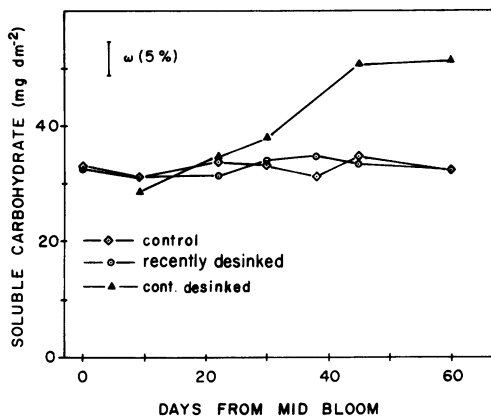


FIG. 3. Concentrations of soluble carbohydrates in leaves of control, recently desinked, and continuously desinked soybean plants. See Figure 1 for statistics.

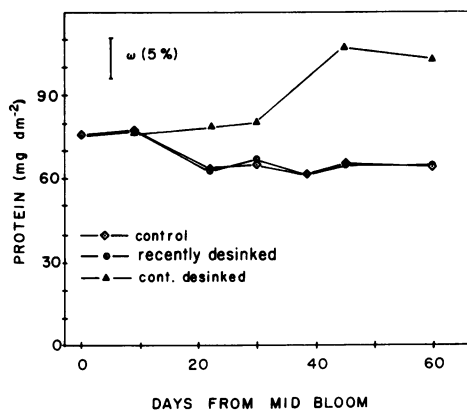


FIG. 4. Protein concentrations in leaves of control, recently desinked, and continuously desinked soybean plants. Means after the first sample date, differing by a value greater than ω , are significantly different at the 5% level (Tukey's test).

twice that of the other two treatments (Fig. 5). Maximum RuBP Case activity occurred at day 30 with declining activities from then until maturity.

Control and treated plants had very similar and gradually increasing Pi contents until day 40 (Fig. 6). Thereafter, the Pi content of leaves of control and recently desinked plants declined to a level similar to that on day 0, while the Pi content of leaves of continuously desinked plants reached a maximum on day 45 and then declined significantly.

Specific leaf weights (Fig. 7) of the continuously desinked plants were significantly higher than those of either the control or recently desinked plants for the last four sampling dates. This difference became more pronounced late in the growing period. In contrast, specific leaf weights of control and recently desinked plants, which were very similar, remained fairly constant during the sampling period, except for a modest but significant increase between days 10 and 30.

DISCUSSION

Soybean leaf photosynthesis during reproductive growth was subject to two contrasting influences: (a) the removal of developing pods had an inhibitory effect on photosynthesis; and (b) there was a decline over time in photosynthesis, irrespective of the presence of developing pods.

Inhibition of leaf photosynthesis by pod removal is consistent with the results of others (14, 18, 19, 23) who have found that the presence of a sink stimulates photosynthesis. This effect has

been attributed to at least three mechanisms: (a) sink alleviation of end product inhibition by soluble carbohydrates (8); (b) sink-promoted reduction of starch accumulation in the chloroplasts (23); and (c) sink-mediated hormonal signals (16).

End product inhibition of photosynthesis by soluble carbohydrate is not clearly supported by the present study. In the continuously desinked plants, the inhibition of photosynthesis may or may not have been caused by the observed soluble carbohydrate accumulation. In the recently desinked plants, photosynthetic inhibition was clearly not related to the soluble carbohydrate

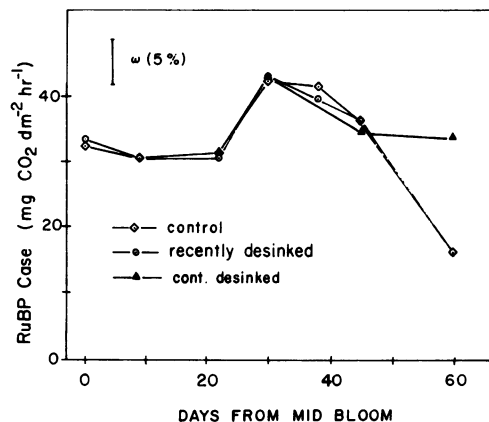


FIG. 5. Activities of RuBP Case of leaves of control, recently desinked, and continuously desinked soybean plants. See Figure 1 for statistics.

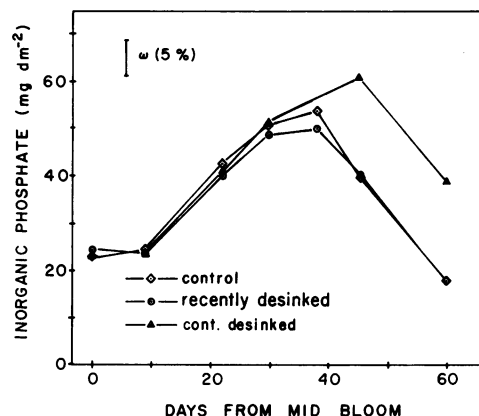


FIG. 6. Pi contents in leaves of control, recently desinked, and continuously desinked soybean plants. See Figure 4 for statistics.

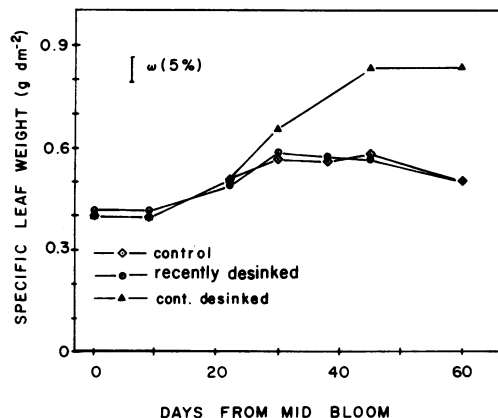


FIG. 7. Specific leaf weights of control, recently desinked, and continuously desinked soybean plants. See Figure 1 for statistics.

accumulation (Figs. 1 and 3). Thus, unless two separate mechanisms are operating, there appears to be no support for the hypothesis. This is in agreement with the observations made by Geiger (7), Claussen and Biller (3), and Nafziger and Koller (21).

Starch accumulation in chloroplasts may also be responsible for decreased photosynthetic rates in soybean leaves (3, 21). In the present work, starch accumulation occurred, especially in recently desinked and continuously desinked plants, but there was no correlation between starch content and photosynthetic rate. The fairly constant photosynthetic advantage of control plants over the two desinking treatments (Fig. 1) showed no counterpart in the leaf starch contents of these treatments (Fig. 2). The time effect on photosynthesis likewise cannot be explained on the basis of starch accumulation. Between 20 and 40 days, e.g. starch increased between 3- and 4.5-fold in the various treatments (Fig. 2) while photosynthetic rate declined very little. Likewise between days 40 and 60, photosynthetic rates dropped to near zero (Fig. 1) while the drop in starch levels was barely significant (Fig. 2). Thus, neither the sink effect nor the time effect appears to be explained by starch contents of the leaves.

The activity of RuBP Case has often been regarded as a good indicator of leaf photosynthetic rate (13) and Thorne and Koller (23) reported that increased sink demand for photosynthate increased the RuBP Case activity of soybean leaves. They ascribed this effect to a hormonal signal from the sink causing RuBP Case activation as reported by Treharne *et al.* (24) for bean plants. In contrast, the present work showed RuBP Case activity not to be altered by the desinking treatments (Fig. 5). In our work, however, sink demand was decreased by the treatments, whereas in the work of Thorne and Koller (23), sink demand was increased by placing all but one leaf of the whole plant in darkness for 6 to 8 days prior to making measurements on the illuminated source leaf. Whether the difference between our results and those of Thorne and Koller is due to this difference in experimental approach, or to the fact that they used growth chamber-grown plants and we used field-grown plants is not known.

Thorne and Koller observed that increased sink demand lowered the concentration of starch in leaves of soybean plants. They attributed the response to an increased starch degradation by phosphorylase. Such activity was stimulated by Pi which they found to accumulate in the leaves. Our study failed to show a consistent relationship between the accumulation of starch and Pi (Figs. 2 and 6), and it is thus difficult to interpret the role of Pi in sink control of leaf starch content.

The observed increase in specific leaf weight of the continuously desinked plants is only partially accounted for by the observed changes in soluble carbohydrates, starch, and protein, and is, therefore, difficult to relate to either of the two observed effects on photosynthesis.

The generally declining photosynthetic rates in all three treatments (Fig. 1) are indicative of progressive senescence of the leaves. Pod removal treatments have previously been described as delaying senescence in soybeans (15) and beans (22) but such senescence was judged by the loss of Chl that does not reflect the function of the leaves. This same delay in Chl disappearance was

also observed (Table I). From day 40 on, the sampled leaves of the control plants were visually yellowing and yet they maintained their photosynthetic rates well above the dark green leaves of the continuously desinked plants until the last sampling date.

The work here reported does not show clearly the cause of either the depodding inhibition of photosynthesis or the photosynthetic decline with time. There are, however, several possible explanations of these effects. Endogenous hormonal levels have been implicated by others (7, 16, 19, 23), but were not determined in this study. Stomatal resistance changes in response to depodding are also possible (16, 19) and may be related to endogenous hormone changes (16).

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