GROWTH AND NODULATION IN SOYBEAN CULTIVATED ON TWO SUBSTRATA AND TREATED WITH FIVE RATES OF METOLACHLOR¹

CRESCIMENTO E NODULAÇÃO DE SOJA CULTIVADA EM DOIS SUBSTRATOS E SUBMETIDA A CINCO DOSES DE METOLACHLOR

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SUMMARY

The effect of metolachlor [2-chloro-N-(2-ethyl-6methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide] at rates of 0.0, 1.5, 3.0, 4.5, and 6.0kg a.i./ha on the growth and nodulation of soybean cv. BR-4 cultivated on two substrata (soil and soil + sand) and inoculated with Bradyrhizobium japonicum (Kirchner 1896) Jordan 1982, 137 (Denotes that this name has been validly published in the oficial publication, International Journal of Systematic Bacteriology), was studied under greenhouse conditions at Lages, SC, Brazil, in 1992-93. The experimental design was completely randomized with four replicates. The experimental unit was a container with two plants. Fifty-two days after the emergence (at R₁ stage - beginning of flowering) the plants were harvested and evaluated for shoot, root, leaf and nodule dry matter, nodule number, and leaf area. The data were submitted to variance analysis and multiple polynomial regression. Increasing rates of metolachlor significantly reduced shoot, leaf and nodule dry matter and leaf area, the data following a linear polynomial regression, while root dry matter and nodule number were not affected. The interaction "rate x substratum" was not significant for all parameters studied. Except for root dry

matter and nodule number, soybean growth was significantly reduced more on soil + sand than on soil, probably as a result of a nutritional effect. Visual phytotoxicity was not observed for all treatments.

Key words: metolachlor, Glycine max (L.) Merrill, growth, nodulation.

RESUMO

Este trabalho foi conduzido com o objetivo de estudar o efeito de cinco doses do herbicida metolachlor (0,0, 1,5, 3,0, 4,5 e 6,0 kg i.a./ha) sobre o crescimento e nodulação de soja cv. BR-4, inoculada com *Bradyrhizobium japonicum* (Kirchner 1896) Jordan 1982, 137 (nomenclatura válida conforme publicação oficial do International Journal of Systematic Bacteriology) e cultivada em dois substratos (solo e solo + areia) em condições de casa de vegetação, em Lages, SC, Brasil, no ciclo 1992/93. O delineamento experimental utilizado foi o inteiramente casualizado com quatro repetições, sendo a unidade experimental o vaso com duas plantas. Cinqüenta e dois dias após a emergência (no

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Received for publication: March 16, 1995. Approved: Jun 21, 1995.

estágio R_i - início do florescimento) as plantas foram colhidas, procedendo-se a determinação de matéria seca de caule, raiz, folhas e nódulos, número de nódulos e área foliar. Os dados foram submetidos à análise de variância e regressão polinomial múltipla. Doses crescentes de meto-lachlor significativamente reduziram a matéria seca de caule, folha e nódulos e área foliar, segundo uma regressão polinomial linear, o mesmo não ocorrendo com a matéria seca de raiz e o número de nódulos. A interação "dose x substrato" não foi significativa para todos os parâmetros avaliados. À exceção da matéria seca de raiz e do número de nódulos, o crescimento foi significativamente menor no substrato solo + areia do que no substrato solo, devido, provavelmente, a efeitos nutricionais. Nenhum tratamento casou efeito fitotóxico visual às plantas de soja.

Palavras-chave: metolachlor, Glycine max (L.) Merrill, crescimento, nodulação.

INTRODUCTION

Soybean is one of the most important agricultural crops in the Brazilian economy, displaying an increase in cultivated area throughout the country. One of the most important factors limiting crop productivity is the presence of weeds and, according to CARTTER & HARTWIG (1967), weed control is an essential procedure. The most critical period of weed competition occurs up until 30-45 days after emergence of the soybean seedlings (BLANCO et al., 1978). Soybean productivity reductions of 27-50% have been reported as a result of weed competition (BLANCO et al., 1978; BURNSIDE, 1972; CARTTER & HARTWIG, 1967; KNAKE & SLIFE, 1965).

The development of new herbicide formulations for weed control in soybean has been intensive, but disregarding the study of possible environmental impacts and effects on plants and soil microbiology (DEUBER et al., 1981).

One of the soil processes most likely to be affected by the application of herbicides is nodulation (DUNIGAN et al., 1972). High rates of trifluralin (KUST & STRUCKMEYER, 1971) and chloramben (OLUMBRE & VEATCH, 1969) reduced the nodulation in soybean. This crop cultivated on a clay soil and treated with trifluralin, vernolate and metribuzin had reduced nodule dry matter (DEUBER et al., 1981). When cultivated in pots, the treatment with trifluralin, pendimenthalin, vernolate and metribuzin reduced the number of nodules on different soils (DEUBER et al., 1981). On a sandy soil, metolachlor and metribuzin reduced the number and dry matter of nodules (MACEDO & OLIVEIRA, 1986). Metribuzin also reduced the growth and nodulation in soybean cultivated in pots,

with a more intense effect on a sandy substrata (MORAES et al., 1989). ROSOLEM et al. (1985) observed that trifluralin inhibited the nodule development at the earlier stages of soybean growth in the superficial layer of treated soil. The combination of trifluralin and alachlor significantly inhibited the growth in soybean cultivated in sand in pots, but when applied separately had no or little effect (PROSCH & WEBER, 1988). GIADINI et al. (1979) observed that the consecutive application over two years of trifluralin, vernolate or alachlor had no negative effect on soybean nodulation. According to ALAA-ELDIN et al. (1981), trifluralin at high concentration and dinoseb acetate at a recommended rate, stimulated soybean nodulation, while nitralin, dinitramine or linuron, especially at high concentration, inhibited nodule formation and even, in some instances, prevented plant growth. Furthermore, in all herbicidal treatments, the dry matter of the whole plant, the yield of pods, and the total N-content were reduced.

The objective of the present work was to study the effects of the application of increasing rates of metolachlor on soybean growth and nodulation.

MATERIALS AND METHODS

The experiment was conducted under greenhouse conditions at Lages, SC, Brazil. On 05/12/92 the seeds of soybean cv. BR-4 were inoculated with **Bradyrhizobium** japonicum, strain SEMIA 5079 x 5080 (produced by Nitral), and sowed in pots filled with 8kg of substratum. After emergence, plants were thinned to two seedlings per pot. The pots were well watered during the experimental period.

The herbicide metolachlor was applicated at preemergence, without incorporation, with a CO₂ backpack sprayer operating at 2.109kg/cm² to deliver 200 l/ha. The herbicide was applied at rates of 0.0, 1.5, 3.0, 4.5, and 6.0 kg a.i./ha, on two substrata, soil (substratum 1) and a 50-50% mixed soil + sand (substratum 2).

The soil used was a dystrophic brown latosol, with a pH in water of 5.84. The chemical and physical properties of the substrata are described in Table 1. The substrata 1 and 2 were fertilised with 200 and 150mEq of P, 100 and 80mEq of K and 0.1 and 0.08mEq of Mo, respectively.

Fifty-two days after emergence (at R₁ stage - beginning of flowering), the plants were harvested and evaluated for shoot, root, leaf and nodule dry matter, nodule number, and leaf area.

The experimental design was completely randomized with four replicates. The experimental unit was a pot with two plants. The data were submitted to variance analysis and multiple polynomial regression.

Table 1. Physical and chemical characteristics of the two substrata used in the present work.

Property	Substr	atum
	Soil	Soil + Sand
Potassium (ppm)	96.00	48.00
Calcium + Magnesium (mEq)	11.30	5.65
Organic matter (%)	5.80	2.90
Clay (%)	70.00	30.00
Sand (%)	1.50	55.00
Silt (%)	27.50	15.00

RESULTS AND DISCUSSION

The increase of metolachlor rate significantly reduced shoot, leaf and nodule dry matter and leaf area, according to a linear polynomial regression (Figure 1), while root dry matter and nodule number were not affected. The interaction "rate x substratum" was not significant for all variables studied, showing that the effect of increasing rates of metolachlor in soybean growth and nodulation followed the same pattern on the two substrata, despite the differences in the physical characteristics. Therefore, the average results of the two substrata for each rate of metolachlor are presented in Figure 1. The highest rate of metolachlor resulted in a reduction of close to 20% of shoot, leaf and nodule dry matter and leaf area, despite the absence of visual phytotoxicity in plants treated with the herbicide.

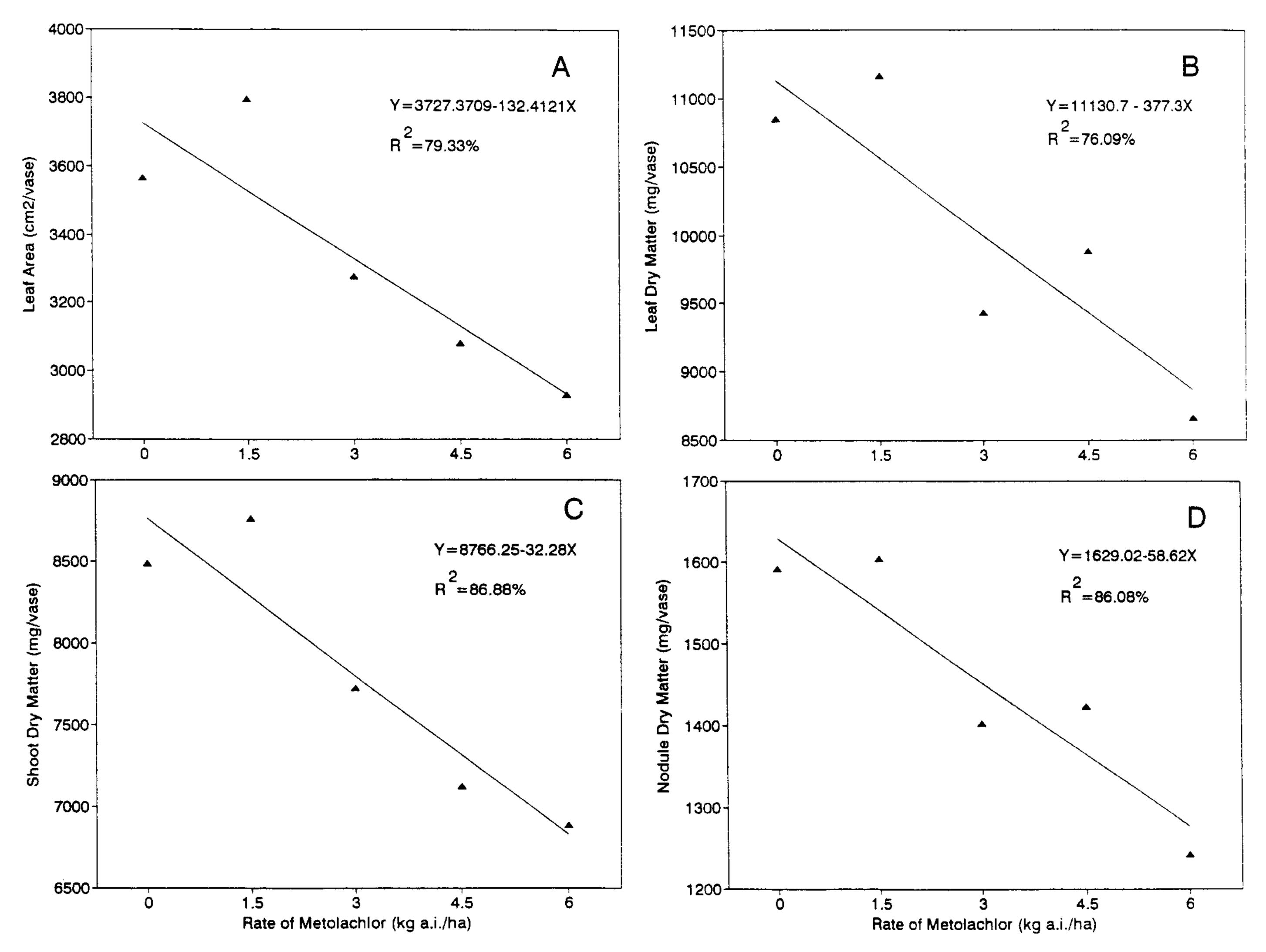


Figure 1. Leaf area (A), leaf (B) and shoot (C) dry weight, and nodule number (D) of soybean cv. BR-4 treated with increasing rates of Metolachor. Lages, SC. Brazil, 1992-93.

Metolachlor affected the nodule dry matter and not the nodule number. Similar results were obtained by DUNIGAN et al. (1972) when they observed a more detrimental effect to tap root than to lateral root nodules in soybean treated with different herbicides. According to these authors as tap root nodules are generally larger and might therefore fix more atmospheric N₂ than lateral root nodules, the nodule dry matter must be a better parameter since with the determination of total number of nodules, each nodule, large or small, receives equal weight in analysis. MORAES et al. (1989) observed a delay on the process of nodulation in soybean treated with high rates of metribuzin, with a resultant reduction of nodule dry matter. Therefore, soybean plants treated with herbicide can produce, on maturity, less total-N as well as lower percentage of total-N content in pods, as a result of a less effective simbiotic fixation of N₂, with a consequent reduction of plant growth and pod yield (ALAA-ELDIN et al., 1981).

Nodulation is a symbiotic relationship, consequently, factors affecting either the plant or bacteria will affect nodule formation. Several authors report a more negative effect of herbicides in soybean plants than on bacterium. Increasing rates of chlorpropham and nitralin reduced nodule fresh weight in soybean but did not reduce the soil population of B. japonicum, indicating that the herbicidal influence on nodulation was due to altered plant physiology reactions, rather than a direct toxicity to B. japonicum (KAPUSKA & ROUWENHORST, 1973). Addition of inorganic N to trifluralin treated soybeans exhibiting decreased nodulation did not correct the growth inhibition (KUST & STRUCKMEYER, 1971). GARCIA & JOHNSON (1969), CARLYLE & THORPE (1947), and KUST & STRUCKMEYER (1971), agree that the detrimental effect of herbicides on legume nodulation is not at the nodule site. They suggest that herbicides induce responses in plants that reduce nodulation.

Metolachlor appears to interfere with nucleic acid and protein synthesis (ASHTON & CRAFTS, 1981) and thus it may interfere with the physiological processes related with nodulation, symbiotic fixation as well as plant growth, with negative effects to soybean development, as observed on the present work (Figura 1). SCARPONI et al. (1992) observed a reduction on dry matter, protein and chlorophyll content of soybean seedlings treated with metolachlor. GREENFIELD (1991) reported no effect on nodule number or mass in soybean treated with metolachlor but observed a yield reduction of 10% compared to manual weeding, showing that the herbicide may affect plant physiology and/or symbiotic fixation without causing a perceptive negative effect on nodulation.

With the exception of root dry matter and nodule number, the growth was significantly reduced on soil + sand in comparison to soil. This may be caused by calcium deficiency on vases with this kind of substratum (Table 2), since they were not supplied with this element to compensate for the addition of sand. As calcium is a macronutrient, essential to nodulation and plant physiology (SALISBURY & ROSS, 1992), its low level may have caused the observed effect.

Table 2. Mean results per substratum of nodulation and growth of soybean treated with different rates of metolachlor. Lages, SC, Brazil, 1992-93.

			Parameter			
Substratum	Leaf area (cm²/vase)		Shoot	Root	Nodule	Nodule
	(CIII / Vase)	Dry matter (mg/vase)				- number
Soil	3,610a*	10,868a	8,633a	3,592a	1,541a	386a
Soil+Sand	3,050 в	9,129 b	6,962 b	3,571a	1,365 b	386a
CV (%)	15.59	11.37	10.52	19.39	13.95	9.02

^{*} Mean separation within columns by Duncan's Multiple Range Test, P = 0.05.

The root dry matter was not affected by the treatments, probably as a result of the limitation of volume of substratum in the vases and the advanced stage of development of plants at harvest (52 days after emergence), resulting in a restriction on root growth.

From the obtained results it can be concluded that increasing doses of metolachlor show a linear patern of inhibitory affects on the growth and nodulation of soybean. However, it should noted that this work was conducted in pots in a greenhouse, and in a limited volume of substratum, which may have enhanced the inhibitory effects of metolachlor on the growth variables affected by the herbicide, compared to a field situation.

REFERENCES

ALAA-ELDIN, M.N., MAHMOUD, S.A.Z., MAKAWI, A. et al. Effect of pre-emergence application of some herbicides on nodulation, nitrogen fixation and growth of soybean. **Pesquisa Agropecuária Brasileira**, Brasília, v. 16, n. 6, p. 833-839, 1981.

ASHTON, F.M., CRAFTS, A.S. Mode of action of herbicides. 2. ed. New York: John Wiley & Sons, 1981. 525 p.

BLANCO, H.G., OLIVEIRA, D.A., ARAUJO, J.B.M. Período crítico de competição de uma comunidade natural de mato em soja. O Biológico, São Paulo, v. 44, p. 299-305, 1978.

- BURNSIDE, O.C. Tolerance of soybean cultivars to weed competition and herbicide. Weed Science, Champaign, v. 20, n. 4, p. 294-297, 1972.
- CARLYLE, R.E., THORPE, J.D. Some effects of ammonium and sodium 2, 4- dichlorophenoxyacetates on legumes and Rhizobium bacteria. **Journal of American Society of Agronomy**, Geneva, v. 39, p. 929-936, 1947.
- CARTTER, J.L., HARTWIG, E.E. The management of soybeans. In: NORMAN, A.G. The soybean. 2. ed. New York: Academic Press, 1967. p.162-226.
- DEUBER, R., CAMARGO, P.N., SIGNORI, L.H. Efeitos de herbicidas e populações de plantas na nodulação e produção de soja (Glycine max (L.) Merrill cv. "Santa Rosa"). Planta Daninha, Campinas, v. 4, n. 2, p. 97-109, 1981.
- DUNIGAN, E.P., FREY, J.P., ALLEN Jr., L.D. et al. Herbicidal effects on the nodulation of *Glycine max* (L.) Merrill. Agronomy Journal, Madison, v. 64, p. 806-808, 1972.
- GARCIA, M.M., JORDAN, D.C. Herbicide action on trefoil symbiosis. Plant and Soil, Haren, v. 30, p. 317-334, 1969.
- GIARDINI, A.R., LOPES, E.S., DEUBER, R. Influência de herbicidas na nodulação da soja (*Glycine max* (L.) Merr.). Planta Daninha, Campinas, v. 2, n. 1, p. 21-32, 1979.
- GREENFIELD, P.L. The influence of methods of inoculation and certain herbicides on nodulation and seed yield of soybean. South African Journal of Plant and Soil, Pretoria, v. 8, n. 3, p. 119-122, 1991.
- KAPUSTA, G., ROUWENHORST, D.L. Interaction of selected pesticides and *Rhizobium japonicum* in pure culture and under field conditions. Agronomy Journal, Madison, v. 65, p. 112-115, 1973.

- KNAKE, E.L., SLIFE, F.W. Giant foxtail seeded at various times in corn and soybean. Weeds, New York, v. 13, n. 4, p. 331-334, 1965.
- KUST, C.A., STRUCKMEYER, B.E. Effects of trifluralin on growth, nodulation and anatomy of soybeans. Weed Science, Champaign, v. 19, n. 2, p. 147-152, 1971.
- MACEDO, E. de C., OLIVEIRA, D.A. Influência de herbicidas na nodulação e desenvolvimento da soja. In: SEMINÁRIO NACIONAL DE PESQUISA DE SOJA, 4, Porto Alegre, 1986. Resumos..., Porto Alegre, 1986. p. 35.
- MORAES, R. de C.P., LOPES, N.F., SILVA, E.A.M. da, et al. Tolerância e nodulação de soja (*Glycine max* (L.) Merrill), cultivada em dois substratos e submetida a quatro doses de metribuzin. Revista Ceres, Viçosa, v. 36, n. 208, p. 492-506, 1989.
- OLUMBRE, J.W.K., VEATCH, C. Organic matter-amibem interaction on nodulation and growth of soybean. Weed Science, Champaign, v. 17, n. 2, p. 264-265, 1969.
- PROSCH, S.D., WEBER, J.B. Trifluralin-substituted amide herbicide interactions. Agronomy Journal, Madison, v. 80, p. 567-570, 1988.
- ROSOLEM, C.A., NAKAGAWA, J., MARCONDES, D.A.S. Efeito da trifluralina na nodulação e absorção de nitrogênio pela soja. Pesquisa Agropecuária Brasileira, Brasília, v. 22, n. 11, p. 1265-1271, 1985.
- SALISBURY, F.B., ROSS, C.W. Plant physiology. 2. ed. Belmont: Wadsworth Pub. Co., 1992. 682 p.
- SCARPONI, L., NEMAT-ALLAH, M., MARTINETTI, L. et al. Metolachlor in corn (Zea mays) and soybean (Glycine max): persistence and biochemical signs of stress during its detoxification. Journal of Agricultural and Food Chemistry, Washington, v. 40, n. 5, p. 884-889, 1992.