NODULATION AND NITROGEN FIXATION IN SOYBEANS TREATED WITH HERBICIDES[1]

RICARDO ANTONIO MARENCO[2], NEI FERNANDES LOPES[3] and PAULO ROBERTO MOSQUIM[4]

Departamento de Biologia Vegetal, Universidade Federal de Viçosa, Viçosa, MG, 36570-000, Brazil.

ABSTRACT- Under greenhouse condition an experiment was carried out to evaluate trifluralin, chlorimuron and clomazone effects on nodulation and nitrogen fixation in soybeans (*Glycine max* (L.) Marr. cv Uberlândia). Nitrogen fixation was assessed by the xylem-solute technique. The samples were collected at 14 day intervals, from 14 up to 126 days after emergence (DAE). Chlorimuron treated plants showed a reduction in the number (N_n) and dry matter of nodules (W_n) up to 28 and 42 DAE, respectively. Trifluralin (800 g a.i./ha) stimulated both Wn and N_n up to 28 DAE, whereas at its highest rate (1600 g a.i./ha) it decreased both W_n and N_n, up to 42 DAE. Clomazone did not affect W_n; however, its application increased the number of nodules in treated plants up to 28 DAE. Finally, the highest rates of herbicides reduced N_2 fixation during the first vegetative growth stages.

Additional index terms: *Glycine max*, ureides, nodule efficiency, chlorimuron, clomazone, trifluralin.

NODULAÇÃO E FIXAÇÃO DE NITROGÊNIO EM SOJA TRATADA COM HERDICIDAS

RESUMO- Os efeitos de varias doses dos herbicides clorimuron, trifluralina e clomazone sobre a nodulação e a fixação de nitrogênio em soja (*Glycine max* (L.) Merr.) foram estudados em casa de vegetação. A fixação de nitrogênio foi avaliada utilizando a técnica da concentração de ureideos no exsudato do xilema. Coletaram-se as amostras a intervalos de 14 dias, sendo a primeira aos 14 e a última aos 126 dias após a emergência (DAE). As plantas tratadas com clorimuron apresentaram redução no número (N_n) e na matéria seca de nódulos (W_n) até os 28 e 42 DAE, respectivamente. Trifluralina em dose de 800 g i.a/ha estimulou W_n e N_n até os 28 DAE. A dose máxima de trifluralina, 1600 g i.a./ha, diminuiu W_n e N_{ni} até os 42 DAE. Clomazone não influenciou W_w , entretanto, aumentou o N_n , até os 28 DAE. A fixação de N_2 foi reduzida pelos herbicides durante os primeiros estádios vegetativos da soja.

Termos adicionais para indexação: *Glycine max*, ureideos, eficiência de nódulos, clorimuron, clomazone, trifluralina.

INTRODUCTION

Weed control is an essential cultural practice in soybean, during the crop cycle. The chemical control of weeds has a great importance due to scarcity of labor, little time available to weed control and large cultivated areas. Trifluralin [2,6- dinitro-N,N-dipropyl-4-(trifluoro-methyl)benzenamine], clomazone (2-(2-chlorophenyl) methyl-4,4-dimethyl-3-isoxazolidinone] and chlorimuron [ethyl 2-[[(4-chloro-6-methoxypyrimidin-2-yl)-aminol carbonyllami-nosulfonyl] benzoate] are herbicides often used in soybean because they are effective to control a broad range of weeds.

In tropical leguminous species, ureides are the main nitrogenous compounds exported from their nodules, they account for 60 to 90% of the total N in the xylem sap. However, these are present only in small quantities in plants that do not depend on N fixation (McClure & Israel, 1979; Peoples et al., 1989). These researches show that the proportion of ureides in the xylem sap may be used as an indicative of the quantity of N_2 fixed.

A small fraction of ureides transported through xylem during transpiration may be translocated to phloem without previous metabolism. However, they undergo metabolic transformation before their translocation from vegetative organs to phloem (Pate et al., 1984).

Although all herbicides receive intensive testing by both the manufacture and federal regulatory agencies before they are released, their effects on the N_2 fixing process in soybean were not studied (Bollich et al., 1995). The use of trifluralin in soybean production may either reduce (Bollich et al., 1988) or not affect nodulation or N_2 fixation (Rennie & Dubetz, 1984). Occasionally, either N_2 fixation or nodulation has been increased in soybean due to trifluralin application (Rennie & Dubetz, 1984; Bollich et al., 1985). Postemergence herbicides, such as acifluorfen and bentazon, may reduce N_2 fixation or nodulation in soybean plants (Ozair & Moshier, 1988).

Effects of chlorimuron and clomazone on nitrogen fixation were not found in the literature. Thus, this study aims to evaluate the effects of trifluralin clomazone and chlorimuron on nodulation, N_2 fixation and nodule efficiency in soybeans.

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2Eng. Agr., D.Sc., Bolsista do CNPq, CP 242, Viçosa, MG, 36570-000, Brasil. Parte da Tese de D.S.

3Eng. Agr., Ph.D., Prof. Titular, Bolsista da CAPES, Depto. de Biologia Vegetal, UFV, Viçosa, MG, 36570-000, Brasil. To whom correspondence should be adressed.

4Biologo, D.Sc., Prof. Adjunto, Depto. de Biologia Vegetal, UFV, Viçosa, MG, 36570-000, Brasil.

MATERIALS AND METHODS

The greenhouse experiment was conducted at Viçosa, Minas Gerais, from October 23, 1990 to March 7, 1991. During this period, temperature ranged from 21 to 23^{o}C at night to maxima of 33 to 39^{o}C during the day. Relative humidity ranged from 38 to 60% in the day to a maximum of 100% during the night. Substrate used for plant development was a Red-Yellow Latosol, collected from a 0- 0.2 m depth, with moderate fertility, clay texture and 3.2% organic-matter. This soil was placed in pots (4 kg/pot), amended with 0.9 g of lime per kg of soil, and fertilized with 400 ppm of P and 50 ppm of K. Amendment and fertilization were done 30 and three days before planting, respectively. Just after fertilization, the soil was sterilized with methyl bromide for 24 h. It was irrigated and kept near to field capacity throughout the study.

Soybean seeds cv. Uberlandia were disinfected with sodium hypochloride (2%) for 5 min, washed with distilled water and dried with paper towels. The seeds were dipped (1 h) into a suspension of freshly cultured *Bradyrhizobium japonicum* cells ($1O^{7}$ cells/mL), just before planting. The suspension contained a mixture of three *B. japonicum* strains: Semia 586, 587 and 5019. The seeds (six/pot) were planted into pots, and after emergence, the seedlings were thinned to two per pot to obtain 15 plants/m^{2}. During the first 30 DAE, 40 mL of a micronutrient solution (Waugh & Fitts, 1966) was applied to each pot, at 10 day intervals.

The experimental design was a randomized complete block with treatments in split-plot with three replications. The whole plots were the sampling dates, and the plots were the herbicide treatments. The herbicides tested were chlorimuron (7.0, 10.5 and 14 g a.i./ha), trifluralin (800, 1200 and 1600 g a.i/ha) and clomazone (800, 1200 and 1600 g a.i/ha); there was also an untreated control. They were applied with a backpack sprayer operated with a boom pressure of 230 KPa and delivering a spray volume of approximately 400 L/ha 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 emergence (DAE).

Nitrogen fixation and nodulation were determined throughout plant development. The plant materials were collected at 14 day intervals, from 14 up to 126 DAE. At each sampling, plant growth stage was recorded (Fehr et al., 1971). Nitrogen fixation was assessed by the xylem-solute technique (Peoples et al., 1989). Xylem exudate was collected from intact root stumps from soybean plants following decapitation of the shoot (Herridge et al., 1988). Sap was collected between 9:00 h and 12:00 h to reduce diurnal variability. Stems were cut midway between ground level and the cotyledonary node with a razor blade. At the first sampling date, the exudate from cut surfaces was collected with capillary pipettes. Thereafter, latex rubber tubing sleeves, 40 mm long with an internal diameter slightly smaller than the stem were placed over the root stumps (Peoples et al., 1989). The collecting period from individual plants was always no longer than 30 min.

The concentration of ureides (allantoin and allantoic acid), nitrate and α-aminoacids in xylem exudates was assessed according to Peoples et al. (1989). The relative abundance of ureide-N in xylem sap (U) was calculated according to Herridge et al. (1990), as: U% = 40OA/(4A+ B+ C); where A, B and C are the molar concentrations of ureides, nitrate and α-amino-N, respectively. The calculation of the proportion of plant N derived from N_2 fixation (P) was based on regressions according to Peoples et al. (1989), as follows: P% = 1.2(U - 4.8) for plants in the vegetative and flowering stages, and P% = 1.5(U - 21.3) for plants during the pod-fill stages.

At each sampling date shoot and root were dried at 75^{o}C until reaching constant mass, immediately weighed, and finely ground. These samples were stored dry until analyzed for N content. The concentration of total nitrogen in samples was determined by Kjeldahl digestion (Lindner, 1944) followed by colorimetric assay for ammonia-nitrogen (Jackson, 1965).

The number of nodules (N_n) per plant was determined at each sampling date. The nodules were removed, dried at 75^{o} until reaching constant mass, and immediately weighed. White or green nodules, inactive nodules, were not considered when nodulation was assessed. The nodule efficiency on N_2 fixation was determined on a per nodule basis, calculated as: $\xi_n = P \times T_n/W_n$; where ξ_n, T_n, and W_n are the nodule efficiency on N_2 fixation (g/g), total plant nitrogen (g) and nodule dry matter (g), respectively.

The data were subjected to analysis of variance after transformation. Data derived from counting were transformed to $(Y+ 0.5)^{1/2}$ whereas data derived from weighing were transformed to log(Y).

RESULTS AND DISCUSSION

Number and dry matter of nodules were affected by both plant age and herbicide treatments ($P \leq 0.01$). Dry matter and number of nodules increased with plant age up to the beginning of the growth stage R_7. The greatest values were observed at 112 DAE. On a per plant basis, the greatest means were 3114 mg/plant for mass of nodules (W_n) and 777 for number of nodules (N_n) (Table 1). From 112 to 126 DAE, W_n and N_n decreased due to plant senescence. In senescent plants, reduced levels of carbohydrates within nodules may increase resistance to oxygen diffusion. As a consequence, nitrogenase activity may be decreased, leading to nodule senescence (Vessey et al., 1988).

Herbicide treatments reduced W_n and N_n up to 42 DAE ($P \leq 0.01$). Chlorimuron and trifluralin affected both dry matter and nodule number. Clomazone, however, only affected the nodule number. Chlorimuron effects were proportional to the applied rate. Chlorimuron reduced W_n from 6.8 to 0.7 mg/plant at 28 DAE (Fig. 1A) and from 376 to 131 mg/plant at 42 DAE (Fig. 1B). The reduction on W_n at 28 DAE was probably a consequence of its effect on N_n. On the average, plants treated

TABLE 1- Effects of plant age on nodule dry matter, nodule number and nodule efficiency on N_2 fixation in soybean plants. Values shown are the means (± SE) of 30 observations (10 herbicide treatments with three replications).

Plant age	Growth stage	Nodule dry matter	Nodule number	Nodule efficiency
DAE		mg/plant	number/ plant	g/g
14	V_3	0	0	0
28	V_7	5± 1	15± 2	3.97± O.68
42	V_{10}	-268± 19	136± 7	0.89± 0.09
56	R_2	754± 26	23O± 11	0.47± O.O1
70	R_3	1582± 4O	5OO± 16	0.34± O.O1
84	R_5	2393± 68	719± 25	0.34± 0.01
98	R_6	2899± 93	777± 28	0.39± O.O2
112	R_7	3114± 78	773± 16	0.40± O.O1
126	R_8	1663± 39	443± 39	1.11± 0.10
F test		**	**	**

DAE: Days after emergence

** Significantly at $P \leq 0.01$

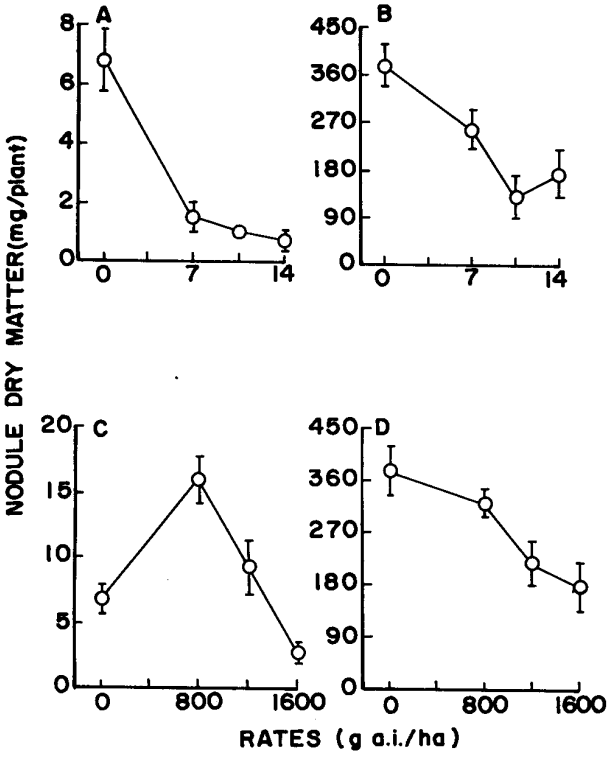

FIGURE 1- Dry matter of nodules in soybean plants treated with herbicides at two sampling dates (A: chlorimuron at 28 DAE; B: chlorimuron at 42 DAE, C: trifluralin at 28 DAE and D: trifluralin at 42 DAE). Each point, mean of three replications;

with chlorimuron (14 g a.i/ha) reduced nodule number from 15.3 to 2.2 nodules/plant at 28 DAE (Fig 2A). At 42 DAE, the number of nodules was not influenced by chlorimuron ($P \leq 0.05$). Therefore, the reduction on W_n,

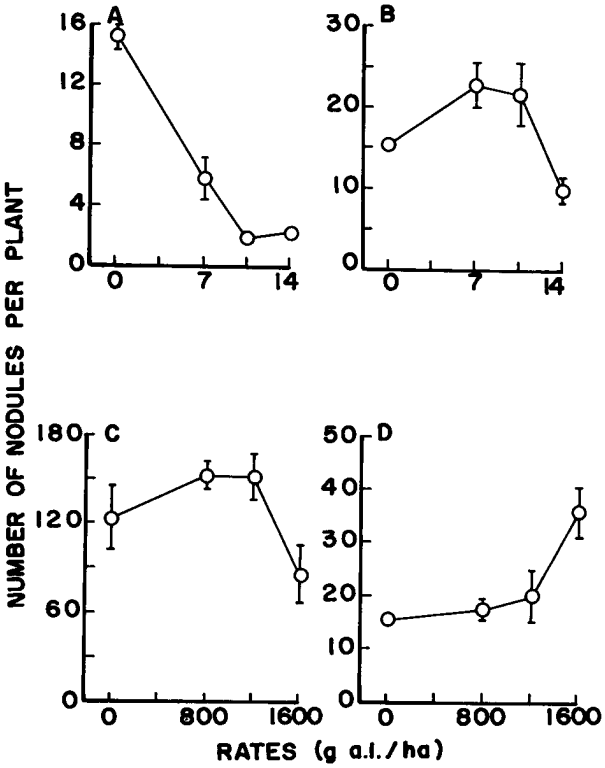

FIGURE 2- Number of nodules in soybean plants treated with herbicides at two sampling dates (A: chlorimuron at 28 DAE; B: trifluralin at 28 DAE; C: trifluralin at 42 DAE; D: clomazone at 28 DAE). Each point, mean of three replications; bar, ± SE.

observed at 42 DAE, was caused by decreasing of nodule size. Chlorimuron may either affect rhizobia or the plant-rhizobia relationship. It may also indirectly interfere with nodulation by interrupting production and translocation of photosynthate to the nodules. Chlorimuron reduced W_n at 42 DAE (Fig. 1B), whereas N_n was only reduced at 28 DAE. So it may be concluded that dry matter accumulation of nodules was greater affected than production of new nodules. Similar effects were observed in soybean plants treated with other overtop herbicides (Ozair & Moshier, 1988).

Trifluralin, at rate of 800 g a.i./ha, stimulated both W_n (Fig. 1C) and N_n (Fig. 2B) at 28 DAE. Trifluralin rates of 800 to 1200 g a.i./ha also showed a trend to increase N_n at 42 DAE (Fig. 2C). Occasionally, stimulating effects of trifluralin on nodulation have been reported (Alaa-Eldin et al., 1981; Bollich et al., 1984). However, this is a no well understood phenomenon in plant physiology. Several herbicides may stimulate plant growth when applied at subtoxic rates (Ries, 1976). At 28 and 42 DAE, the highest rate of trifluralin (1600 g a.i./ha) decreased both W_n (Fig. 1C,D) and N_n (Fig. 2B,C). Several researchers (Dunigan et al., 1972; Bollich et al., 1988) have reported that trifluralin reduces nodulation in soybean treated plants. Kust and Struckmeyer (1971) observed disruption at the cellular level in tissues of soybean treated with trifluralin. This disruption could affect assimilate production and translocation to the nodules, and as consequence, nodulation could be reduced.

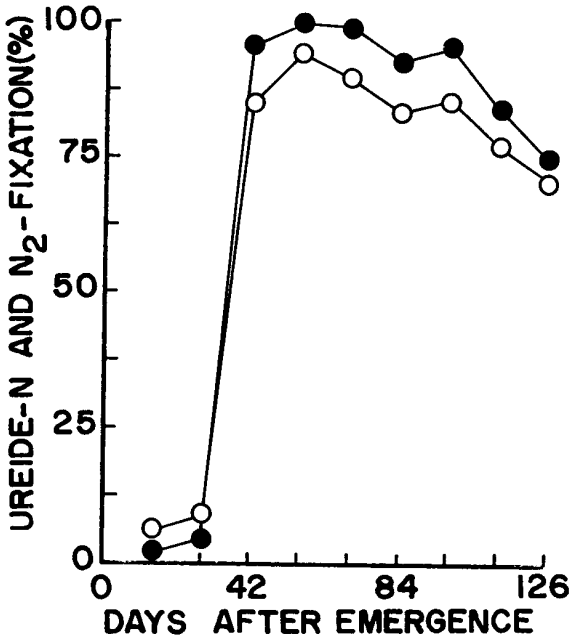

FIGURE 3- Effects of plant age on the relative abundance of ureide-N in root-bleeding xylem sap (o) and on the proportion of N derived from N_2 fixation (e) of soybean plants. Values shown are the means of the 30 observations. At each point, the bar (± SE) is smaller than the point height.

Clomazone did not affect nodule dry matter accumulation ($P \leq 0.05$). However, nodule number was greater ($P \leq O.O1$) in treated plants at 28 DAE (Fig. 2D). No explanation was found for the increase in number of nodules; since no hormonal properties have been attributed to clomazone.

The relative abundance of ureide-N in the xylem sap was affected by both plant age ($P \leq 0.O1$) and herbicide treatments ($P \leq O.05$). The relative ureide-N values rapidly increased during the first 42 DAE. On the average, the ureide-N proportion was greater than 85% from 42 to 98 DAE. After 98 DAE the ureide-N in the xylem sap declined with plant age (Fig. 3). Although nodule activity normally decline after R3 stage, the current photosynthate supply to nodules may not be the primary factor limiting N_2 fixation (Vance, 1991). The higher ureide-N values observed during reproductive stages of soybean is coherent with results obtained in previous reports (McClure & Israel, 1979; Herridge et al., 1990). Peat et al. (1981) suggested that increase in N_2 fixation during reproductive stages may be due to hormonal stimulus coming from flower buds.

The relative ureide-N in the xylem sap was reduced by the herbicide treatments during the vegetative stages ($P \leq 0.05$). Trifluralin and clomazone decreased the relative ureide-N at 28 DAE. Chlorimuron, however, extended its detrimental effect up to 42 DAE. The highest rate of chlorimuron reduced ureides proportion, from 14 to 6%, at 28 DAE (Fig. 4A), and, from 88 to 71%, at 42 DAE (Fig. 4B). It may be observed that at 42 DAE, the reduction caused by 7 g a.i./ha was lower than that caused at 28 DAE. This is probably due to greater plant tolerance with time.

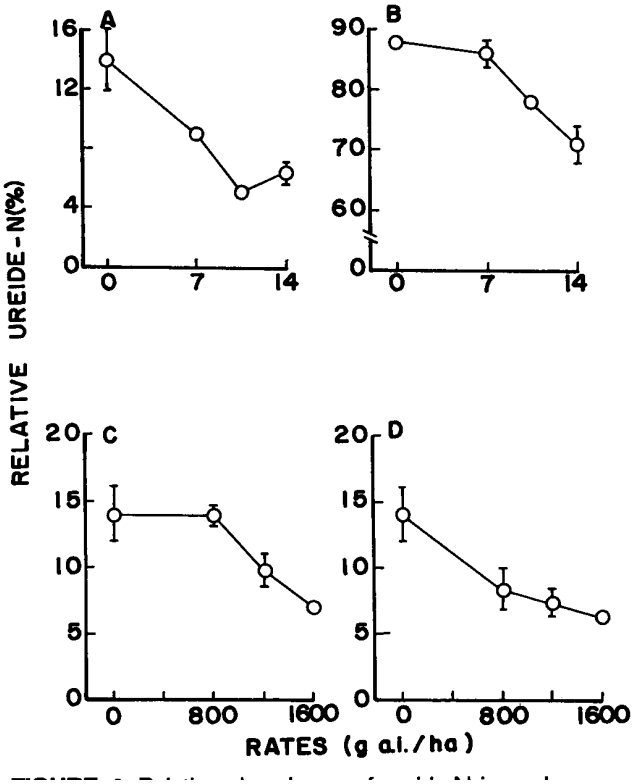

FIGURE 4- Relative abundance of ureide-N in soybean root xylem exudate treated with herbicides at two sampling dates. (A: chlorimuron at 28 DAE; B: chlorimuron at 42 DAE; C: trifluralin at 28 DAE and D: clomazone at 28 DAE). Each point, mean of three replications; bar, ± SE.

The effect of chlorimuron on ureides concentration may be a consequence of its effect on plant growth. This was suggested because foliar injuries caused by chlorimuron were visible up to three weeks after its application (35 DAE). During this same period reduction in nodulation and ureides concentration were also observed. Chlorimuron, therefore, could indirectly interfere with N_2 fixation by affecting production or transfer of photosynthate to the nodules. The supply of photosynthate is essential in order to nitrogenase activity be maintained within nodules (Wong and Evans, 1971).

Trifluralin reduces the ureide-N from 14 to 7% at 28 DAE (Fig. 4C). Thereafter, there was no effect of trifluralin on N_2 fixation (P 0.05). Although trifluralin reduced W_n at 42 DAE, this effect did not influence the ureide concentration, as could be expected. It suggests that at 42 DAE, reduction on nodulation in trifluralin treated plant was compensated by increasing nodule efficiency on N_2 fixation. Occasionally, soybean plants treated with trifluralin have had enhanced its nodulation with or without effect on N_2 fixation (Alaa-Eldin et al., 1981; Bollich et al., 1984).

Dry matter of nodules was not reduced significantly ($P \leq 0.05$) by clomazone, even when it reduced ureides-N from 14 to 6% at 2e DAE (Fig. 4D). Thus, it may be suggested that this herbicide may influence the nodule efficiency on N_2 fixation.

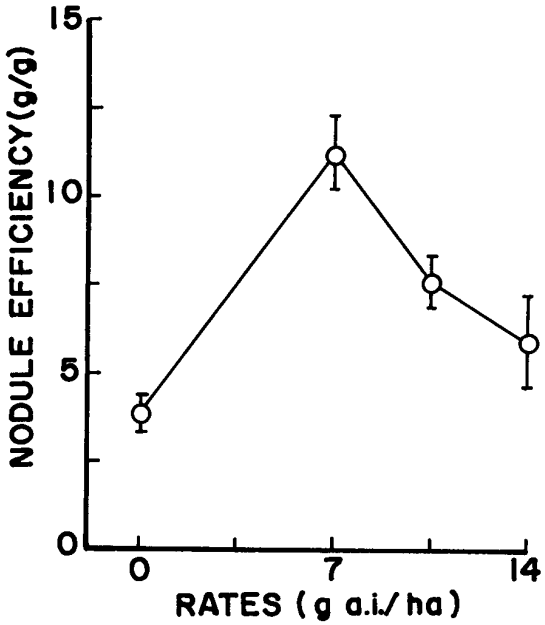

FIGURE 5- Efficiency of nodules on nitrogen fixation in soybean plants treated with chlorimuron at 28 DAE. Each point, mean of three replications; bar, ± SE.

There was a positive correlation between nodulation and the ureides concentration during the first 42 DAE. The r values were 0.71 and 0.49 ($P \leq 0.01$) at 28 and 42 DAE, respectively. Similar results were observed by Herridge et al. (1990). At 56 DAE and thereafter the r values were lower and no significant ($P \leq 0.05$). It occurred because a high ureide concentration was observed early in the plant cycle, at the Vlo vegetative growth stage. Thus, at reproductive growth stages increasing in dry matter of nodules were not coupled with increasing on N_2 fixation.

The proportion of plant N derived from nitrogen fixation increased rapidly during the first 42 DAE. it nearly remained constant from 42 to 98 DAE, and thereafter, it declined with plant age (Fig. 3). The effect of herbicides on the N derived from N-fixation were similar to those observed on the concentration of ureides in the xylem exudate. The N_2 fixation nodule efficiency (En) was influenced by both plant age (P eO.01) and herbicide treatments. Herbicide effects, however, were only significant ($P \leq 0.01$) at 28 DAE. On the average, the greater values were observed at the beginning and at the end of the plant cycle (Table 1). The smaller ξ_n value, 0.34 g/g, were observed at 70 and 84 DAE (Table 1). They coincided with a period of intensive dry matter accumulation in the nodules, as compared with N accumulation in the plant. This was consistent with the lack of correlation between W_n and ureide-N during the pod-fill stages. Increasing in W_n from R_3 to R_6 stages were not coupled with increasing in ureide-N, which caused a declining on in'

The effect of herbicide treatments on ξ_n was only as a result of chlorimuron application. In chlorimuron treated plants the greatest ξ_n value (11.2 g/g) was observed at a rate of 7 g a.i/ha (Fig. 5) at 28 DAE. At the highest chlorimuron rate (14 g a.i./ha) the in values were similar to the untreated control (Fig. 5). It occurred because the rates of chlorimuron influenced in different ways nodule dry matter and total plant nitrogen.

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