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Herbicide effects on the growth and nodulation potential of *Rhizobium trifolii* with *Trifolium subterraneum* L.

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Abstract

A study was made of the effect of the herbicides 2,4-D, amitrole, atrazine, chlorsulfuron, diclofop-methyl, diquat, glyphosate, paraquat and trifluralin on the nodulation of sub-clover (*Trifolium subterraneum* L. 'Clare'), the growth of *R. trifolii* TA1 in liquid nutrient medium and the ability of herbicide-treated inoculum to successfully nodulate sub-clover plants. As concentrations of amitrole, diclofop-methyl and glyphosate in the rooting environment increased from 0 to 20 mg ai L^{-1}, nodulation decreased linearly. The other herbicides at these concentrations caused more severe decreases in nodulation. Growth of *R. trifolii* TA1 in nutrient broth was significantly retarded by all concentrations of diquat, 2 mg ai L^{-1} of paraquat, 10 mg ai L^{-1} of glyphosate and 2 mg ai L^{-1} of chlorsulfuron. Other herbicides did not suppress rhizobial growth. Inoculation with TA1 that had been grown in the presence of amitrole, atrazine or glyphosate and then washed free of the herbicide decreased nodulation of sub-clover, indicating that these herbicides may physiologically influence the nodulating potential of certain strains of Rhizobium. The remaining herbicides showed no indications of this effect.

Introduction

Many cereal cropping regions of temperate Australia rely mainly upon spontaneous regeneration of pasture legumes for the input of nitrogen into the soil. Reports have appeared in the literature of herbicide-induced declines in nodulation of legumes (Bollich *et al.*, 1985; Dunigan *et al.*, 1972; Eberbach and Douglas, 1983; Fletcher *et al.*, 1957; Fletcher *et al.*, 1956; Garcia and Jordan, 1969; Kust and Struckmeyer, 1971; Mallik and Tesfai, 1985; Olume and Veatch, 1969) and declines in rates of symbiotic nitrogenase activity (Bollich *et al.*, 1985; Cardina *et al.*, 1986; Eberbach and Douglas, 1983; Mallick and Tesfai, 1985; Torstensson, 1975). Herbicide-induced declines in nodulation may be the result of injury to the legume's root system or to *Rhizobium* before or during infection. Also, the decline in nitrogenase activity may be due to a herbicide-induced reduction in supplies of photosynthates to the nodules, physiological damage to the plant root or nodules or, physiological damage to *Rhizobium* either before or after inoculation.

Recommended herbicide dosage rates have been shown to have a neglible effect on the growth of rhizobia (Cardina *et al.*, 1986; Moorman, 1986; Roslycky, 1985). Although the effect of herbicides on bacterial growth is relevant, rhizobia may lose the ability to induce nodulation when exposed to pesticides before they lose the ability to multiply (Curley and Burton, 1975). Fletcher *et al.* (1956; 1957) showed that phenoxy herbicides at recommended rates could be detrimental to nodulation. Similarly, recommended rates of 2,4-DB on trefoil plants (Garcia and Jordan, 1969) and trifluralin on soybean plants (Kust and Struckmeyer, 1971) reduced lateral root growth and nodule formation. In

both of these reports, morphological examination of root nodule tissue indicated extensive herbicide-induced damage to xylem vessels but little damage to nodular bacteroids. More recent work has established that herbicide-affected legumes suffered a reduction in nitrogenase activity but not necessarily in total plant weight (Mallik and Tesfai, 1985; Torstensson, 1975). Results of Torstensson (1975) further indicated that it may be possible for the nodule-bacteroid complex to suffer some physiological disturbance without the plant showing any obvious signs of injury.

The objectives of this study involving commercial formulations of herbicides were to: 1) estimate the effects of herbicides added to liquid nutrient media on the nodulation of sub-clover; 2) observe the ability of the *R. trifolii* TA1 grown in herbicide-amended nutrient solutions to nodulate sub-clover plants.

Materials and methods

Herbicides

The herbicides investigated were 2,4-D [(2,4-dichlorophenoxy) acetic acid], amitrole (1*H*-1,2,4-triazol-3-amine), atrazine [(6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine], chlorsulfuron (2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzene sulfonamide), dichlofop-methyl {2-[4-(2,4-dichlorophenoxy)-phenoxy]-methyl propionate}, diquat (6,7-dihydrodipyrido[1,2-a2′,1′-c]pyrazinediium ion), glyphosate [*N*-(phosphonomethyl)glycine], paraquat (1,1′-dimethyl-4,4′-bipyridinium ion) and trifluralin [2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl) benzenamine]. Research grade chlorsulfuron was obtained for use in this study as a commercial formulation was at the time unavailable. All other herbicides were used as commercial formulations.

Herbicides were sterilized by filtration using Gelman Metricel Glass fibre membrane filters. They were then diluted to the appropriate concentrations with sterile distilled water. For all herbicides except chlorsulfuron, a concentration of 2 mg ai L^{-1} of nutrient solution was calculated to result in approximately the equivalent concentration of active ingredient in the top one cm of soil following recommended field applications. For chlorsulfuron, this concentration was calculated to be 0.2 mg ai.l^{-1}.

Bacteria

R. trifolii TA1 inoculum for experiment 1 was prepared from cultures of bacteria incubated at 28°C for 4 days on yeast extract mannitol (YEM) agar plates. Sterile saline solution (0.9% w/v) was added to each plate and then the bacterial suspensions were transferred to a flask and diluted to an approximate concentration of 10^5 bacteria per ml. The number of bacteria per ml was determined before inoculation by using the viable count technique. For experiment 2, a loopful of TA1 was taken from a culture that had been growing on plates as in experiment 1 and used to inoculate a flask containing Bergersen's (1961) broth. When growing exponentially, the new culture was used in experiment 2.

Seedlings

Seeds of *T. subterraneum* cv Clare for both experiments were sterilized and germinated as previously described (Eberbach and Douglas, 1983). Agar slopes in 20 mm × 150 mm glass test tubes were prepared for plant growth by using Gibson's (1963) technique. Seedlings with straight radicles 2.0–2.5 cm in length were selected for use. Each radicle was inserted through a perforation in the aluminium foil cap of a tube and manipulated so that it came into contact with the agar surface. Tubes containing the seedlings were placed in a growth room with light intensity of 140 μE m^{-2} s^{-1}, a photoperiod of 12 hours light at 26°C and a dark period of 12 hours at 15°C.

Experiment 1

Seedlings were grown for three days, during which time the roots elongated to approximately 120 mm and the cotyledons opened. 28 mL of Jensen's nutrient solution (Vincent, 1970) amended with 5 mg L^{-1} of N as NH_4NO_3 was added to each tube. Each plant was inoculated with 1 mL of the bacterial suspension described previously after

which the herbicide solutions were added which, except for chlorsulfuron, enabled the concentrations of active ingredient to be 0, 2, 5, 10 or 20 mg ai L^{-1} of nutrient solution. Concentrations of chlorsulfuron were 0, 0.2, 0.5, 1.0 or 2.0 mg ai L^{-1} of nutrient solution. Nodule numbers were recorded every two weeks for a period of eight weeks. Four replications of each treatment were used.

Experiment 2

This experiment was similar to that described by Grossbard (1975).

Herbicide treatment of bacteria. Sterile dilutions of the herbicides were transferred to 28 ml of Bergersen's (1961) broth contained in 150 ml side-arm erlenmeyer flasks to achieve final concentrations of 0, 2, 5, 10 or 20 mg ai L^{-1} except in the case of chlorsulfuron, where the final concentrations were 0, 0.2, 0.5, 1.0 and 2.0 mg ai L^{-1}. One ml aliquots of TA1 growing exponentially in Bergersen's broth were added to the flasks which were then incubated in the dark for 7 days at 28°C. Each treatment was performed in duplicate. Bacterial growth was measured every 24 hours turbidimetrically using a Klett-Summerson photo-electric colorimeter. Approximate bacterial numbers were determined from a calibration curve of TA1 grown in Bergersen's broth over a period of 168 hours, where measurement of the turbidity of the suspension was related to viable cell numbers determined at the time of the turbidimetric measurement.

Preparation of inoculum. After the bacteria had grown in the presence of herbicide for seven days, two 6 mL aliquots of each suspension were taken and transferred to 10 mL centrifuge tubes. The suspensions were centrifuged at 9,250 *g* for 15 minutes at 10°C. The supernatant was discarded and the harvested cells resuspended in 6 mL sterile saline solution (0.9% w/v). One tube of each treatment was then set aside and not washed any further (single wash treatment). The other tube was recentrifuged and resuspended a further four times (repeated washings treatment).

Inoculation and nodulation of plants. Seedlings grown in tubes as described in experiment 1 were inoculated with the herbicide-treated bacteria. Progressive nodulation numbers for the plants were recorded as in experiment 1. Four replicates were used for each bacterial treatment.

Statistical analysis

Prior to statistical analysis, the nodulation data collected from the experiments were tested for homogeneity of variance by using Bartlett's Test (Sokal and Rohlf, 1969). While data from experiment 2 were homogeneous, nodule data from experiment 1 required transformation into the square root form to be homogeneous. The data were analysed by regression analysis, the results of which are reported in Tables 2 and 5. Data for bacterial growth were analysed by a oneway analysis of variance with repeated measures, and where the F-test indicated a significant difference ($P < 0.05$) between means, Fisher's least significant differences (LSD) were constructed at the 5% level (Sokal and Rohlf, 1969). After statistical analysis, nodulation results were converted into the percentage of the control form to aid presentation.

Results and discussion

In this study, for all herbicides apart from chlorsulfuron, commercial formulations were used as we wanted to determine the influence that the commercially-applied product as opposed to the pure active ingredient has over the selected legume-rhizobia symbiosis.

Experiment 1

In most cases, the observed response of subclover inoculated with TA1 to the herbicide-amended nutrient solutions was a reduction in the number of nodules per plant (Table 1). Diquat, atrazine and 2,4-D inhibited nodulation at all concentrations. These observations agree with those of other workers who studied 2,4-D (Fletcher *et al.*, 1957) and atrazine (Torralba Redondo *et al.*, 1986). Paraquat-treated plants exhibited minor nodu-

Table 1. Nodulation of Sub-clover c.v. Clare inoculated with *R. trifolii* TA1 and grown in herbicide-amended nutrient solutions

Herbicide concentration in nutrient solution (mg L^{-1})	Nodulation (% of control)[a]				
	Glyphosate	Paraquat	Diquat	Trifluralin	Diclofop-methyl
0	100	100	100	100	100
2	73	2	0	2	78
5	92	0	0	1	32
10	26	0	0	0	17
20	3	0	0	0	18
	Atrazine	Amitrole	2,4-D	Chlorsulfuron[b]	
0	100	100	100	100	
2	0	60	0	11	
5	0	36	0	4	
10	0	22	0	7	
20	0	19	0	1	

[a] After statistical analysis, the nodulation results for each treatment were transformed to percentage of the control to aid in presentation.
[b] Rates of chlorsulfuron were one tenth of those used for the other herbicides.

lation when the herbicide was applied at 2 mg ai L^{-1} but higher application rates caused total nodule inhibition.

Amitrole, diclofop-methyl and glyphosate caused nodulation to decrease approximately linearly as the herbicide concentration increased from 0 to 20 mg ai L^{-1}. All concentrations of trifluralin and chlorsulfuron caused severe reductions in nodule numbers. Data obtained with glyphosate and trifluralin agree with results reported by Mallik and Tesfai (1985) and, Brock (1972) and Parker and Dowler (1976) respectively. However the present results with diclofop-methyl show that this herbicide in the current study affected nodulation more than that reported by Peters and Ben Zbiba (1979). Simple regression equations developed relating nodulation to herbicide concentration in the rooting media are reported in Table 2. For each of these herbicides, linear regression of the square root transformation of the number of nodules formed per plant to herbicide concentration was significant at $P < 0.01$.

Regression equations presented in Table 2 were used to predict the minimum concentration of active ingredient of each herbicide required in the liquid nutrient medium to totally inhibit nodulation of this legume-rhizobia combination. These estimates are given in Table 3. Estimates for concentrations of herbicides that relate to recommended field application rates distributed evenly through the top 1 cm of soil were approximately 2 mg ai L^{-1} with one exception (chlorsulfuron 0.2 g L^{-1}) and hence, the predictions in Table 3 suggest that at field application rates, these her-

Table 2. Regression equations relating the number of nodules formed per sub-clover plant to the herbicide concentration in Jensen's nutrient solution

Herbicide	Equation[a]	R^2(Adj)	F-test	SE[b] of y	SE of b
Amitrole	$\sqrt{y} = 7.50 - 0.27x$	0.74	53.2***[c]	1.2	0.04
Chlorsulfuron	$\sqrt{y} = 4.09 - 2.15x$	0.33	9.7**	2.1	0.69
Diclofop-methyl	$\sqrt{y} = 9.33 - 0.41x$	0.82	89.5***	1.2	0.04
Glyphosate	$\sqrt{y} = 6.83 - 0.28x$	0.88	120.9***	0.8	0.02
Trifluralin	$\sqrt{y} = 6.21 - 0.43x$	0.42	17.8***	3.5	0.10

[a] Equations are in the form of $\sqrt{y} = a + bX$, where y = number of nodules; a = intercept; b = gradient of line; X = herbicide concentration.
[b] SE, standard error.
[c] ** Significant at the probability of less than 1.0%, *** significant at the probability of less than 0.1%.

Table 3. Predicted herbicide concentration in nutrient media necessary to completely inhibit nodulation of sub-clover c.v. Clare

Herbicide	Herbicide concentration ($mg\,l^{-1}$)
Amitrole	27.5
Chlorsulfuron	1.90
Diclofop-methyl	23.0
Glyphosate	24.5
Trifluralin	14.6

bicides would cause little damage to legume nodulation. Further, Fletcher *et al.* (1956, 1957) have shown that soil may reduce the activity of herbicides, and hence, results obtained in the present experiment where plants were grown in nutrient solution are indicative only of the potential of the herbicides to interfere with the formation of nodules on the roots of sub-clover.

Experiment 2

Growth of inoculum. Most fast growing *Rhizobium* species grow satisfactorily in the presence of mineral salts, glutamate, biotin and thiamine (Chakabartis *et al.*, 1981). Yeast extract-based mediums supply a variety of sources for nutrients and growth factors (Elkan and Kwik, 1968; Graham, 1963) while a minimal medium made with only specific additives is more likely to highlight metabolic disorders of bacteria resulting from herbicide-induced metabolic damage. Therefore for the present study Bergersen's (1961) broth was selected as the growth medium to be used for the TA1 inoculum due to it's nutrient specificity.

The growth of TA1 in pure culture was completely inhibited by $2\,mg\,ai\,L^{-1}$ of diquat and significantly retarded ($P < 0.05$) by concentrations of 2 to $20\,mg\,ai\,L^{-1}$ of paraquat, 10 and $20\,mg\,ai\,L^{-1}$ of glyphosate and $2\,mg\,ai\,L^{-1}$ of chlorsulfuron (Figs. 1a, b, c and d). No other herbicide when present at the selected concentrations had an effect on bacterial growth.

When applied to a minimal medium, paraquat has been reported to inhibit the production of branched-chain amino acids in *Escherichia coli* by the induction of stringency, resulting in inhibition of bacterial growth (Seither and Brown, 1984). Growth of some rhizobia strains has exhibited a relatively high tolerance to paraquat in media that contained a multiple nutrient source (Moorman, 1986; Roscylsky, 1985). The possibility exists for the paraquat-induced decline in the growth of TA1 (Fig. 1b) in the present study to be due to the inability of treated bacteria to synthesize branched-chain amino acids that were absent from the growth media. No reports of the effects of diquat on the growth of *Rhizobium* are available, but Wallnoefer (1968) found that this herbicide was more effective than paraquat in causing an inhibition of the growth of various bacteria and fungi *in vitro*.

The growth-retarding effect of glyphosate on TA1 grown in Bergersen's broth is consistent with reports of Jaworski (1972) relating to *R. japonicum* USDA 71 and Faizah *et al.* (1980) with various species of rhizobia. Results presented in Fig. 1c for the growth of TA1 suggest that glyphosate at concentrations above a critical level ($10\,mg\,ai\,L^{-1}$) may act by increasing the lag phase. Similarly, an extension in the lag phase of *Pseudomonas* sp. grown in media where glyphosate was the only form of phosphate, has been reported (Moore *et al.*, 1983). As glyphosate acts to inhibit 5-enolpyruvylshikimate-3-phosphoric acid synthetase (Amrhein, 1980) resulting in the inhibition of the synthesis of aromatic amino acids, the lack of such supplements in the growth medium used in the present study may have affected the growth of TA1. Where, however, the supply of aromatic amino acids in media was sufficient, as with yeast extract based media, growth of *R. japonicum* strain 110SK and 138ES was not influenced by the presence of glyphosate (Moorman, 1986).

Data in Fig. 1d indicate that cells of *R. trifolii* TA1 were able to sustain normal growth in the presence of 0.2, 0.5 and 1.0 mg of chlorsulfuron per litre of nutrient medium. Results obtained with plants that resist the phytotoxic effects of this chemical (Hageman and Behrens, 1984; Sweetster *et al.*, 1982) suggest that the resistance observed in the present study may have been due to the ability of cells to rapidly detoxify the herbicide. When the chlorsulfuron concentration was increased to $2\,mg\,ai\,L^{-1}$, some inhibition of growth occurred, suggesting that if a detoxification mechanism was present in the bacterial cells, it was unable to transform a sufficient quantity of the herbicide applied

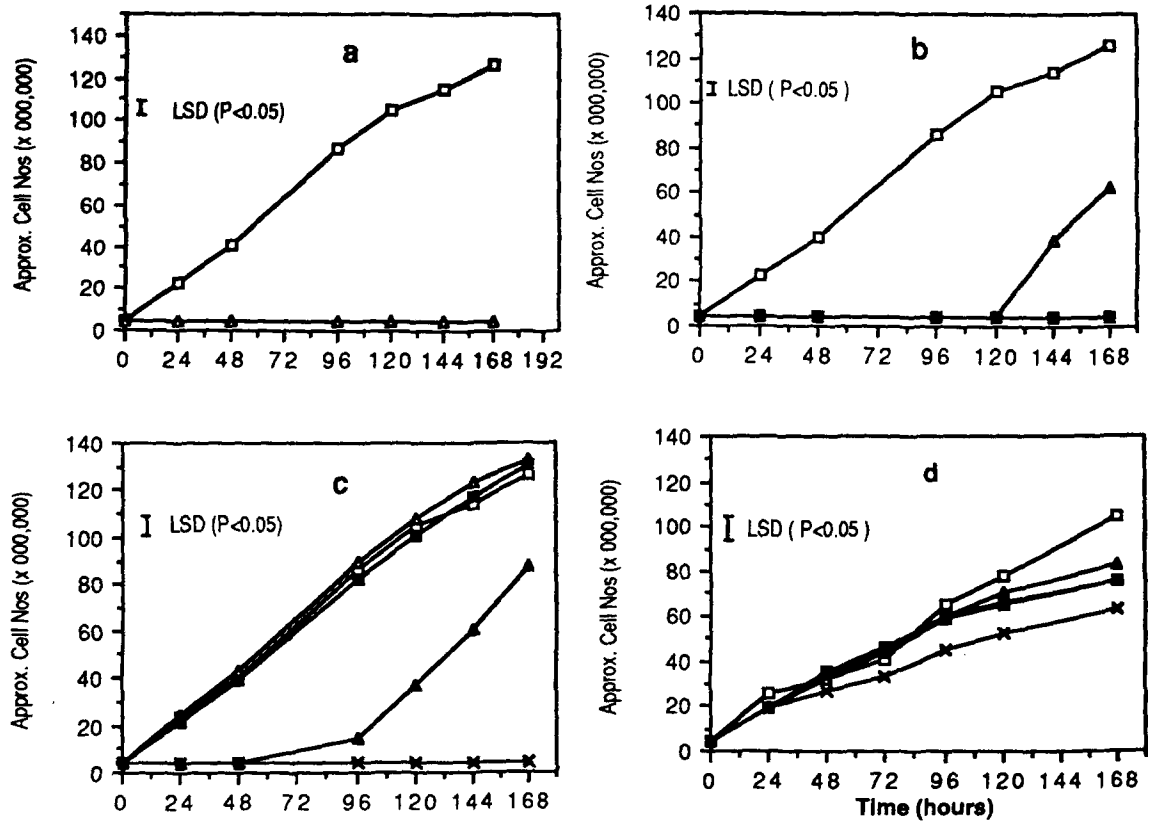

Fig. 1. The influence of diquat (**1a**), paraquat (**1b**), glyphosate (**1c**) and chlorsulfuron (**1d**) on the growth of *R. trifolii* TA1 grown in Bergersen's broth. Herbicide application rates for paraquat, diquat and glyphosate are denoted as 0 (□), 2 (△), 5 (■), 10 (▲) and 20 (×) mg ai L^{-1}. For chlorsulfuron the herbicide application rates are denoted as 0 (□), 0.2 (△), 0.5 (■), 1.0 (▲) and 2.0 (×) mg ai L^{-1}.

at this high rate to non-toxic metabolites for normal growth to occur.

Nodulation of plants. Two inoculum washing procedures as described by Grossbard (1975) were used in this experiment; a single wash procedure to separate the inoculum from the herbicide-amended growth medium and a repeated wash procedure, to remove as much of the residual herbicide associated with the inoculum as practicable. Hence, the former procedure was used to indicate the damage that any herbicide carried over with the inoculum may have on nodulation, while the latter procedure attempted to highlight any herbicide-induced physiological change that had occurred to the bacteria with regard to their potential to nodulate.

Single wash. After the single wash treatment, herbicide carryover associated with all herbicide-treated inocula was sufficient to alter the plants' potential to nodulate (Table 4). Regression equations relating nodule numbers per plant to herbicide concentrations of the inoculum growth media are presented in Table 5. Chlorsulfuron, 2,4-D and trifluralin stimulated nodulation when present in the inoculum growth medium at concentrations of 0.2, 1.0 and 2.0 mg ai L^{-1} respectively. The carryover of 2,4-D and trifluralin associated with the inoculum grown in progressively higher concentrations of these herbicides had a significantly ($P < 0.05$) depressive effect on plant nodulation, while the increased carryover in chlorsulfuron had no effect on plant nodulation (Tables 4 and 5). Levels of carryover of 2,4-D in this instance of observed nodulation stimulation may have been low enough to stimulate root growth as reported elsewhere (Ashton and Crafts, 1981). As Fletcher *et al.* (1956) demonstrated that rates of

Table 4. Nodulation of sub-clover c.v. Clare inoculated with herbicide-treated *R. trifolii* TA1 given repeated (R.W.) or single washings (S.W.) before inoculation

Herbicide concentration in growth medium mg L^{-1}	Nodulation (% of control)[a]							
	Glyphosate		Paraquat		Amitrole		Trifluralin	
	R.W.[b]	S.W.[c]	R.W.	S.W.	R.W.	S.W.	R.W.	S.W.
0	100	100	100	100	100	100	100	100
2	55	61	61	81	67	67	79	116
5	51	62	—[d]	—	70	81	129	98
10	55	65	—	—	67	76	87	62
20	48	37	—	—	58	61	98	39
	2,4-D		Diclofop-methyl		Chlorsulfuron[e]		Atrazine	
	R.W.	S.W.	R.W.	S.W.	R.W.	S.W.	R.W.	S.W.
0	100	100	100	100	100	100	100	100
2	103	173	84	85	79	133	85	82
5	126	46	88	92	107	109	96	69
10	97	19	79	85	94	95	75	72
20	85	21	84	50	109	102	76	63

[a] After statistical analysis, nodulation results for each treatment were transformed to percentage form to aid presentation.
[b] R.W. bacteria washed four times in sterile saline solution (0.9% w/v) after growing in the herbicide-amended nutrient broth.
[c] S.W. bacteria washed once only with sterile saline solution (0.9% w/v) after growing in the herbicide-amended nutrient broth.
[d] Nodulation study not performed for levels of paraquat above 2 mg ai L^{-1}.
[e] Rates for chlorsulfuron treatment were one tenth of those used for the other herbicides.

Table 5. Regression equations depicting the ability of *R. trifolii* TA1, grown in the presence of herbicides and singly or repeatedly washed before inoculation, to nodulate sub-clover plants

Herbicide	Equation[a]	R^2(Adj)	F-test	SE[b] of y	SE of b
2,4-D SW[c]	y = 57.7 − 2.9x	0.43	15.8***[e]	23.4	0.73
2,4-D RW[d]	y = 51.3 − 0.31x	0.02	0.6	13.8	0.43
Amitrole SW	y = 69.4 − 1.06x	0.25	6.8**	11.7	0.41
Amitrole RW	y = 69.1 − 1.12x	0.39	13.1**	9.9	0.31
Atrazine SW	y = 73.3 − 1.14x	0.48	16.9***	8.6	0.28
Atrazine RW	y = 76.9 − 0.92x	0.22	6.5*	11.6	0.36
Chlorsulfuron SW	y = 93.3 − 8.70x	0.09	2.7	16.9	5.30
Chlorsulfuron RW	y = 83.0 − 3.84x	0.01	1.3	10.9	3.42
Diclofop-methyl SW	y = 83.2 − 2.07x	0.54	23.2***	13.7	0.43
Diclofop-methyl RW	y = 77.8 − 0.34x	0.01	0.8	11.6	0.38
Glyphosate SW	y = 64.7 − 1.38x	0.25	7.1*	15.2	0.52
Glyphosate RW	y = 59.7 − 1.12x	0.21	5.7*	15.3	0.49
Trifluralin SW	y = 52.9 − 1.51x	0.33	10.4**	15.0	0.47
Trifluralin RW	y = 42.5 − 0.28x	0.04	0.4	14.7	0.46

[a] Equations are in same form as those in Table 2.
[b] SE, standard error.
[c] SW Bacteria from the single wash treatment.
[d] RW Bacteria from the repeated wash treatment.
[e] * Significant at the probability of less than 5%; ** significant at the probability of less than 1%; *** significant at the probability of less than 0.1%.

0.01 ppm of 2,4-D in plant growth media were capable of a depressive influence on nodule initiation with some legumes, the carryover reported here would need to be very low indeed. All other herbicides reduced nodulation significantly ($P < 0.05$) as their concentrations in the inoculum growth media increased (Table 5).

Repeated washing. Inocula grown in the presence of 2,4-D, chlorsulfuron, diclofop-methyl or trifluralin and repeatedly washed to remove associated carryover herbicide prior to inoculation had no significant ($P < 0.05$) effect on plant nodulation (Table 5). This suggests that with these particular herbicides, herbicidal activity observed in the single wash category was entirely due to the effect of the carryover herbicide on the plant and that the bacteria's potential to nodulate the host plant when grown in the presence of herbicide over a short time period was unaffected.

Inocula grown in the presence of amitrole, atrazine or glyphosate and repeatedly washed before inoculation showed a decreased ability to cause nodulation ($P < 0.05$) (Table 5). This was not necessarily due to carryover-herbicide but possibly to herbicide-induced physiological damage occurring to the bacteria prior to nodule initiation. The observation regarding atrazine in the current study differs from observations of Grossbard (1970) with a different rhizobia strain-legume species. This may however, reflect the diverse array of effects that have been noted with herbicides on different strain-species combinations.

Reports have appeared in the literature supporting the observation that in some cases herbicides may affect the legume-*Rhizobium* symbiosis by affecting the bacterial member without seeming to affect the legume. For example, analysis of Torstensson's (1975) data revealed that in some legume-herbicide combinations (white clover-bentazon), reduction in plant dry weight closely correlated with an observed reduction in acetylene reduction activity (ARA). In other combinations (field bean-bentazon) plant dry weight remained unaffected by herbicide treatment while a 40% reduction in ARA occurred. Glyphosate has also been observed to induce a similar reduction in nitrogenase activity and nodulation of soybeans while not affecting legume growth or total plant nitrogen content (Mallik and Tesfai, 1985). In addition, ^{15}N-isotope dilution techniques have revealed that legumes may compensate for lower nitrogenase activity by assimilating more soil inorganic nitrogen so that total nitrogen contents of herbicide-affected plants may appear unaltered while nitrogenase activity is observed to decline (Rennie and Dubetz, 1984). Therefore, the total N content of legumes should not be used as an index of herbicide damage.

The present results, and those reported by other authors suggest that in some cases the *Rhizobium* is likely to be the member of the symbiotic pair that is damaged by the herbicide, either prior to infection, during infection or in the bacteroidal state within the nodule after infection. This being the case, then not only should further work be directed towards the herbicidal influence on bacterial nodulation initiation capacity, but also towards the potential of the ensuing symbionts to efficiently fix atmospheric nitrogen.

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