LEAF CHLOROPHYLL CONCENTRATION AND NITROGEN CONTENT IN SOYBEAN PLANTS TREATED WITH HERBICIDES[1]

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ABSTRACT - Under greenhouse condition an experiment was carried out to evaluate trifluralin, chlorimuron and clomazone effects on nitrogen content and chlorophyll concentration in soybeans (*Glycine max* (L.) Merr. cv Uberlandia), throughout the plant cycle. The samples were collected at 14-day intervals being the first sample collected at 14 days after emergence. Herbicides affected N concentration of plant parts only during the vegetative growth stages of soybeans. Trifluralin and clomazone applications reduced N concentrations in the stems, leaves and roots. Chlorimuron, however, tended to increase leaf and nodule N concentrations. Pod, pod wall and seed N concentrations were not influenced by the herbicides. Leaves, stems and roots had greater N concentrations at the beginning of the vegetative cycle. There were no effects of herbicides on chlorophyll concentrations. It was estimated that the soil gained 4.7 g/m^2 of N during the plant cycle, as result of nitrogen fixation.

Additional index terms: *Glycine max*, N_2-fixation, pigment, chlorimuron, clomazone, trifluralin.

CONTEÚDO DE NITROGENIO E TEOR DE CLOROFILA FOLIAR EM PLANTAS DE SOJA TRATADAS COM HERBICIDAS

RESUMO - Um experimento foi conduzido sob condições de casa-de-vegetaçao para avaliar os efeitos de trifluralina, clorimuron e clomazone sobre o acúmulo de nitrogênio na planta e teor de clorofila nas folhas, em soja (*Glycine max* (L.) Merr. cv. Uberlândia), ao longo do ciclo da cultura. As amostras foram coletadas a intervalos regulares de 14 dias, a partir da emergência. O teor de N nos órgaos foi máximo nos estádios iniciais da fase vegetativa. Somente nesta fase houve efeito dos herbicidas nos teores de N. Os tratamentos com trifluralina e clomazone reduziram o teor de N em caules, folhas e raízes. Clorimuron, entretanto, tendeu a aumentar o teor de N em folhas e nódulos. Os herbicidas nao influenciaram o teor de N dos legumes, pericarpos e sementes, nem a concentraço de clorofila nas folhas. Estimou-se que o solo teve um ganho de 4.7 g/m^2 de N durante o ciclo da cultura, como resultado da fixaçao de nitrogênio.

Termos adicionais para indexaçâo: *Glycine max*, fixaçao de N, pigmento, clorimuron, clomazone, trifluralina.

INTRODUCTION

Several herbicides may influence either uptake or translocation of mineral nutrients in plants. Soybeans treated with 3 mg/kg of trifluralin developed leaves with less chlorophyll content than untreated plants (Behran et al., 1979). Kust and Struckmeyer (1971) suggested that soybean plants treated with 74 to 110 mg/m^2 of trifluralin may develop nitrogen-deficiency symptoms if grown in soil low in available nitrate.

The leaf chlorophyll content decreased from 345 to 138 mg/m^2 when soybean plants were treated with 3 mg/kg of trifluralin, whereas 1 mg/kg of trifluralin showed a trend to increase leaf chlorophyll concentration (Behran et al., 1979). Cowpeas (*Vigna unguiculata*) treated with trifluralin showed greater leaf chlorophyll content than untreated plants (Behran et al., 1979).

Clomazone reduced chlorophyll concentration in eight-day-old soybean seedlings, during the first 56 h of greening (Argenta & Lopes, 1991). However, it did not affect chlorophyll content in 30-day-old soybean plants (Argenta & Lopes, 1992). The leaf chlorophyll content decreased in several soybean cultivars treated with imazaquin (Cayon et al., 1990). Imazaquin and chlorimuron are herbicides that inhibit the biosynthesis of branched chain amino acids (leucine, isoleucine and valine), clomazone affects carotenoid biosynthesis, whereas trifluralin directly disrupt cell division (Duke, 1990). Trifluralin, clomazone and chlorimuron are herbicides frequently used in soybean because they are effective to control a wide range of weeds.

Since there is little available information on the physiological effects of herbicides on leguminous plants, the present study was undertaken to evaluate the effects of trifluralin, chlorimuron and clomazone on nitrogen content and chlorophyll concentration in soybean plants.

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MATERIAL AND METHODS

The experiment was carried out under greenhouse condition, at Viçosa, MG, from October 23, 1990 to March 7, 1991. During this period, temperature ranged from 21 to 23^oC at night to maxima of 33 to 39^oC during the day. Relative humidity ranged from 38 to 60% in the day to a maximum of 100% at night. Soybean seeds (*Glycine max* (L.) Merrill cv. Uberlandia) were inoculated with *Bradyrhizobium japonicum*, and then sown in pots (six/pot). The substrate used for plant development was a Red-Yellow Lotosol, collected from a 0-0.2 m depht, with moderate fertility, clay texture and 3.2% organic-matter. It was amended with 0.9 g of lime per kg of soil, fertilized with 400 mg/kg of P and 50 mg/kg of K, and kept near to field capacity throughout the study. After emergence the seedlings were thinned to two per pot to obtain 15 plant/m^2. Other experimental conditions were according to Marenco et al. (1993).

The experimental design was a randomized complete block with treatments in split-plot replicated three times. The whole plots were the sampling dates, and the plots were the herbicide treatments. The herbicides tested were chlorimuron (0.70, 1.05 and 1.40 mg/m^2 a.i), trifluralin (80, 120 and 160 mg/m^2 a.i) and clomazone (80, 120 and 160 mg/m^2 a.i); there was also an untreated control. Herbicide were applied with a backpack sprayer operated with a boom pressure of 230 KPa and delivering a spray volume of approximately 40 mL/m^2 through flat fan nozzles. Trifluralin was incorporated immediately after application. Clomazone was applied to soil surface just after planting, whereas chlorimuron was applied 14 days after emergency (DAE).

Leaf chlorophyll was determined throughout the plant cycle at 14-day intervals. The first samples were taken at 21 and the last at 119 DAE. From individual plants, five- 9 mm-diameter discs were taken from the third upper leaf. The discs were immediately weighed, and then chlorophyll concentration determined (Arnon, 1949). At each sampling date the plant growth stage was recorded (Fehr et al., 1971).

The leaves (blade only), stems (stems and petioles), nodules, roots and pods (or pod walls and seeds) from each plant were separated, dried, in an oven set at 75^oC until reaching constant mass (approximately 48 h), and weighed. Whole plant part samples were finely ground, thoroughly mixed, and then stored dry in closed containers until analyzed for N content. Nitrogen was determined by Kjeldahl digestion, followed by colorimetric assay for ammonia-N (Jackson, 1965). The total plant N derived from N_2 fixation (N_f) was calculated as: $N_f = P \times T_n$, where, P is the proportion of N derived from N_2 fixation and T_n is the total plant N content. Thus, the plant N derived from soil uptake (N_s) was calculated as: $N_s = T_n - N_f$. The proportion of N derived from N_2 fixation, at each sampling date, was obtained from a previous report (Marenco et al., 1993). The data were subjected to analysis of variance, data derived from weighing were previously transformed to log (Y).

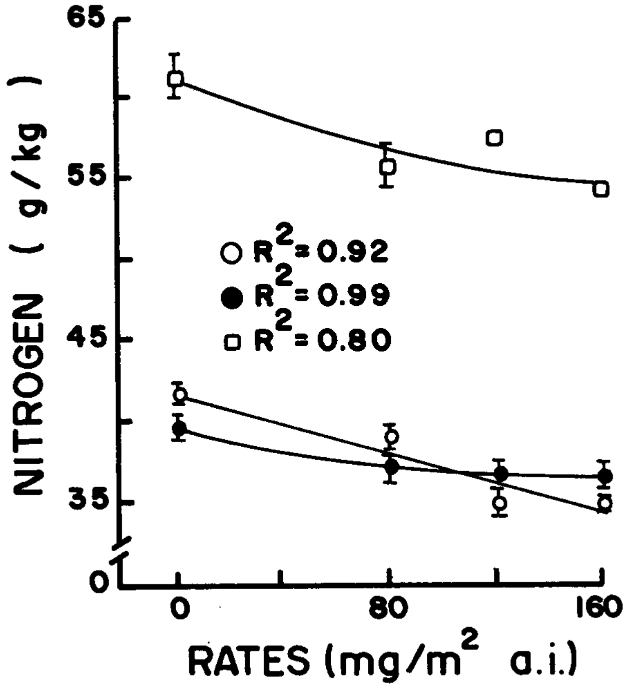

FIGURE 1- Nitrogen concentration in parts of soybean plants treated with trifluralin. Effects in the roots (❍) and stems (❶) at 14 days after emergence (DAE), and in the leaves (☐) at 28 DAE. Each point, mean of three replications; bar, ± SE.

RESULTS AND DISCUSSION

Nitrogen concentrations in the stems, leaves and roots decreased rapidly with plant age up to 42 DAE (P < .01). The N concentration remained nearly constant in the leaves and stems from 56 to 70 DAE, whereas it increased in both the stems, from 8.6 to 12.3 mg/g, and the leaves, from 36.3 to 41.2 mg/g, between the R_4 and R_6 growth stages (Table 1). This suggests that the seed N requirements at both R_5 and R_6 growth stages were smaller than the available N from N_2 fixation, the exceeding N being accumulated in leaves and stems. Batchelor et al. (1984) also observed greater N concentration in leaves of soybeans between the R_2 and R_5 growth stages. There is evidence that vegetative store proteins may be rapidly synthesized or degraded according to other plant part nutrient requeriments (Staswick, 1989). In contrast to stems and leaves, the N concentration in the roots decreased slightly throughout the reproductive growth stages.

N concentration was greater in leaves than in roots. In addition, it was greater in roots than in stems, during the most part of the plant cycle (Table 1). On the average, N concentration in the leaves ranged from about 24 to 80 g/kg, at the R_8 and V_3 growth stages, respectively (Table 1). These results are similar to those previously

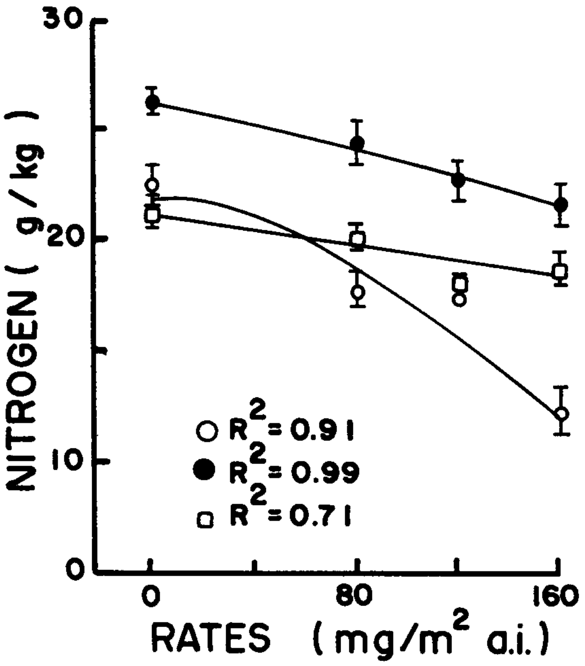

FIGURE 2- Nitrogen concentration in parts of soybean plants treated with trifluralin. Effects in the stems at 28 days after emergence (❍) (DAE), and effects in the roots at 28 (❶) and 42DAE (☐). Each point, mean of three replications; bar, ± SE.

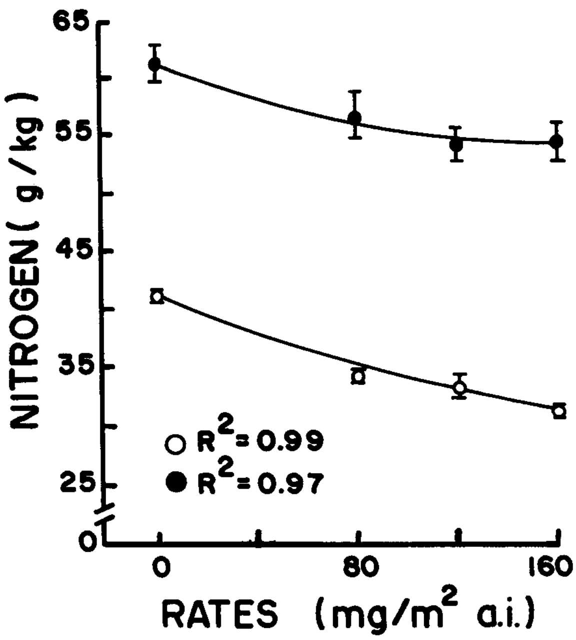

FIGURE 3- Nitrogen concentration in parts of soybean plants treated with clomazone. Effects in the roots at 14 (❍) and in the leaves at 28 days after emergence (❶). Each point, mean of three replications; bar, ± SE.

reported (Ohlrogge, 1960; Batchelor et al., 1984). On the other hand, N concentration ranged from 8.2 to 38

TABLE 1- Nitrogen concentration of soybean plant parts (g/kg) during plant development at 14-day intervals. Means of 30 observations.

DAE	Stage	Stems	Leaves	Roots	Nodules	Pods	Pericarps	Seeds
14	V_3	38.0	79.1					36.1
28	V_7	18.8	59.2					23.0
42	V_{10}	9.7	34.3	20.0				39.9
56	R_2	9.5	37.0	24.4				32.6
70	R_4	8.6	36.3	18.7	40.3			26.9
84	R_5	10.6	41.2	16.7	37.8			34.2
98	R_6	12.3	37.6	17.8		38.0	23.4	52.5
112	R_7	9.1	29.6	16.0	34.5	-	12.7	54.8
126	R_8	8.2	24.4	16.2	34.0	-	11.8	60.0
	SE	0.4	0.8	0.6	0.6	1.4	0.6	0.6
	CV (%)	12.16	6.93	10.06	5.75	18.33	15.26	4.01
	F test	**	**	**	**	**	**	**

DAE: Days after emergence.

** : Significant at P<0.01 by the F test.

g/kg in the stems, and from 16 to 36 g/kg in the roots, values observed at the R_8 and V_3 growht stages, respectively (Table 1). In soybean plants, the N concentration may decreases from 98 to 50 g/kg in leaves, and from 98 to 30 g/kg in stems during the first 20 days after plant emergence (Ohlrogge, 1960).

Herbicides influenced both stem and leaf N concentration up to 28 DAE ($P < 0.01$), whereas they affected root N concentration up to 42 DAE ($P<0.05$). Trifluralin application decreased N concentration in both the roots, from 41.4 to 34.8 g/kg, and the stems, from 39.6 to 36.0 g/kg, at 14 DAE. However, it only reduced significantly ($P < 0.05$) the N concentration in the leaves at 28 DAE, from 61.3 to 54.3 g/kg (Fig. 1). Trifluralin reduced linearly N concentration in the roots at both 28 DAE, from 26.2 to 22.6 g/kg, and 42 DAE, from 21.4 to 17.4 g/kg. In addition, it reduced curvilinearly the N concentration in the stems, from 22.5 to 11.2 g/kg, at 28 DAE (Fig. 2). These results are consistent with the reduction on N_2 fixation caused by trifluralin during the most part of the vegetative period of soybeans (Marenco et al., 1993). Behran et al. (1979) also observed reduction in both N concentration, of shoots and roots, and N uptake in soybean plants treated with 3 mg/kg of trifluralin. Kust & Struckmeyer (1971) reported anatomical disorders and found occlusions in xylem elements, and suggested that trifluralin or its metabolites may affect the activity of enzymes needed for respiratory metabolism. In the present study, higher rates of trifluralin reduced lateral root growth, which probably influenced nutrient uptake.

Clomazone reduced the N concentration in the leaves from 61.3 to 53.5 g/kg at 28 DAE (Fig. 3). It also reduced N concentration in the roots at both 14 DAE, from 41.4 to 31.5 g/kg (Fig. 3), and 28 DAE, from 26.0 to 21.0 g/kg (Fig. 4). Its influence on stem N concentration tended to be curvilinear, decreasing from 22.5 to 17.0 mg/kg at 28 DAE (Fig. 4). The effects of clomazone on plant part N

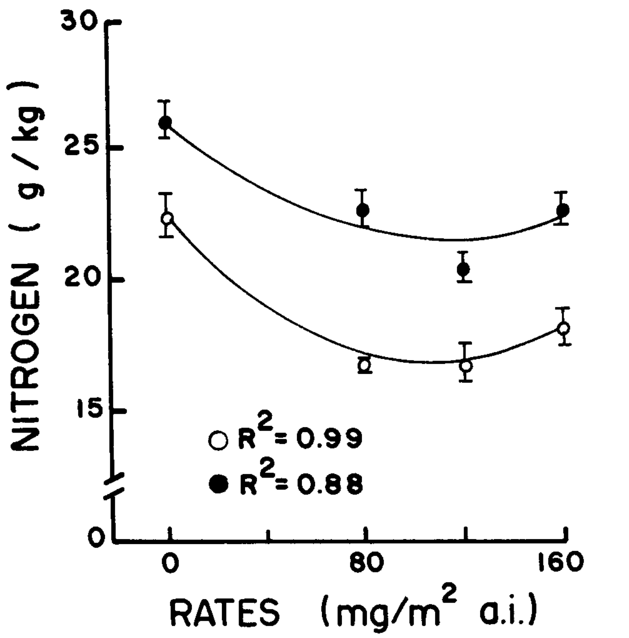

FIGURE 4- Effects of clomazone on nitrogen concentration in stems (❍) and roots (●) of soybean plants at 28 days after emergence. Each point, mean of three replications; bar, ± SE.

concentrations are consistent with its effect on N -fixation (Marenco et al., 1993).

In contrast to trifluralin and clomazone, chlorimuron increased significantly ($P<0.05$) N concentration in both leaves, from 61.3 to 68.0 g/kg at 28 DAE, and nodules from 38.3 to 43.5 g/kg at 42 DAE (Fig. 5). The effect of chlorimuron was unexpected since this herbicide reduced N_2 fixation during the first 42 DAE (Marenco et al., 1993). Therefore, the greater N concentration in chlorimuron treated plants was probably due to a greater soil N uptake. During the vegetative period, it was observed a trend for greater root stump exudate production in chlorimuron treated plants, which could explain at least partially the chlorimuron effects on leaf and nodule N concentration, if root stump exudate production is related to plant part N concentration.

At 56 DAE and thereafter, there was no effect of herbicides on N concentrations in any plant part ($P<0.05$). Therefore, there was no effect of herbicides on pod walls and seed N concentration. This was probably due to higher levels of N_2 fixation observed during the reproductive stages (Marenco et al., 1993), which diminished the detrimental effects of herbicide applications. On the other hand, older plants are usually less susceptible to herbicide injuries.

During the pod fill stages, from 98 to 126 DAE, N concentration increased in the seeds from 52.5 to 60.0 g/kg, but it decreased in the pod walls (Table 1). This, at least partially, occurred because still at the R_6 reproductive growth stages, there was production of seeds in lateral branches, which accumulated dry matter and N up to the end of the plant cycle.

The total plant N content was reduced by trifluralin and clomazone up to 28 DAE. Therefore, only herbicide

TABLE 2- Nitrogen content of soybean plants treated with chlorimuron, trifluralin and clomazone at 14 and 28 days after emergence. Means of three replications.

Herbicides	Rates	Days after emergence	
		14	28
	mg/m^2 a.i.	mg/plant	
Control	0.00	38.7	184.2
Chlorimuron	0.70	36.3	156.7
Chlorimuron	1.05	37.2	150.9
Chlorimuron	1.40	39.4	149.1
Trifluralin	80.00	28.5	123.6
Trifluralin	120.00	29.7	119.6
Triflularin	160.00	22.6	100.1
Clomazone	80.00	34.6	137.1
Clomazone	120.00	32.0	137.8
Clomazone	160.00	30.6	129.4
Tukey ($P < 0.05$)		6.6	37.2

effects at 14 and 28 DAE are presented (Table 2). Chlorimuron, which was applied at 14 DAE, only influenced N content at 28 DAE (Table 2). Dry matter accumulation was reduced by trifluralin, chlorimuron and clomazone (Marenco, 1993). Thus, the reductions on N content caused by trifluralin and clomazone were due to their effects on both N concentration (Fig. 1-4) and

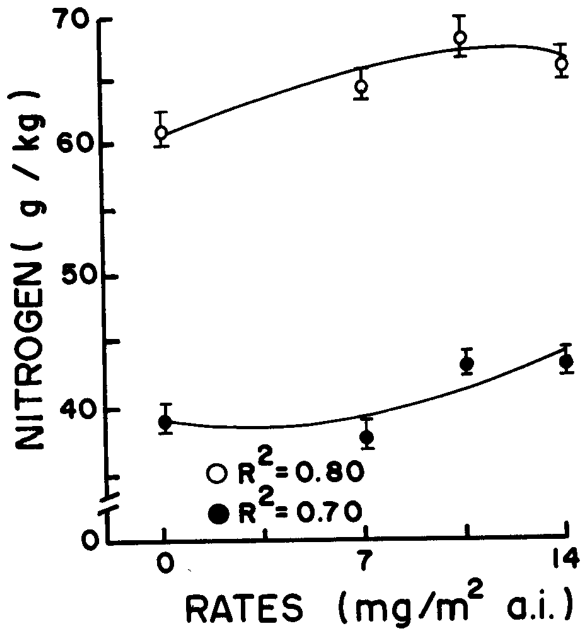

FIGURE 5- Leaf and nodule nitrogen concentration of soybean plant treated with chlorimuron. Effects in the leaves at 28 (❍), and in nodules at 42 days after emergence (●). Each point, mean of three replications; bar, ± SE.

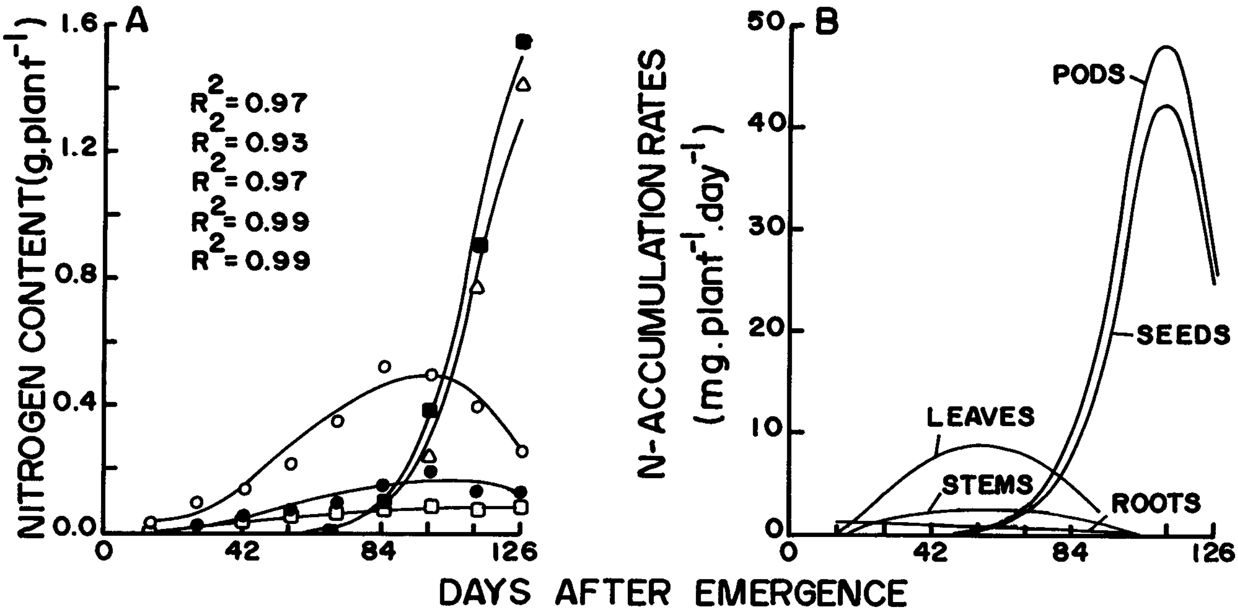

FIGURE 6- Nitrogen content (A) and rates of nitrogen accumulation (B) in soybean plant parts as a function of plant age. Leaves (❍), stems (➊), roots (☐), pods (■), and seeds (∆). Each point, mean of 30 observations.

dry matter accumulation. As chlorimuron did not reduce N concentration, N content could only be diminished due to its detrimental effects on dry matter accumulation.

At 42 DAE and thereafter, there was no effect of herbicides on N accumulation in any plant part ($P<0.05$). Therefore, the data were pooled to determine the plant age effect on plant part N accumulation, and to estimate N remobilization from vegetative to reproductive parts. Nitrogen content in both leaves and stems increased ($P<0.01$) with plant age until 98 DAE, R_6 growth stage, and then declined. The roots accumulated N during the most part of the plant cycle (Fig. 6A), because of a similar pattern on dry matter accumulation (data no shown). These results are consistent with the greater availability of leaf and stem N to be translocated to reproductive structures, when compared with N accumulated in the roots (Peoples & Gifford, 1990). Nitrogen accumulated in pods and seeds followed a logistic pattern, increasing rapidly between 88 and 120 DAE, R_5 to R_7 growth stages (Fig. 6A). In a per plant basis, the greatest N accumulation rates were 8.77 in the leaves, 2.76 in the stems, 1.21 in the roots, 47.97 in the pods and 42.03 mg/plant per day in the seeds (Fig. 6B). The greatest value for seed N accumulation rate observed in this experiment is similar to that obtained by Spaeth & Sinclair (1983).

Since during the pod filling stages the oldest leaves translocate great part of their N to developing seeds, before shedding, an estimated of N remobilization may be done. On a per plant basis, the shoot translocated to reproductive parts 0.296 g of the total N accumulated in the pods (1.446 g), between the R_5 and R_8 growth stages (Table 3). The proportion of N remobilized came from N previously fixed was 16% (0.233 g/1.446 g). At the R_8 growth stage, 1.737 g of N, 84% of the total N accumulated by the plant, was derived from N_2 fixation. It represents an estimate of 26 g/m^2 of N derived from N_2 fixation. These results agreed with those of Thurlow & Hiltbold (1985) who estimated that soybeans may derive greater than 70% of their N requirements from N_2 fixation. In this experiment, high levels of N_2 fixation during the pod filling stages coincided with small dry matter reductions in both stems and leaves (Marenco, 1993). Thus, these results confirm those obtained by Martignone et al. (1987) who suggested that under high level of N, most of the N remobilized from shoot to pods may be derived from soluble N forms rather than structural proteins.

TABLE 4- Chlorophyll (Chl) concentrations in leaves of soybeans throughout the plant cycle at 14-day intervals. Means (SE) of 30 observations.

DAE	Chl-a	Chl-b	Chl(a+b)	Chl(a+b)	Chlorophyll a/b ratio
	g/kg fresh matter			mg/m^2	+ 21
35	2.06 ± 0.05	1.53 ± 0.06	3.59 ± 0.10	331 ± 9	1.38 ± 0.03
49	2.57 ± 0.04	1.73 ± 0.06	4.30 ± 0.08	387 ± 7	1.52 ± 0.05
63	2.49 ± 0.05	1.85 ± 0.05	4.34 ± 0.06	399 ± 6	1.37 ± 0.04
77	2.58 ± 0.05	1.69 ± 0.05	4.27 ± 0.07	374 ± 7	1.58 ± 0.04
91	2.12 ± 0.07	1.00 ± 0.04	3.12 ± 0.10	280 ± 8	2.16 ± 0.06
105	1.87 ± 0.08	1.10 ± 0.07	2.97 ± 0.14	264 ± 11	1.77 ± 0.05
119	1.18 ± 0.03	0.81 ± 0.01	1.98 ± 0.04	223 ± 4	1.46 ± 0.03
CV (%)	16.00	18.00	14.00	12.56	17.46,
F test	**	**	**	**	***

** Signigficant at P<0.01 by the F test.

DAE= Days after emergence

TABLE 3- Nitrogen translocated from leaves and stems to pods (g/plant) during pod filling stages of soybean plants.

Plant parts	DAE growth stages	Total N accumulated: Total N	N-Derived from N_2 fixation[1]	N-uptaken from soil[1]
Stems +leaves	84 (R_5)	0.676 a	0.531 a	0.145 a
	126 (R_8)	0.380 b	0.298 b	0.082 b
Differential of N		-0.296	-0.233	-0.063
Pods	84 (R_5)	0.103 c	0.090 c	0.013 c,
	126 (R_8)	1.549 d	1.336 d	0.213 d
Differential of N		1.446	1.246	0.200
Whole plant	126 (R_8)	2.067	1.737	0.330

DAE = Days after emergence

Means in columns followed by different letter are significantly different at P < 0.01 by the Tukey test.

At the R_8 stage (126 DAE), the total plant N accumulated was distributed as follows: 0.518 g in the non- reproductive parts, 1.419 g in the seeds and 0.130 g in the pod walls (Fig. 6A). The proportion of N derived from N_2 fixation was 78% for vegetative parts and 86% for pods (Table 3). Thus, the whole plant N accumulated, excluding seeds, came from N_2 fixation was 0.516 g/plant. In addition, the seed N derived from soil uptake was 0.199 g/plant. Thus, 0.317 g/plant (0.516-0.199), equal to 4.76 g/m^2, represents an estimate of the N added to the soil in one soybean crop cycle. Yaacob and Blair (1983) concluded that, on the average, one soybean crop cycle may add 3.5 g/m^2 of N to the soil.

Although leaf N concentration was lower in trifluralin and clomazone herbicide treated plants, during the vegetative stages, the herbicides did not affect significantly (P<0.05) the leaf chlorophyll concentration. Therefore, the chlorophyll data were pooled to determine the effect of plant age on leaf chlorophyll concentration (Table 4). As N but not chlorophyll concentration was affected by herbicides, it may be suggested than the pool of soluble N rather than the structural proteins was more affected by the herbicide treatments.

The leaf chlorophyll concentrations were greater from 49 (stage R_2) to 77 DAE. During the whole study period, chlorophyll **a** concentration ranged from 1.18 at 119 DAE, to 2.58 mg/g fresh matter at 77 DAE; whereas chlorophyll **b** ranged from 0.81 at 119 DAE, to 1.85 mg/g FW at 63 DAE. The total chlorophyll ranged from 1.98 mg/g FW (223 mg/m^2) to 4.34 mg/g (399 mg/m^2), whereas the chlorophyll **a/b** ratio ranged from 1.37 to 2.16 during the whole study period (Table 4). Finally, it may be concluded that under these plant growth experimental conditions, the herbicide treatments affected plant part nitrogen, but not chlorophyll concentration during the first vegetative stages. The plants recovered from herbicides effects with time, and therefore, there was no difference between treated and control plants at the reproductive stages.

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