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NITROGEN PARTITIONING AND NET PHOTOSYNTHESIS IN SOYBEAN GENOTYPES DIFFERING IN SEED FILLING DURATION AND N SOURCE

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GUFFY R. D., VASILAS B. L. and HESKETH J. D. *Nitrogen partitioning and net photosynthesis in soybean genotypes differing in seed filling duration and N source.* BIOTRONICS **21**, 1–10, 1992. Four soybean cultivars differing in the duration of the seed filling period (*SFP*) were evaluated in the field for N and dry weight accumulation, N_2-fixation and net leaf photosynthetic CO_2 exchange rates (*CER*). N_2-fixation was measured by isotope dilution, using a non-nodulating (non-nod) near-isogenic line of the cultivar Harosoy as a control. There were consistent differences between the non-nod and its sister cultivar for seed yield, N accumulation and N redistribution, but both genotypes had the same *SFP* duration and final shoot biomass. Reduced N accumulation in the non-nod resulted in less dry weight partitioned to seeds but no reduction in the length of the *SFP*.

Cultivars with long *SFP*'s had greater yield, more N and dry weight accumulation between the beginning of seed fill and maturity, and a slower decline in leaf *CER* during seed fill, compared to short SFP cultivars. However cultivars varied with respect to vegetative dry weight, N accumulation, N redistribution, the proportion of total N allocated to seeds, and the peak *CER*, independent of differences in *SFP*. Neither the amounts of accumulated N or fixed N_2 explained differences in the *SFP*.

Key words : N_2-fixation ; carbon budget ; nitrogen budget ; legume ; *Glycine max* (L.) Merr.

INTRODUCTION

The duration of the seed filling period (*SFP*) in soybean [*Glycine max* (L.) Merr.] and various aspects of the photosynthetic CO_2 exchange rate (*CER*) and nitrogen assimilation have been reported to be related to yield (*5, 10, 14, 16, 25, 26, 30*). Genetic variation for the duration of the seed filling period (*7, 12, 21*), *CER* (*17*) and N accumulation and distribution (*27, 31*) has been shown, but correlations with yield have been low (*12, 16, 25*).

N losses from vegetative tissue coincide with N gains in the seed (*3, 15, 31*), but considerable genetic variation in accumulation and distribution of N occurs (*27*). During seed fill, nitrate reduction and N_2–fixation decline (*29*), and N is redistributed within the plant to meet N demands in the seed. The loss of N from vegetation has been proposed as a mechanism for controlling senescence in soybean (*23, 24*), but this has been questioned (*20, 22*). Comparisons of N harvest indices among a number of non–nod strains suggested differences in the efficiency of N redistribution from vegetation to seeds (*18*).

Seed growth and N accumulation during the filling period may depend upon the concurrent photosynthetic supply (*30*). Leaf *CER* values declined rapidly during seed fill (*11*), even when long–season cultivars were induced to flower early by temporary short–day treatments (*8*). Boon–Long *et al.* (*1*) found that leaf *CER* declined faster in a cultivar with a short *SFP*, compared to a genetically–related one with a long *SFP*. Thus *SFP* and *CER* decline are closely correlated, but the causal mechanism has not been determined.

Clearly, the processes of seed development and whole plant senescence are related to *CER*, N assimilation, and N redistribution, as proposed by the Sinclair –de Wit (*24*) model. However, it is not clear if genotypic differences in the duration of the *SFP* can be explained on this basis. The purpose of this research was (a) to quantify the relationship between N assimilation, *CER* and seed growth in soybean genotypes known to differ in the *SFP* and (b) to examine the role of N partitioning and *CER* on the duration of the *SFP*.

MATERIALS AND METHODS

Soybean cultivars with relatively long [Harosoy and Bansei (Ames)] and short (PI 416.773 and PI 404.166) seed filling periods were evaluated for leaf net photosynthetic carbon dioxide exchange rates (*CER*) and nitrogen assimilation rates in irrigated field plots in 1985. Bansei (Ames), PI 416.73 (Aka Wase from Japan) and PI 404.166 (Krasnoarmejskaja from the Soviet Union) were cultivars selected from the USDA germplasm collection in Urbana IL. PI 404.166 has been classified as a maturity group (*MG*) III cultivar; the others as *MG* II. These genotypes were chosen based on differences found in the seed filling period (*SFP*) during two years of field evaluations at Urbana using the developmental growth stages of Fehr and Caviness (*9*). The number of days to flowering (*R1*), beginning seed fill (*R5*) and physiological maturity (*R7*) were recorded on a plot basis, with the *SFP* defined as the number of days fron *R 5* to *R 7*. The experimental plot areas for N_2–fixation and *CER* studies were near each other on the experimental farm.

Nitrogen experiment

In addition to the four cultivars, a near–isogenic backcross derived line (isoline) of Harosoy background (courtesy of Dr. R. L. Bernard), but lacking root nodules (non–nod), was included to serve as a control, since it couldn't fix N_2.

The experimental design, culture practices, treatment applications and tissue

sampling techniques were described previously (*13*). The soil was treated with a pre-emergence herbicide, Ethalfluralin [N-ethyl-N-(2-methyl-2-propenyl)-2, 6-dinitro-4-(tri-fluormethyl) benzenamine] but was not fertilized. A ramdomized complete block design was used with four replications. Each plot consisted of a single row of each cultivar, 1.8 m in length, adjacent to a non-nod isoline row, with 0.76 m between rows. The experiment was planted 28 May 1985 on a Flanagan silt loam (fine, montmorillonitic, mesic, Aquic Argiudoll) soil. Ten atm% excess ^{15}N-enriched $(NH_4)_2SO_4$ (*13*) was applied at a rate of 10 kg N ha^{-1} immediately after planting. The fertilizer was applied in solution to the soil surface with a tractor-mounted spray system and followed by 2 cm water applied by an overhead sprinkler irrigation system. Irrigation was provided as needed during the growing season. Plots were thinned to a uniform plant density of 20 plants m^{-1} row when the first trifoliolate was fully expanded.

Abscised plant tissue, mostly leaves and petioles, were collected from cages in 1 m of row twice weekly. Whole shoots were harvested by cutting plants at the soil surface from 1 m of row when cultivars reached *R1, R5* and *R7*. At the same time an adjacent row of the non-nod isoline was harvested. At *R 7* harvested plants were separated into seeds and vegetative tissues plus the pod walls (*VTPW*). All plant samples were dried at 60℃, weighed, ground (<1 mm) and sub-sampled for N determinations. Total N was determined using a semi-micro steam distilation procedure (*2*) and the N isotope ratio by mass spectrometry. N_2-fixation was estimated by isotope dilution methods of Legg and Sloger (*19*). An analysis of variance was performed on the data to estimate the least significant difference (*LSD*) at $P=5\%$.

Photosynthesis experiment

The four soybean cultivars were planted 24 May in a randomized block experimental design with four repelications (We included the non-nodulated Harosoy strain; unfortunately the seed from a different source was contaminated with seed from nodulated plants). Plots consisted of three rows 4.6 m long with 0.76 m between rows, on the same soil type and using the same cultural methods as given above for the nitrogen experiment.

CER measurements were made twice weekly from *R1* (mid-July) to *R7* (mid-September), using a hand-held, hinged leaf chamber (*11*) made of clear plastic with a 38 mm circular window covered with polypropylene. The chamber was clamped over 113.4 mm^2 of the center leaflet of a healthy, mature trifoliolate. Such trifoliolates were four nodes below that of the highest fully expanded leaf on the main stem. Gordon *et al.* (*11*) found maximum *CER* values at this node during reproductive growth. Two measurements were made on separate plants in each plot. Leaf temperture, measured with a fine thermocouple in contact with the underside of the leaflet, averaged 2-3℃ above ambient (20-35℃) over the course of the experiment. Leaf temperatures were kept at 35℃ or below with an infra-red filter and by precooling the air supply.

Subsamples of air entering and leaving the leaf chamber were directed to an infra-red gas analyzer for determining the difference in CO_2 content. *CER* was

measured in full sunlight, or in ambient sunlight supplemented with a 400 W sodium vapor lamp, to give quantum flux densities greater than 2000 μmol photons $m^{-2}s^{-1}$ (400–700 nm wavelengths), to minimize effects of differences in *PAR* at the surface of the leaf on *CER*. Gas exchange measurements were continued until maximum *CER* was achieved; this took as much as 20 min for some leaves.

Ten consecutive plants in the center row of each plot were randomly selected for measurements of growth stage. A plot was defined to be at one of three growth stages (*R1*–one open flower at any node on the main stem, *R5*–a 3 mm bean in a pod on one of the four uppermost mainstem nodes, or *R7*–first brown pod on the mainstem) when five or more plants showed the necessary characteristics. An analysis of variance was performed on the *CER* data for each sample date. Mean daily *CER* values for each cultivar were plotted against days after *R5*; regression polynomials were estimated for the data.

RESULTS

Measurements were made at the three standard reproductive growth stages *R1*, *R5* and *R7* (see above for definitions). Generally, vegetative growth and pod set is complete at about *R5* and the near-linear phase of bean dry matter accumulation begins. Bean dry matter accumulation is almost complete by *R7*.

Nitrogen experiment

Harosoy and its non-nodulated backcross near-isoline (non-nod) started to flower at 43 days after planting, but both *R5* and *R7* were delayed three days in the isoline (Table 1). Hence the *SFP*'s (*R5* to *R7*) for both were 39 days. Both lines had accumulated about the same amount of dry matter and N (Table 2) by *R1*, but Harosoy produced more N by *R5*. Both produced the same amount of dry matter, including abscised leaf material, by *R7*, but Harosoy accumulated much more N. Seed yield was lower in the non-nod by *R7* but non-seed plant

Table 1. Dry weights at different reproductive stages of growth.

Cultivar	Reproductive Growth Stages				Dry Weights Whole Shoots*VTPW***			Seed	Total
	R1	*R5*	*R7*	*SFP**	*R1*	*R5*	*R7*	*R7*	*R7*
	(days)				($g\ m^{-2}$)				
Harosoy	43	68	107	39	85	367	352	208	559
Harosoy non-nod	43	71	110	39	93	327	406	146	552
Bansei (Ames)	51	72	112	40	155	366	374	154	528
PI 416.166	64	84	114	30	200	343	310	107	415
PI 404.166	77	91	118	27	452	491	472	114	585
LSD (P=0.05)	2	2	2	3	83	32	61	30	86

* Days from *R5* to *R7*.

** Vegetative tissue plus pod walls, including abscised leaves and petioles.

Table 2. Nitrogen concentration and content in various plant parts at different growth stages.

Cultivar	Ab*	Shoots**	*VTPW****		Seed	Shoots**	*VTPW****		Seed	Total
	R7	*R1*	*R5*	*R7*	*R7*	*R1*	*R5*	*R7*	*R7*	*R7*
	N ($g\ kg^{-1}$)				(N $g\ m^{-2}$)					
Harosoy	19	37	30	15	62	3.1	10.9	5.1	12.8	17.9
Harosoy non-nod	14	35	24	9	51	3.3	7.5	3.8	7.4	11.2
Bansei (Ames)	19	37	30	15	62	5.0	10.1	5.3	9.8	15.1
PI 416.773	17	28	26	13	69	5.5	8.6	4.0	7.3	11.3
PI 404.166	19	26	23	14	64	9.7	10.8	6.5	7.2	13.7
LSD ($P=0.05$)	1	1	1	2	1	0.9	1.3	0.9	1.9	2.4

* Abscised leaves and petioles.
** Whole shoots including abscised tissue.
***Vegetative tissues plus pod walls, including abscised leaves and petioles collected from *R1* to *R7*.

Table 3. The amount of nitrogen redistributed from vegetative tissues between *R5* and *R7*, the rate of depletion of N during this period, the ratio of N at *R5* to that at maturity, the percent of seed N redistributed from leaves, and the ratio of seed to total N.

Cultivar	Redistributed Nitrogen	Ratio of Total N at *R5* to mature seed N	percent of Seed N redistributed from leaves		Ratio of mature seed N to total N accumulated
	($g\ m^{-2}$)	($mg\ m^{-2}\ d^{-1}$)	(%)		
Harosoy	5.80	146.2	85.4	45.5	71.3
Harosoy non-nod	3.7	92.7	101.5	49.8	66.0
Bansei (Ames)	4.72	118.0	103.8	48.4	64.6
PI 416.773	4.61	153.6	119.2	63.7	64.2
PI 404.166	4.35	158.5	156.3	54.5	52.3
LSD ($P=0.05$)	1.44	34.7	26.5	ns	4.5

mass was as much higher (but not significantly higher at the $P=0.05$ level, 54 vs. a *LSD* value of 61), compared to the nodulated Harosoy. Accumulation of non-seed dry matter was largely complete in Harosoy by *R 5* (Table 2). However, for the non-nod, the vegetative dry weight, including pod walls (*VTPW*, Table 2) increased by 79 g m^{-2} (24%) after *R5*.

The concentration of N in mature seeds of the non-nod line was lower (Table 2, 5.1 vs. 6.2%N), as was that in abscised leaves (1.4 vs. 1.9%N), compared to Harosoy. In this same comparison, more N was redistributed from vegetative plant parts to seed in Harosoy, at a faster rate (Table 3) and Harosoy allocated a larger proportion of its total N to seeds.

Cultivars with short and long *SFP*'s required about 30 and 40 days to get

Table 4. N_2-fixation and the percent nitrogen derived from N2-fixation found in seeds, vegetative tissues and pod walls (*VTPW*, see Tables 1 and 2), and total shoots at *R7*.

Cultivar	N_2-fixation (g m^{-2})			Percent N from		N_2-fixation
	Seeds	*VTPW*	Total Shoot	Seeds	*VTPW*	Total Shoot
Harosoy	6.2	1.1	8.0	48.2	40.9	44.8
Bansei (Ames)	3.7	0.6	4.9	38.2	26.4	33.4
PI 416.773	2.9	0.4	4.0	39.6	25.5	36.3
PI 404.166	3.2	0.8	5.3	51.8	34.1	42.4
LSD (P=0.05)	1.44	0.20	1.88	9.4	8.2	11.2

from *R5* to *R7*, respectively (Table 1). Harosoy produced the most seed mass; the short *SFP* cultivars the least (Table 1). There were no differences between PI 404.166 and the long *SFP* lines in total shoot biomass at *R7*.

Comparing tissue N concentrations (gN g^{-1}dry mass, or [N]) in the two short *SFP* cultivars with those in the other two nodulated lines, [N] was lower at *R1* and *R5*, [N] in abscised tissue was the same and seed [N] was higher (Table 2). Cultivar differences in accumulated shoot N at *R5* did not relate to differences in *SFP*, total accumulated N or seed yield (Tables 1 and 2).

Harosoy fixed more of its total N than other cultivars (Table 4). Although the short *SFP* cultivars yielded less; their seeds contained as much fixed N as the Harosoy seed.

Net leaf photosynthetic CO_2 exchange rate (CER)

In order to compare *CER* values during seed fill and account for differences in phenology, mean values for each cultivar are plotted against days from R5 (Fig. 1). Harosoy exhibited peak *CER* values just after *R5*, when seeds have been reported to accumulate dry matter at the maximum linear rate (*29*). *CER* declined rapidly thereafter, especially in the last 10 days before *R7*. The *CER* pattern for Harosoy was similar to that reported earlier (*11*). *CER* values for Bansei, which had the same *SFP* as Harosoy, Table 1, tended to be lower and declined sooner (Fig. 1a). We found virtually no net C fixation in the upper Bansei leaves 5 days prior to *R7* (Fig. 1a).

Both the short *SFP* cultivars tended to have peak *CER* values just prior to *R 5*, before the Harosoy *CER* peaked (Fig. 1b).

DISCUSSION

Differences in plant growth between Harosoy and its non-nodulated (non-nod) sister isoline should primarily be due to differences in the supply of N. Good early season growth in a non-nodulated line is common for the Urbana location under irrigation or good rainfall, but it may not be at locations with low soil N or during a drought stress.

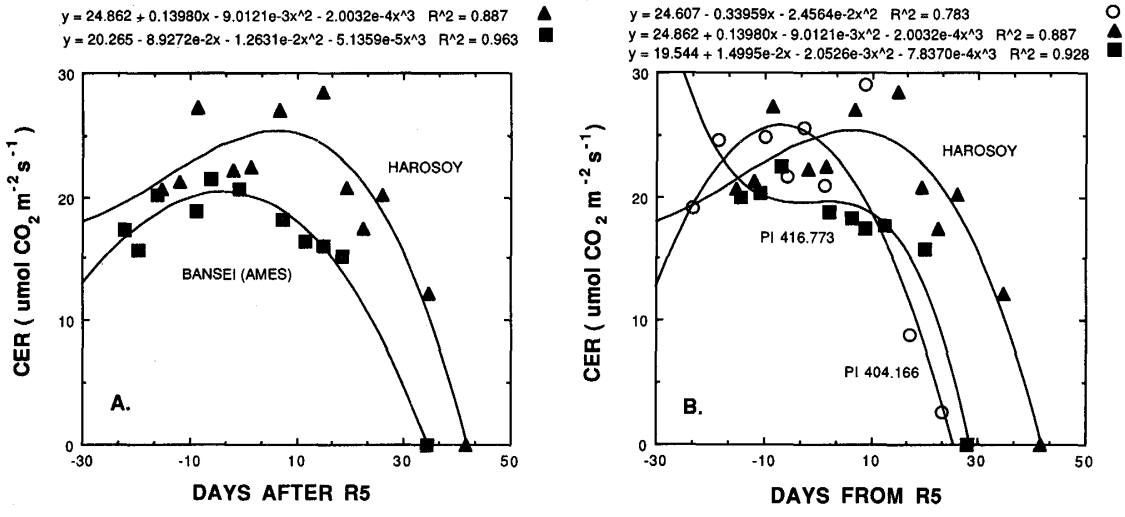

Fig. 1 Leaf CO_2 exchange rates [*CER*, mean of two determinations, μmol CO_2 m^{-2} s^{-1}, *LSD* ($P=0.05$)=3] from flowering to maturity for: A. Harosoy and Bansei (Ames) and B. Horosoy, PI 404.166, and PI 416.773. Maturity, or *R7*, occurred at 32 [Bansei(AMES)], 39 (Harosoy), 27(PI 404.166) and 30 (PI 416.773) days after flowering, or *R5*, for the strains shown.

Severe N stress during seed fill, induced by removing N from the nutrient solution the plants were growing in, has been shown to accelerate the decline in *CER* (1) and to reduce the effective seed filling period (*4, 6*). Their treatments differed from our own in that their N stresses were imposed after pod set was complete. In our experiments the non-nod began to accumulate less N during flowering and pod set, resulting in fewer pods being set. However, the similarity in total shoot biomass produced in the nodulated and non-nod isolines in our experiments suggested that *CER* in the non-nod was not greatly depressed, if total biomass partitioned to roots were the same. Ignoring roots, it would appear that less total shoot dry weight was partitioned to seeds in the non-nod, and the *SFP* was not shortened.

During seed fill, the non-nod accumulated and abscised less N than Harosoy. The lower N concentration of the abscised material reflected that throughout the plant, rather than an increase in N partitioning from senescing leaves to seeds (Table 3). Since not all vegetative N is available for redistribution, the larger vegetative dry weight in non-nod plants may have tied up more N, accounting for the observed differences in N redistribution and partitioning. Instead of reductions in the *SFP* and the enhancement of associated senecing processes, yield and seed N were reduced. Seed N seems to be more sensitive to N supply than the *SFP* or time to *R7*. These results are consistent with the observation that the *SFP* is a relatively stable genetic trait across different environments (*7,*

21, 25). While the Sinclair–de Wit model (*23, 24*), which tied senescence closely to the redistribution of N from vegetation to seeds, may ultimately prevail in the long run, in many cases the rate and amount of N redistribution seem to be adjusted over a fairly wide range of accumulated N values (in our experiment 11 to 18 g N m^{-2}) to keep the duration of seed fill and the rate of senescence constant. Sheehy (*22*) suggested that plants might adjust N supply and demand to delay senescence ; Derman *et al.* (*3*) and Nooden (*20*) reported evidence suggesting that both N redistribution and senescence are controlled by growth regulators generated in growing pods or seeds.

Streeter (*28*) observed that the N content of abscised tissue under N stress may be as low as 9 g N kg^{-1} dry weight, as we found for *VRPW* in the non–nod at *R7* (9 g N kg^{-1} dry weight, Table 2) but did not find for abscised leaves and petioles (Ab, *R7*, Table 2). The minimum N content of abscised tissue may vary with the severity of the stress and growth stage.

Although the four cultivars varied considerably in N and dry weight at *R1*, these differences were not indicative of such values after *R1*, or of the *SFP*, peak *CER*, or how *CER* declined after *R5*. The amount of total N at *R5* was a poor predictor of the duration of the *SFP* or seed yield, due to variations in N uptake, N redistribution, or *CER* during the *SFP*. Short *SFP*'s were associated with rapid decreases in *CER* after *R5*.

For Harosoy, high yield, a long *SFP* and a slow decline in leaf *CER* during seed fill was not incompatible with vigorous N redistribution and a high proportion of total N committed to seeds. Jeppson *et al.* (*18*) also reported that high yielding cultivars had the largest amounts of total N going to seeds.

The twice–weekly collection of abscised leaf material gave better estimates of various components of the plant N budget than what might have been achieved earlier in other reports for field–grown plants. However, the high availability of soil N in our experiments would have affected N_2 fixation and its distribution in the plant, as well as the N budget for our non–nod. The amount of available soil N and adjustments in N required for seed growth in the non–nodulated plants in our experiments were such that both nodulated and non–nodulated Harosoy plants matured at the same time. The relationship between N redistribution to the seed and senescence may be more complex than hypothesized in earlier models.

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