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Fruit removal in soybean induces the formation of an insoluble form of ribulose-1,5-bisphosphate carboxylase/oxygenase in leaf extracts*

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Abstract. In some soybean (*Glycine max* (L.) Merr.) cultivars, fruit removal does not delay the apparent loss of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco, EC 4.1.1.39) activity and abundance or the decline in photosynthesis. Analysis of leaf extracts from defruited plants indicated a time-dependent increase in both Rubisco activity and abundance in a 30000·*g* pellet fraction in cultivars which had been reported to lose all Rubisco protein from the supernatant fraction. Attempts to solubilize the pelleted Rubisco by increasing the buffer volume/tissue ratio or by adding alkylphenoxypolyethoxyethanol (Triton X-100), ethylenediaminetetraacetic acid (EDTA), or NaCl were unsuccessful. However, treatment of the pellets with denaturants such as 8 M urea or 5% (w/v) sodium dodecyl sulfate (SDS) did release Rubisco from the pellet. Redistribution of protein to the pellet fraction appeared to be specific for Rubisco since the amount of ribulose-5-phosphate kinase (EC 2.7.1.19) found in the pellet fraction of leaf extracts of control and defruited plants was small and constant over time. The loss of soluble Rubisco, and the concomitant increase in insoluble Rubisco, in response to fruit removal varied with genotype and was reproducible in both field and greenhouse environments. In addition, the effect was influenced by node position and light; lower and-or shaded leaves exhibited less Rubisco in the pellet fraction than leaves from the top of the plant that was fully exposed to sunlight. When isolated by sucrose-density-gradient centrifugation, the insoluble Rubisco was found to co-purify with a 30-kDa (kilodalton) polypeptide. These results indicate that alteration of the source/sink ratio by removing fruits results in the formation of an insoluble form of Rubisco in leaf extracts of soybean. Whether or not Rubisco exists as an insoluble complex with the 30-kDa polypeptide in intact leaves of defruited plants remains to be determined.

Key words: Fruit removal and Rubisco – *Glycine* (Rubisco and fruit removal) – Leaf senescence – Photosynthesis and fruit removal – Ribulose-1,5-carboxylase/oxygenase – Ribulose 5-phosphate kinase – Senescence (leaf)

Introduction

Under field conditions, leaf senescence in soybean is correlated with seed development and consists in a progressive loss of chlorophyll, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; EC 4.1.1.39) activity, and general photosynthetic competence. There are several conflicting reports in the literature regarding the effect of fruit removal or male sterility on photosynthesis and loss of Rubisco (Mondal et al. 1978; Wittenbach 1982, 1983a; Huber et al. 1983; Crafts-Brandner et al. 1984; Heitholt and Egli 1985; Schweitzer and Harper 1985; Crafts-Brandner and Egli 1987a, b). Continuous fruit removal or male sterility alter the rate but not the timing of the decline in photosynthesis associated with leaf senescence. It has been reported that absence of fruits causes photosynthesis to decline at faster rates (Mondal et al. 1978; Wittenbach 1982, 1983a; Huber et al. 1983; Crafts-Brandner and Egli 1987a) or slower rates (Heitholt and Egli 1985; Crafts-Brandner and Egli 1987a, b) compared to control plants with fruits.

As with photosynthesis, fruit removal or male sterility alter the rate but not the timing of declines in Rubisco activity. In a series of detailed studies Wittenbach (1982, 1983a, b) reported that fruit removal resulted in loss of activity and abundance of Rubisco at a faster rate than for controls and that the loss of Rubisco was associated with de-novo synthesis of vegetative storage proteins. Subsequent reports indicated that the effect of fruit

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Abbreviations: kDa = kilodalton; PGA kinase = 3-phosphoglyceric acid kinase (EC 2.7.2.3); Rubisco = ribulose-1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39); Ru5P kinase = ribulose-5-phosphate kinase (EC 2.7.1.19); SDS-PAGE = sodium dodecyl sulfate-polyacrylamide gel electrophoresis

removal on loss of Rubisco differed among soybean cultivars (Crafts-Brandner and Egli 1987a).

In the present study, we have found that leaf extracts from defruited plants that did not contain Rubisco in the supernatant fraction did contain a substantial quantity and activity of Rubisco in a 30000·*g* pellet fraction. Considering that Rubisco activity is usually measured by assaying supernatants of centrifuged leaf extracts, this result could explain the variability reported in the literature regarding the effect of fruit removal or male sterility on loss of Rubisco.

Material and methods

Plant culture. Results from both field and greenhouse experiments are presented in this report. Soybean (*Glycine max* (L.) Merr.) seeds were obtained from the Kentucky Foundation Seed Project. For the field experiments, soybean seeds were planted on 19 May 1988 and 24 May 1989 at Spindletop Research Farm near Lexington, Ky., USA. For the 1988 field experiment, twelve cultivars from a range of maturity groups were planted in a randomized complete block design with three replications. "Maturity group" refers to the geographical adaptability of a cultivar in terms of flowering and maturity (maturity group 00 cultivars are grown in the northern USA and maturity group VI cultivars are grown in the southern USA). Seeds were planted at 26 seeds·m^{-1} in single-row plots that were 6.1 m long with 0.7 m row spacing. A 0.5-m section of the row of each plot was used for the fruit-removal treatment. In a separate experiment, also in 1988, the cv. Ozzie was planted in a randomized complete block design with three replications. Seeds were planted in three-row plots at 26 seeds·m^{-1} with 0.35 m row spacing. Fruit-removal treatments were applied to a 0.5-m section of the middle row. For the 1989 field experiment, the experimental design was a completely randomized design with three replications. Six 6.1-m rows of the cultivar Essex were planted at 26 seeds·m^{-1} with 0.7 m row spacing. Randomly selected 0.5-m row sections within the plot were used for fruit-removal and control treatments. For the greenhouse experiment, the experimental design was a completely randomized design with three replications. Seeds of the cultivar Ozzie were planted in 4.0-dm^3 pots as previously described (Crafts-Brandner and Egli 1987b). Field experiments were irrigated as required to maintain nonlimiting soil moisture. For the fruit-removal treatments in field experiments, small (1 mm) fruits and flowers were removed every 3 d such that all fruits were continuously removed from the plants. For the greenhouse experiment, treatments consisted of 1) 70% removal or 2) 70% fruit removal plus 90% shade applied at the time of fruit removal. Shade was applied with 90% shade cloth (E.C. Geiger, Harleysville, Penn., USA).

Sampling. For most of the measurements, the second or third leaf below the uppermost leaf that was unrolled (edges not touching; Fehr and Caviness 1977) was sampled. Leaves were sampled on sunny days between 11:00 and 13:00 Easten daylight-saving time. In one experiment (1988 experiment with the cv. Ozzie) the eleventh leaf below the uppermost unrolled leaf was also sampled. For all measurements, two middle leaflets per replicate were sampled and the same leaflets were used for both photosynthesis and enzyme measurements. For enzyme and electrophoretic analysis, leaflets were transported to the laboratory on ice and leaf punches (113 mm^2) were stored at −80° C until analysis.

Activity of Rubisco. For each Rubisco assay, two leaf discs were homogenized in a glass homogenizer in 2.0 ml of medium containing 100 mM N-[2-hydroxy-1,1-bis (hydroxymethyl)ethyl]glycine (Tricine)-NaOH (pH 8.0), 5 mM $MgCl_2$, 0.1 mM EDTA, 5 mM dithiothreitol (DTT), 5 mM *iso*ascorbate, 1 mM phenylmethyl sulfonyl fluoride (PMSF), 20 μM leupeptin and 1% (w/v) casein. Extracts were centrifuged for 5 min at 30000·*g*, the supernatant was removed, and the pellet was washed with 2 ml of homogenization medium and centrifuged again. After the second centrifugation, the supernatant was discarded and the pellet was resuspended in 2 ml of homogenization medium. The Rubisco activity in the supernatant of the washed pellets was negligible in all treatments. Activity of the enzyme before and after activating by incubation for 10 min in 10 mM Mg^{2+} and 10 mM $NaCO_3$ was determined at 30° C using 50-μl aliquots of supernatant or resuspended pellet as described in Salvucci and Anderson (1987) except that Triton X-100 and casein were not included in the assay medium. Assays were terminated and incorporation of $NaH^{14}CO_3$ into acid-stable products was determined.

Ribulose-5-phosphate (Ru5P) kinase activity. Leaf discs were homogenized and supernatants were prepared as described for Rubisco activity. Supernatants were rapidly desalted at 4° C on a column (8 mm diameter, 40 mm high) of Sephadex G50–300 (Pharmacia LKB Biotechnology, Piscataway, N.J., USA). Ribulose-5-phosphate kinase (EC 2.7.1.19) activity was determined spectrophotometrically at 30° C as Ru5P-dependent ADP production coupled to NADH oxidation. The assay medium used was as described by Salvucci and Ogren (1985). Ten microliters of desalted extract were used for each assay and assays were initiated with Ru5P. Activities were corrected for Ru5P-independent ADP production.

3-Phosphoglyceric acid (PGA) kinase activity. Activity of PGA kinase (EC 2.7.2.3) was determined spectrophotometrically at 30° C as ATP-dependent formation of 1,3-diphosphoglyceric acid coupled to NADH oxidation via glyceraldehyde-3-phosphate dehydrogenase, as described by Makino et al. (1983). Ten microliters of desalted extract were used for each assay and assays were initiated by addition of ATP. Assays were corrected for ATP-independent 1,3-diphosphoglyceric-acid production.

Antibody production and Western analysis. The production of monospecific polyclonal antibodies against Rubisco and Ru5P kinase and the isolation of the immunoglobulin G (IgG) fraction was as described in Crafts-Brandner et al. (1990). The antisera for Rubisco and Ru5P kinase used in this study were previously shown to be highly specific for the respective antigen, and the Rubisco antiserum was shown to have a much higher titer for the small subunit relative to the large subunit of the protein (Crafts-Brandner et al. 1990).

For Western analysis, leaf discs were extracted in 50 mM Tris-NaOH (pH 7.65) 5 mM DTT, 1 mM PMSF and 20 μM leupeptin, and supernatants and pellets were prepared as described for Rubisco assays. Equal volumes of supernatant and pellet fractions were subjected to 9–18% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Chua 1980), electroblotted overnight onto nitrocellulose, and probed with both anti-Rubisco and anti-Ru5P kinase IgG as described by Salvucci and Ogren (1985). An alkaline-phosphatase-conjugated second antibody was used to make visible the 55- and 14-kDa subunits of Rubisco and the 43-kDa subunit of Ru5P kinase.

Photosynthesis. Photosynthesis, transpiration, and stomatal resistance were measured with an LI 6000 (LI-COR, Lincoln, Neb., USA) portable photosynthesis system. A 1073-ml leaf chamber was used and gas-exchange parameters were determined on 1250 mm^2 of leaf area.

Fractionation of pellets of leaf extracts. Leaf material was homogenized as described for Rubisco assays except that casein was not included in the buffer. Extracts were layered on 40% Percoll containing 50 mM Tricine, 1 mM EDTA, and 5 mM DTT. After centrifugation at 3000·*g* for 8 min in an HB-4 (Sorvall Instruments, Wilmington, Del., USA) rotor, the membranous material at the Percoll interface was isolated and layered on linear gradients of 1–2 M sucrose in 50 mM Tricine, 1 mM EDTA and 5 mM DTT

Table 1. Distribution of Rubisco activity in supernatants and pellets of leaf extracts of defruited soybean plants of cultivars spanning a range of maturity groups

Cultivar	Maturity group	Rubisco activity[a] ($\mu mol \cdot m^{-2} \cdot s^{-1} \pm SE$)		
		Supernatant	Pellet	% in pellet
McCall	00	85.4±18.1	38.3±10.8	31
Maple Arrow	00	79.2± 9.2	41.2± 4.6	34
Ozzie	0	1.6± 0.2	74.9± 5.3	98
Dawson	0	36.9±17.6	56.6±14.8	60
Hardin	I	0.6± 0.0	41.3± 3.0	99
Anoka	I	0.6± 0.0	44.7± 4.1	99
Elgin	II	2.0± 0.6	62.0± 2.1	97
Harosoy	II	16.7± 4.4	45.1± 2.6	73
Century	II	1.3± 0.4	40.3±12.9	97
Williams 82	III	1.7± 0.6	29.0± 4.9	94
Harper	III	1.9± 0.9	34.4± 3.6	95
Essex	V	0.6 ±0.0	42.6± 1.1	99

[a] Rubisco activity extracted from leaves of field-grown defruited plants in 1988; control plants with pods were at growth stage R7 (Fehr and Caviness 1977) at the time of sampling

with a 2.3 M sucrose cushion. The gradients were centrifuged at 285000·*g*, for 3 h in a SW-40 rotor (Beckman Instruments, Palo Alto, Cal., USA) and fractionated prior to assay for Rubisco activity. For extracts from either young vegetative plants or from control plants with fruits the Rubisco activity remained at the top of the gradients. For extracts from defruited plants the Rubisco activity was found in a clearly visible light-green band at the interface between 2 and 2.3 M sucrose. This fraction was stored at −80° C.

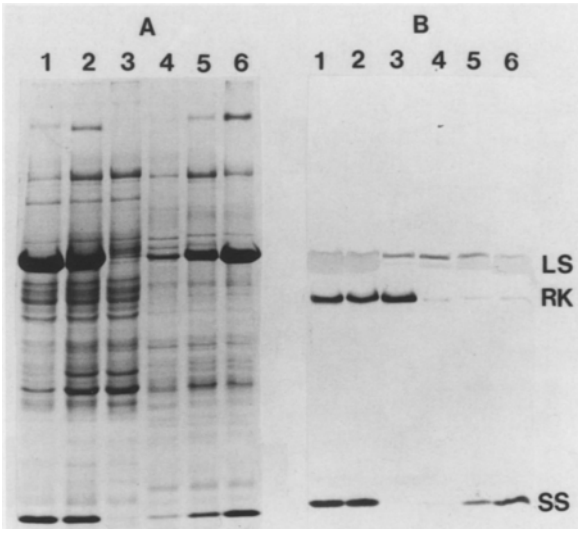

Fig. 1 A, B. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (**A**) and Western-blot (**B**) analysis of supernatant and pellet fractions of leaf extracts of young, vegetative and of defruited soybean plants. *Lanes 1* and *4* represent supernatant and pellet, respectively, of young, vegetative plants of cv. Ozzie. *Lanes 2* and *5* represent supernatant and pellet, respectively, of defruited plants of cv. McCall. *Lanes 3* and *6* represent supernatant and pellet, respectively, of defruited plants of cv. Ozzie. Defruited plants were sampled at the same time and from the same experiment as reported in Table 1. Equal amounts of leaf area, buffer volume, and sample aliquot were used for each treatment. The Western blot was probed with anti-Rubisco and anti-Ru5P kinase antisera. *LS* and *SS* denote the 55- and 14-kDa subunits of Rubisco, respectively, and *RK* denotes the 43-kDa subunit of Ru5P kinase

Results and discussion

The effect of continuous fruit removal on distribution of Rubisco between the pellet and supernatant fractions in leaf extracts from several cultivars representing a range of maturity groups is presented in Table 1. Leaf samples for each cultivar were taken when control plants of that cultivar were past growth stage R7 (R7 = at least one pod on the main stem has reached its mature pod color; Fehr and Caviness 1977) and had just a few yellow leaves attached to the plants. Rubisco activity in the supernatants ranged from 0 to 85 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the different cultivars. These differences were found to be reproducible in a second growing season (data not shown).

The level of Rubisco activity found in the resuspended pellets of the cultivars was also found to vary, ranging from 30 to 99% of the total activity. There was no consistent trend among the maturity groups relative to the effect of fruit removal on the distribution of Rubisco activity in the extracts.

Two cultivars, McCall and Ozzie, that differ markedly in response to fruit removal were used to determine the relationship between Rubisco activity and quantity in the leaf extracts. Leaf extracts from young, vegetative Ozzie plants were also included for comparison. Western-blot analysis of the extracts (Fig. 1) indicated that the relative quantity of Rubisco in the supernatant and pellet fractions was similar to the distribution of Rubisco activity (Table 1) for the two cultivars. There were large and comparable amounts of soluble Rubisco in extracts from leaves of young, vegetative Ozzie and defruited McCall plants, but leaf extracts of defruited Ozzie plants contained no detectable soluble Rubisco small subunit and had only a slight reaction with the Rubisco large subunit. The amount of Rubisco in the pellet fractions was essentially the opposite of that in the supernatant; pellets of extracts from young vegetative plants, defruited McCall plants, and defruited Ozzie plants contained, in this sequence, a small, intermediate, and large amount of Rubisco.

Using chloroplast extracts from spinach (*Spinacia oleracea* L.), McNeil and Walker (1981) reported that a large amount of Rubisco activity was associated with pelleted thylakoids when Mg^{2+} was present in the lysis buffer. This association between Rubisco and thylakoids could be readily broken by deletion of Mg^{2+} or inclusion of 0.2% (v/v) Triton X-100 in the medium used for osmotic shock. To determine whether the insoluble Rubisco from leaf extracts of defruited soybean plants was the result of a loose association with pelleted membranes, the tissue-homogenization procedure was modified. Treatments such as increased buffer-volume: tissue ratio and inclusion of 10 mM EDTA (in the absence

Table 2. Effect of leaf-homogenization procedure on the relative amount of Rubisco activity found in the pellet of leaf extracts of defruited soybean plants, cv. Ozzie

Homogenization buffer	Rubisco activity[a] (% in pellet)
Standard[b] −1.0 ml	98
Standard −5.0 ml	96
Standard −10.0 ml	98
Standard +10 mM EDTA, minus $MgCl_2$ −1.0 ml	98
Standard +0.1% (v/v) Triton X-100 −1.0 ml	97
Standard +0.5% (v/v) Triton X-100 −1.0 ml	98
Standard, wash pellet with 1.0 M NaCl −1.0 ml	90
Standard +1.0 M NaCl −1.0 ml	79
Standard[c] +8.0 M urea −1.0 ml	0
Standard[c] +5% (w/v) SDS −1.0 ml	0

[a] Rubisco activity extracted from 113 mm^2 of leaf area from field-grown defruited plants. Plants were sampled in 1988 at the time when control plants with pods were at growth stage R7 (Fehr and Caviness 1977). For all treatments the total activities (pellet plus supernatant) were within 5% of each other

[b] Standard buffer was as indicated in *Material and methods* except that casein was not included in the homogenization medium

[c] Rubisco amount estimated by SDS-PAGE of supernatant and pellet (data not shown)

Table 3. Transpiration and leaf resistance of leaves of defruited and control soybean plants, cv. Essex[a]

Days after fruit removal	Transpiration (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$ ±SE)		Leaf resistance ($s \cdot cm^{-1}$ ±SE)	
	Control	Defruited	Control	Defruited
5	9.5±0.3	9.2±0.2	0.4±0.0	0.5±0.0
7	15.5±0.7	13.8±0.5	0.5±0.0	0.6±0.0
10	11.8±0.7	9.8±0.2	0.4±0.0	0.7±0.0
13	9.9±0.3	10.0±0.2	0.5±0.0	0.5±0.0
15	10.5±0.4	9.2±0.2	0.4±0.0	0.7±0.0
18	9.9±0.4	8.9±0.4	0.5±0.0	0.7±0.1
20	10.3±0.9	6.2±0.6	0.9±0.2	2.2±0.3
24	9.4±0.4	7.0±0.4	0.7±0.0	1.2±0.1
25	6.7±0.2	5.0±0.3	0.9±0.0	1.6±0.2
27	6.2±0.5	3.3±0.5	0.8±0.2	2.4±0.4
31	2.6±0.2	2.4±0.2	2.8±0.3	3.3±0.3
34	2.0±0.3	2.8±0.3	3.8±0.4	2.8±0.6

[a] Measurements made on the same leaves and at the same time as photosynthesis measurements reported in Fig. 1. Experiment was conducted in the field in 1989

of $MgCl_2$), 0.1% or 0.5% (v/v) Triton X-100 did not release Rubisco from the pellet (Table 2). Washing the pellet with 1 mM NaCl or inclusion of 1 M NaCl in the homogenization buffer released only a small amount of the Rubisco activity. Complete solubilization, in contrast, was achieved with treatment with denaturants such as 8 M urea or 5% (w/v) SDS. These data indicated that the formation of insoluble Rubisco did not result from a loose association of Rubisco with the pelleted membranes.

The formation of insoluble Rubisco as a function of time after fruit removal was determined using Essex, a soybean cultivar that loses all soluble Rubisco in response to continuous fruit removal. The photosynthesis rate of defruited plants was slightly lower than for controls by a relatively constant amount between 5 and 18 d after fruit removal and appeared to decline at a faster rate for defruited plants between 18 and 27 d after fruit removal (Fig. 2). Fruit removal did affect stomatal function as indicated by increased leaf resistance and decreased transpiration compared to a controls (Table 3), and thus, differences in photosynthesis between the treatments could be partially attributed to stomatal closure. These results for the effect of fruit removal on photosynthesis were, in general, in agreement with results reported by Wittenbach (1982, 1983a).

In control plants, the majority of the Rubisco activity was in the supernatant and this activity declined with time (Fig. 3). The pellets of extracts from controls contained about 25% of the total activity at each sampling time. The distribution of Rubisco activity in defruited plants was distinctly different from that in the controls. The activity in the supernatant decreased to zero between 10 and 25 d after fruit removal whereas the activity in the pellet increased during this time. The pellet contained all of the activity after approx. 25 d after fruit removal. The level reached by 35 d after fruit removal was equivalent to 50–60% of the total (pellet plus supernatant) activity present in the extract prior to fruit removal. The loss of soluble Rubisco activity from leaves of the defruited plants was not well correlated with the decline in photosyntheis (Fig. 2).

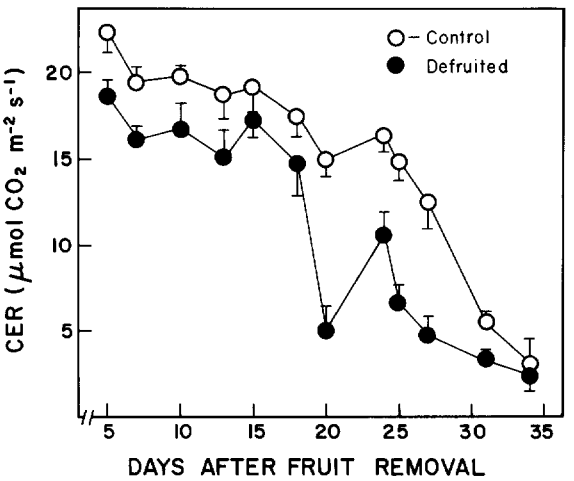

Fig. 2. Effect of fruit removal on CO_2-exchange rate (*CER*) of soybean leaves. Data were collected during 1989 from field-grown plants of cv. Essex. Data points represent the mean ±SE for three replications

In Wittenbach's experiments, loss of photosynthesis by defruited plants was not well correlated with loss of Rubisco in a growth-chamber study (Wittenbach 1982) whereas the two traits were closely correlated in

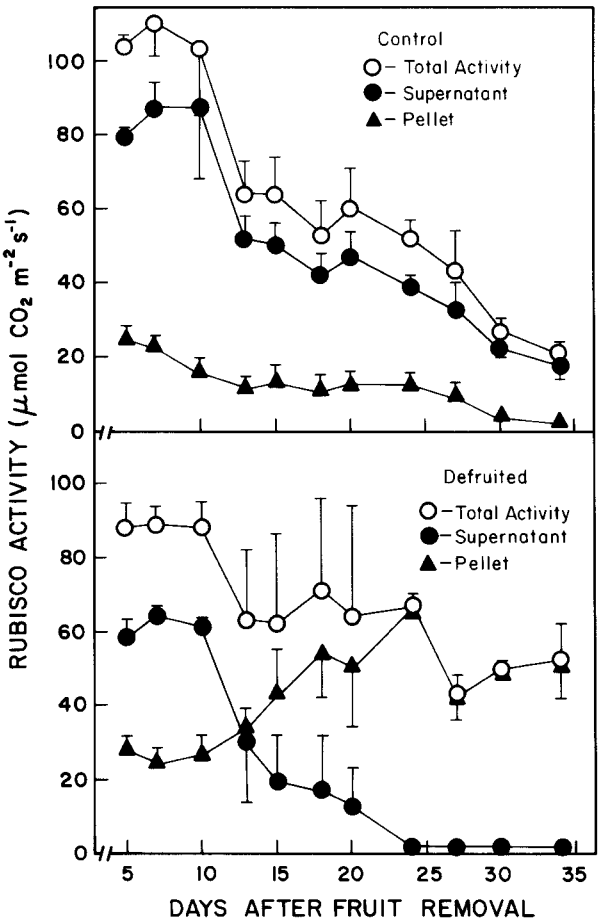

Fig. 3. Effect of fruit removal on distribution of Rubisco activity in leaf extracts of leaves of control (*upper panel*) and defruited (*lower panel*) soybean plants, cv. Essex. Activity was determined on the same experimental material as in Fig. 2. Data points represents the mean ± SE for three replications

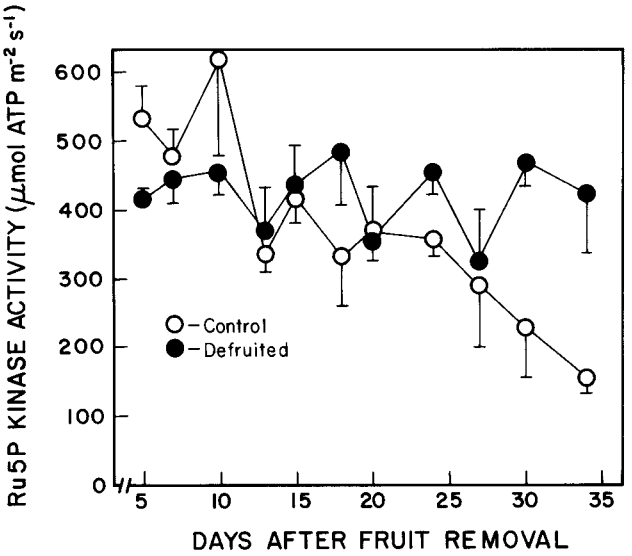

Fig. 4. Effect of fruit removal on Ru5P-kinase activity in the supernatant of leaf extracts from upper-canopy soybean leaves, cv. Essex. Activity was determined on the same experimental material as described in Fig. 2. Data points represents the mean ± SE for three replications

Table 4. Effect of leaf position on distribution of Rubisco activity in leaf extracts of defruited soybean plants, cv. Ozzie, grown in the field in 1988

Leaf position (node)[b]	Sampling time[a]	Rubisco activity (μmol·m^{-2}·s^{-1} ± SE)		
		Supernatant	Pellet	% in pellet
2	R7	1.0 ± 0.0	71.1 ± 12.2	99
11	R7	48.3 ± 2.2	13.4 ± 5.0	22
2	R7 + 2 weeks	1.0 ± 0.1	62.1 ± 4.2	98
11	R7 + 2 weeks	28.2 ± 8.1	8.1 ± 0.3	22

[a] Plants were sampled when control plants with fruits were at growth stage R7 and R7 + 2 weeks (Fehr and Caviness 1977)
[b] Node position below the uppermost leaf that was unrolled

a subsequent field study (Wittenbach 1983a). These results, in addition to our present results, indicate that changes in both Rubisco level or form, as well as alterations in stomatal function, may have influenced photosynthesis in defruited plants.

To test whether other soluble proteins were affected by fruit removal, Ru5P-kinase activity was determined in supernatants in leaf extracts of control and defruited plants (Fig. 4). This activity roughly followed soluble Rubisco activity in the controls (Fig. 3) but for defruited plants it remained essentially constant over the entire sampling time. Western-blot analysis of Ru5P kinase in supernatants and pellets confirmed the large amount of activity in the supernatant of extracts from defruited plants, and indicated that very little Ru5P kinase was located in the pellets of extracts from both young vegetative plants and defruited plants that had a large amount of Rubisco in the pellets (Fig. 1). Similar to the results for Ru5P kinase, high activities of PGA kinase were also found in supernatants of leaf extracts from defruited plants that had no soluble Rubisco activity (data not shown). Therefore, it appeared that the formation of insoluble Rubisco in leaf extracts from defruited plants was not a general effect on all Calvin-cycle enzymes.

The distribution of Rubisco in supernatants and pellets of extracts from defruited plants was also influenced by node position and-or light. For example, lower leaves of field-grown plants contained a much lower percentage of the total Rubisco activity in the pellet compared with leaves from the top of plant (Table 4). In addition, greenhouse-grown plants that had 70% of the fruits removed also lost all soluble Rubisco from leaves from the top of the plant (Table 5). However, if these plants were placed in 90% shade the distribution of Rubisco activity was altered such that 40% of the activity was retained in the supernatant. These results indicate that, in addition to the genetic component, environmental factors

Table 5. Effect of shade on distribution of Rubisco activity in leaf extracts of defruited soybean plants, cv. Ozzie, grown in the greenhouse

Treatment	Rubisco activity[a] ($\mu mol \cdot m^{-2} \cdot s^{-1} \pm SE$)		
	Supernatant	Pellet	% in pellet
70% fruit removal	0.4±0.2	17.8±3.7	98
70% fruit removal/ 90% shade	8.9±4.4	12.9±4.3	59

[a] Leaves were sampled when control plants with fruits were at growth stage R7 (Fehr and Caviness 1977). The shade treatment was applied at the time of fruit removal

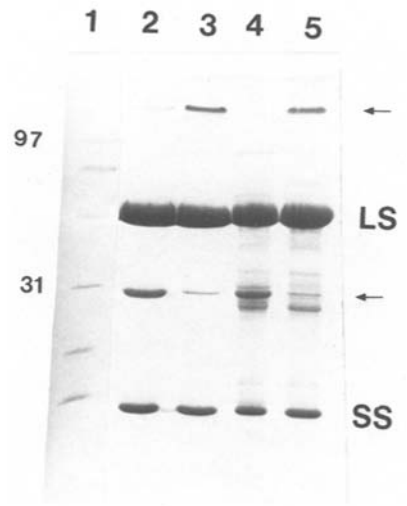

Fig. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of pellet fractions from leaf extracts of defruited soybean plants, cv. Ozzie, prior to and after sucrose-density-gradient fractionation. Extracts were previously shown to contain no soluble Rubisco activity (Table 1). Sucrose-gradient samples were taken from the interface between 2 and 2.3 M sucrose; this fraction contained all of the Rubisco activity loaded on the gradient. Samples were either boiled for 3 min or not boiled prior to loading on the gels. *Lane 1*, Molecular-mass (Mr) standards; *lane 2*, sucrose-gradient fraction, boiled; *lane 3*, sucrose-gradient fraction, not boiled; *lane 4*, resuspended pellet prior to sucrose-gradient fractionation, boiled; *lane 5*, resuspended pellet prior to sucrose-gradient fractionation, not boiled. *LS* and *SS* indicate the large and small subunit of Rubisco, respectively. *Arrows* indicate the polypeptide that co-purifies with Rubisco on the sucrose gradients

also influence the distribution of Rubisco in leaves of defruited soybean plants.

The insoluble Rubisco was partially purified by sucrose-density-gradient centrifugation of the pellet fractions of leaf extracts. When this procedure was carried out using extracts from young vegetative plants, or from plants with developing fruits, all of the Rubisco activity was found at the very top of the gradient. When pellet fractions from defruited plants were subjected to this treatment, a light-green band containing Rubisco activity was clearly visible at the 2/2.3 M sucrose interface. As indicated by SDS-PAGE analysis (Fig. 5) this fraction contained Rubisco and a 30-kDa polypeptide in approximately stoichiometric amounts. The polypeptide apparently aggregates as indicated by the high-molecular-mass (approx. 120000) band observed if the samples were not boiled prior to electrophoresis. Thus, the formation of insoluble Rubisco as a result of fruit removal is apparently associated with an interaction between Rubisco and the 30-kDa polypeptide. Rubisco activity of the isolated complex had a specific activity of 0.65 $\mu mol \cdot (mg\ protein)^{-1} \cdot min^{-1}$. Specific activity of the insoluble Rubisco prior to activation by Mg^{2+} and CO_2 was 0.13 $\mu mol \cdot (mg\ protein)^{-1} \cdot min^{-1}$. Therefore, despite being insoluble, the isolated Rubisco exhibited a surprisingly high specific activity and was able to activate in the presence of Mg^{2+} and CO_2.

In summary, our results demonstrate that loss of photosynthesis during senescence is associated with quite different phenomena in control plants compared to defruited plants. Fruit removal results in the appearance of an insoluble Rubisco in leaf extracts. This is dependent upon both cultivar and environment and is apparently related to a specific association between Rubisco and a newly produced polypeptide. Other Calvin-cycle enzymes are active and remain in the soluble phase of leaf extracts. It would be premature to speculate at this time about the possible physiological importance of this observation; we are currently in the process of purifying and raising antibodies to the 30-kDa polypeptide in an attempt to resolve this question. The results presented in this study, however, do indicate that source/sink manipulations in soybean (and possibly other plants) can profoundly affect the distribution of Rubisco in leaf extracts. They provide an explanation for the variable results in the literature concerning the magnitude of loss of Rubisco in response to fruit removal, and indicate that this factor must be considered in studies of source/sink manipulation.

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