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Effect of herbicide residues in a sandy loam on the growth, nodulation and nitrogenase activity (C_2H_2/C_2H_4) of *Trifolium subterraneum*

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Abstract

The herbicides 2,4-D, amitrole, atrazine, diclofop-methyl, diquat, paraquat and trifluralin were applied at rates of 0, 2, 5 and 10 μg ai. g^{-1} to a sandy loam soil and allowed to degrade for 120 days. After this period, subterranean clover seedlings were transplanted into treated soil and the effect of herbicide residues on plant growth, number of nodules formed and nitrogenase activity was investigated. At all rates of atrazine and chlorsulfuron, and at all rates of amitrole in excess of 2 mg ai g^{-1} of soil, sufficient herbicide remained to be lethal to the seedlings. When amitrole was applied at the rate of 2 mg ai g^{-1} of soil, plant growth, nodulation and nitrogenase activity of plants were reduced. Residues of diquat reduced all plant parameters studied while, residues of 2,4-D reduced plant growth and nodule formation, but plant nitrogenase activity was unaffected. Residues of trifluralin had no effect on plant growth parameters but the number of nodules formed per plant was reduced. Residues of paraquat and diclofop-methyl had no effect on any of the plant parameters studied.

Introduction

The cereal cropping regions of temperate Australia depend on the growth of pasture legumes during the ley phase of the rotation for a major addition of nitrogen to the soil. Herbicides are essential for weed control management in these regions, and in some cases, they may pose a potential hazard to the establishment of nodulation and to the nitrogenase activity of legumes (Mallik and Tesfai, 1985).

The immediate effects of herbicides on the symbiotic characteristics of legumes have been investigated (Bertholet and Clark, 1985; Bollich *et al.*, 1985; Cardina *et al.*, 1986; Dunigan *et al.*, 1972; Eberbach and Douglas, 1989; Kust and Struckmeyer, 1971; Lindström *et al.*, 1985; Mallik and Tesfai, 1985; Torstensson, 1975). Although these studies relate the response of plants to the presence of herbicides, only a few reports are available where the phytoxicity of residual levels of herbicides has been studied (Anderson, 1985; Anderson and Barrett, 1985; Eberbach and Douglas, 1983; Fink, 1972).

Papers dealing with the effects of herbicides on the legume-Rhizobium symbiosis have focussed attention on the potential of rhizobia to grow in the presence of herbicides. If growth appears satisfactory, it is generally assumed that the Rhizobium would not be the member of the symbiosis that is damaged by the herbicide. However, Curley and Burton (1975) inferred that rhizobia exposed to herbicides may lose the potential to induce nodulation before losing their

potential to multiply. Also, experiments have been published (Mallik and Tesfai, 1985) that describe herbicide-induced reduction in legume nitrogenase activity in cases where no differences in total N content between the control and plants grown in herbicide-treated soil were observed. In these reports, as the total N content of the plant tissue was unaffected by the presence of herbicides, the reduced nitrogenase activity associated with the plant nodules was not considered to be of great importance. However, ^{15}N-isotope dilution studies have revealed that plants may compensate for lower nitrogenase activity in nodules by assimilating more soil inorganic nitrogen. Total nitrogen content of herbicide-treated plants may therefore appear to be unaffected when plant nitrogenase activity is in fact reduced as a result of herbicide interference (Rennie and Dubetz, 1984).

The objective of this present study was to assess the phytotoxic effect of residues of selected herbicides, when applied as the commercially available formulations, on the growth, nodulation and nitrogenase activity of subterranean clover plants growing in a sandy loam which is used extensively for grain production in a cropley rotation in north-western Victoria.

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'*-(1methylethyl)-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), diclofop-methyl {2-[4-(2,4-dichlorophenoxy)-phenoxy]-methyl propionate}, diquat (6,7-dihydrodipyrido[[1,2-∝2′1′-*c*]pyrazinediium ion, 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 unavailable at the time of the study. All other herbicides were used as commercial formulations.

The field rate for each herbicide was calculated as being equivalent to the maximum recommended user rate, assuming an even distribution of the applied herbicide through the top 1-cm of soil. All herbicides were appropriately diluted with glass-distilled water so that on addition to the soil their final concentrations were 0, 1, 2.5 or 5 times the calculated field rate. Therefore, the application rates of all herbicides, except chlorsulfuron, were 0, 2, 5 and 10 μg active ingredient (ai) g^{-1} soil while for chlorsulfuron the rates were 0, 0.2, 0.5 and 1.0 μg ai g^{-1} soil.

Soil

A sandy loam classified as a Calcic luvisol (Dudal, 1970) from Walpeup, Victoria was selected for use as the test soil. Some properties of this soil have been previously reported (Eberbach and Douglas, 1983). Samples weighing 1 kg were placed in plastic-lined trays at an average depth of 2 cm. Herbicides were added to achieve the desired concentrations and in doing so, the moisture content of the soil was raised to 12%. The soil samples were stirred to ensure that the herbicides were well incorporated, and the trays were placed outside in a position that allowed the samples to experience local weather conditions for 120 days. These conditions were: average daily temperature range, 12–27°C; total precipitation, 142 mm; mean daily sunshine, 8–9 h day^{-1}; relative humidity, 44–70%. After this period, the soil samples in each tray were thoroughly mixed, and 200 g (oven dry) were transferred to plastic pots (8 cm in diameter and 10 cm in height). Each treatment was replicated four times.

It is worth noting that the soil in these trays would not have been subject to normal field leaching processes. Therefore, it may not be valid to extrapolate results obtained in this study to the field situation.

Plants

Seeds of *Trifolium subterraneum* (subterranean clover) cv Clare were sterilized and germinated as outlined by Eberbach and Douglas (1983). Seedlings with straight radicles 2.0–2.5 cm in length were planted in the soil at the rate of one

per pot. Polyalkathene beads were placed on the soil surface to reduce evaporation. The pots were watered initially to field capacity and then maintained at 50% of this level for the duration of the experiment. Each pot was given an initial starter dose of KNO_3 at the rate of 2 μg N g^{-1} of soil. Seven days after planting, each plant was inoculated with a mixture of commercially available strains of *Rhizobium trifolii* (WA 67 and WV 290) and the selected strain TA1. Nitrogen-free nutrient solution (Rüegg and Alston, 1978) was applied once every four weeks at the rate of 2 mL pot^{-1}.

The plants were grown in a growth cabinet set at a photoperiod of 12 h at 26°C with light intensity of 140 $\mu Em^{-2}s^{-1}$ and a dark period of 12 h at 20°C. When the plants reached 18 weeks of age, they were carefully removed from the pots and soil was washed from their roots. Nodule numbers and root dry weights were then recorded.

Nitrogenase activity

A non-destructive acetylene reduction assay procedure as described by Hopmans *et al.* (1982) was used to measure nitrogenase activity. C_2H_4 production from the plants was determined after the plants had grown for 9, 13 and 15 weeks. A Packard Becker gas chromatograph, Model 419 equipped with a flame ionization detector (130°C) and a Poropak N (80–100 mesh) 200 × 0.3 cm stainless steel column (90°C) was used for all measurements. The injector port temperature was 120°C and the N_2 carrier gas flow rate was 30 mL min^{-1}.

Statistical analysis

Prior to statistical analysis, all data were tested for homogeneity of variance using Bartlett's Test (Sokal and Rohlf, 1981). Nodulation, root and shoot weight data were found to be homogeneous, but all nitrogenase activity data required transformation into the square root form to be homogeneous. The nodulation and root and shoot data were analysed by linear regression analysis, nitrogenase activity data were analysed by a one-way analysis of variance with repeated measures. Where the F-test indicated a significant difference ($P < 0.05$) between individual means, Fisher's least significant differences (LSD) (Sokal and Rohlf, 1981) were constructed at the 5% level. For the herbicides which affected nodulation behaviour of the plants, simple linear regression equations were developed to relate the number of nodules formed to root weight and to compare nitrogenase activity of plants at 15 weeks of age in relation of the number of nodules formed.

Results and discussion

As the purpose of this experiment was to determine if residues of selected herbicides could remain in soil in sufficient quantities of available forms to influence the legume's symbiotic behaviour, a technique was used which ensured that the plant root system was exposed to these residues distributed throughout the soil. This technique was considered to be preferable to one using herbicide additions well in excess of those likely to be experienced in field situations. Additionally, in this study pregerminated seeds having radicles of 2.0–2.5 cm length were used in preference to ungerminated seeds. It is possible that by using pregerminated seeds, phytotoxic injury to the developing seedlings may have occurred at an earlier stage of growth than may otherwise occur. This phenomenon has previously been suggested by Heath *et al.* (1984). Use of ungerminated seeds may have given different results to those reported in the present study.

Atrazine and Chlorsulfuron

Following the four-month degradation period, soil residues of atrazine and chlorsulfuron remained in sufficient concentration in available forms to be lethal to clover seedlings (results not presented). Similar evidence of phytotoxic activity has previously been reported for residues of atrazine (Sheets, 1970) and chlorsulfuron (Mercie and Foy, 1985). Data from various studies have suggested that atrazine may persist in phytotoxically active concentrations in field soils for between 4 and 12 months depending on the initial rate of application (Sheets and Harris, 1965). The persistence of chlorsulfuron in soils

has been reported to vary widely with the half-life of this chemical ranging from 1 to 2 months in field soils (Walker and Brown, 1983), and seven to eight months in dry alkaline soils incubated at low temperatures in laboratory studies (Thirunarayanan *et al.*, 1985). In the current study, sufficient residues of atrazine and chlorsulfuron persisted in available forms in the sandy loam soil over the four-month degradation period to affect survival of the clover seedlings. It was therefore thought that the environmental conditions experienced by the treated soils over this period did not promote rapid deactivation of these herbicides. In a previous study, Eberbach and Douglas (1989) showed that seedlings of this cultivar of subterranean clover when grown in nutrient solution were sensitive to root applications of chlorsulfuron; Mårtensson and Nilsson (1989) have, however, shown that alfalfa and red clover seedlings can grow in soils treated with chlorsulfuron at concentrations of up to 2 g ai ha^{-1}. Therefore, susceptibility of legumes to residues of chlorsulfuron may vary among species.

Amitrole

Residues of the herbicide amitrole when applied at rates in excess of 2 mg ai g^{-1} of soil were lethal to the seedlings (Tables 1, 2 and 3). Although the rate of survival of plants grown in soils treated with amitrole at 2 mg ai g^{-1} soil was unaffected by residues of the herbicide, the growth, nodulation and nitrogenase activity of these plants were considerably less than those of untreated plants (Tables 1, 2 and 3; Fig. 1a). Amitrole is reported to decompose rapidly in warm moist soil (Ashton and Crafts, 1981) but in alkaline soils, degradation of this herbicide has been reported to be slow (Walker, 1980). Hence, the activity of the residues of this herbicide may have been enhanced by the slightly alkaline pH of the soil (pH 7.2). Additionally, since significant concentrations of amitrole remained, it could be speculated that the environmental conditions were not favourable for soil degradation activity.

Diclofop-methyl

Soil residues of diclofop-methyl were not observed to affect any of the plant parameters examined in this study (Tables 1, 2, 3 and 4; Fig. 1b). Residues of this herbicide were presumed to be either present in insufficient concentrations to have a significant effect on any of the parameters studied, or not available in a form that could be taken up by the plant root system. Soil applications of diclofop-methyl have been reported to

Table 1. Effect of herbicide residues on the root weight of subterranean clover plants, and associated regression models

Initial herbicide concentration in soil ($\mu g\, g^{-1}$ soil)	Root weight (g)					
	2,4-D	Diclofop -methyl	Diquat	Paraquat	Trifluralin	Amitrole
0	0.299	0.299	0.299	0.299	0.299	0.299
2	0.265	0.224	0.080	0.231	0.238	0.065
5	0.274	0.181	0.149	0.244	0.237	–[a]
10	0.220	0.227	0.104	0.282	0.148	–

Herbicide	Equation[b]	R^2(ajd)	F-value	SE[c] of y	SE of b
2,4-D	$y = 0.294 - 0.007x$	0.26	5.61*[d]	0.04	0.003
Diclofop-methyl	$y = 0.263 - 0.007x$	0.07	2.13	0.07	0.005
Diquat	$y = 0.224 - 0.015x$	0.22	5.55*	0.10	0.006
Paraquat	$y = 0.268 - 0.004x$	0.01	0.01	0.07	0.004
Trifluralin	$y = 0.290 - 0.014x$	0.15	3.87	0.11	0.007

[a] Residues lethal to seedlings.
[b] Equations are in the form of $y = a + bx$ where y = root weight; a = intercept; b = gradient of regression line; x = herbicide concentration.
[c] SE, standard error.
[d] * Significant at the probability of less than 5%.

Table 2. Effect of herbicide residues on the shoot weight of subterranean clover plants, and associated regression models

Initial herbicide concentration in soil ($\mu g\ g^{-1}$ soil)	Shoot weight (g)					
	2,4-D	Diclofop -methyl	Diquat	Paraquat	Trifluralin	Amitrole
0	0.878	0.878	0.878	0.878	0.878	0.878
2	0.811	0.731	0.336	0.587	0.707	0.229
5	0.765	0.722	0.530	0.877	0.571	–[a]
10	0.666	0.705	0.424	0.856	0.586	–

Herbicide	Equation[b]	R^2(ajd)	F-value	SE[c] of y	SE of b
2,4-D	$y = 0.868 - 0.021\times$	0.24	5.11*[d]	0.12	0.009
Diclofop-methyl	$y = 0.825 - 0.015\times$	0.10	2.82	0.14	0.009
Diquat	$y = 0.692 - 0.033\times$	0.13	3.28	0.28	0.018
Paraquat	$y = 0.774 - 0.007\times$	0.04	0.44	0.17	0.011
Trifluralin	$y = 0.809 - 0.028\times$	0.11	2.99	0.25	0.016

[a] Residues remaining were lethal to the seedlings.
[b] Equations are in the same form as those in Table 1 except that y = shoot weight.
[c] SE, standard error.
[d] * Significant at the probability of less than 5%.

Table 3. Effect of herbicide residues on the number of nodules formed on subterranean clover plants, and associated regression models

Initial herbicide concentration in soil ($\mu g\ g^{-1}$ soil)	Nodule number					
	2,4-D	Diclofop -methyl	Diquat	Paraquat	Trifluralin	Amitrole
0	93	93	93	93	93	93
2	77	87	35	60	60	24
5	75	79	48	69	44	–[a]
10	55	80	41	69	41	–

Herbicide	Equation[b]	R^2(ajd)	F-value	SE[c] of y	SE of b
2,4-D	$y = 89.82 - 3.52\times$	0.38	8.92*[d]	14.9	1.18
Diclofop-methyl	$y = 90.52 - 1.34\times$	0.02	1.26	18.6	1.19
Diquat	$y = 72.32 - 3.96\times$	0.24	5.96*	25.3	1.62
Paraquat	$y = 73.56 - 0.76\times$	0.05	0.22	25.1	1.61
Trifluralin	$y = 80.95 - 4.84\times$	0.45	13.87**	20.3	1.30

[a] Residues lethal to seedlings.
[b] Equations are in the same form as those in Table 1 except that y = number of nodules.
[c] SE, standard error.
[d] * Significant at the probability of less than 5%; ** probability of less than 1.0%.

have a phytotoxic effect on emerging seedlings (Crowley *et al.*, 1978) and root applications of this herbicide have reduced the nodulation of clover plants grown in nutrient culture (Eberbach and Douglas, 1989). Diclofop-methyl is rapidly hydrolysed to diclofop which has been shown to be rapidly inactivated in soil by either adsorption or rapid degradation (Smith, 1977). In either instance, little diclofop-methyl would be expected to be biologically active in the soil used in the present study after 120 days.

Paraquat

Soil residues of paraquat in this study had no effect on any of the plant parameters studied (Table 1, 2, 4 and 4; Fig. 1c). As root applications of this herbicide have been previously shown to reduce nodulation of subterranean clover grown in nutrient culture (Eberbach and Douglas, 1989), it is likely that the lack of effect of residues of this herbicide on the plants examined was due to immobilization of this com-

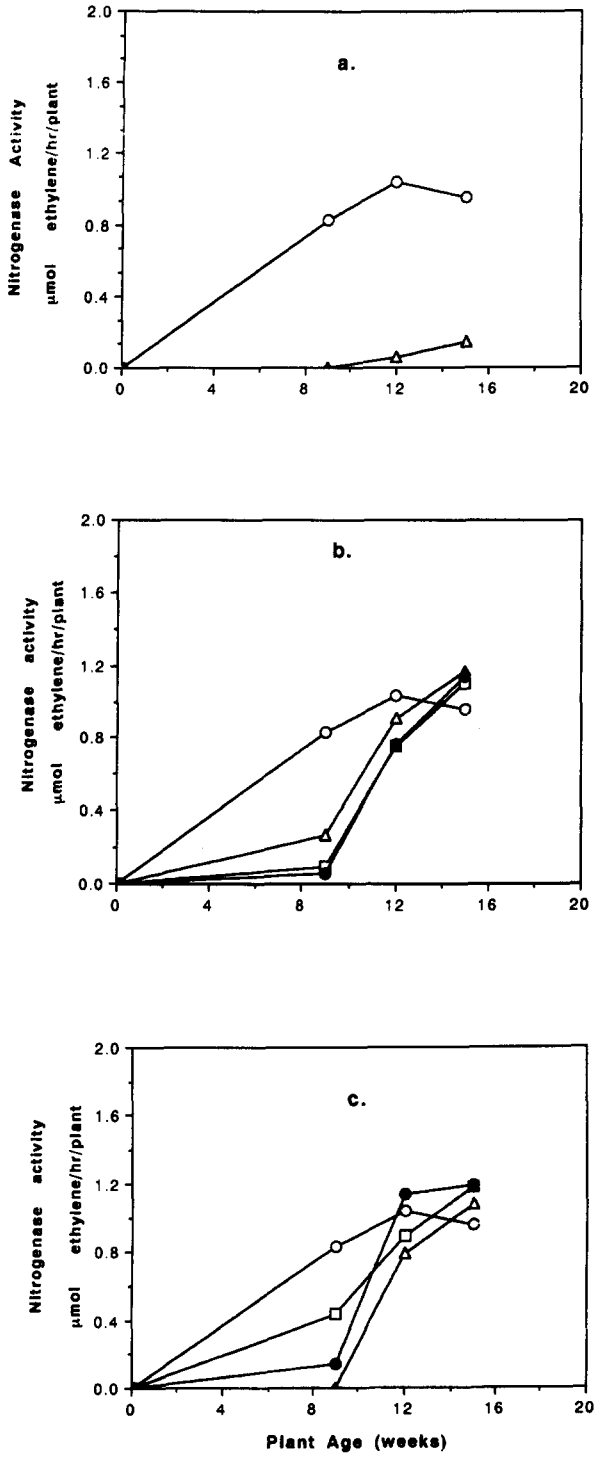

Fig. 1. The effect of the herbicides amitrole (**a**), diclofop-methyl (**b**) and paraquat (**c**) on the nitrogenase activity of subterranean clover plants at 9, 12 and 15 weeks of age. The initial herbicide application rates are denoted as 0(○), 2(△), 5(□) and 10(●) μg ai g^{-1} soil.

Table 4. Level of significance of effect of herbicide residues and plant age at the time of the assay on plant nitrogenase activity

Herbicide	Herbicide residue (A) effect	Plant age (B) effect
2,4-D	–[a]	***[b]
Amitrole	***	***
Diclofop-methyl	–	***
Diquat	**	***
Paraquat	–	***
Trifluralin	**	**

[a] –Non significant effect.
[b] * Significant effect at probability of less than 5%; ** Significant effect at probability of less than 1%, *** Significant effect at probability of less than 0.1%.

pound by soil. This result supports other data indicating that bipyridylium herbicides are bound tenaciously by soil components and are therefore considered biologically inert in most soils (Ashton and Crafts, 1981; Riley *et al.*, 1976).

2,4-D

The residues of 2,4-D significantly ($P < 0.05$) reduced root and shoot weight (Tables 1 and 2) and nodulation (Table 3), but had no apparent effect on the nitrogenase activity of the mature clover plants (Table 4; Fig. 2a). Degradation of 2,4-D in soil has been demonstrated to be dependent on microbial activity and the disappearance of the herbicide *per se* is closely associated with conditions favouring aerobic microbial activity (Jorgensen and Hamner, 1948; Ogle and Warren, 1954). In the current study, 2,4-D residues remaining in soil may be due to inadequate microbial activity over the four-month period, either as an inherent trait of the soil or due to unfavourable environmental conditions over this period. Fletcher *et al.* (1956; 1957) demonstrated the significant part played by soil in reducing the phytotoxic activity of acropetally imbibed 2,4-D on while clover plants and, hence, it is apparent that properties of the soil used in the current study were not sufficient to adsorb all 2,4-D.

Infection of the roots of legumes by Rhizobium takes place through root hairs (Date, 1970; Torrey and Zobel, 1977) so that the process of nodulation of legumes is undoubtedly linked to the expansion of the various parts of the root system (Pate, 1977). Therefore it is possible that a herbicide which induces a reduc-

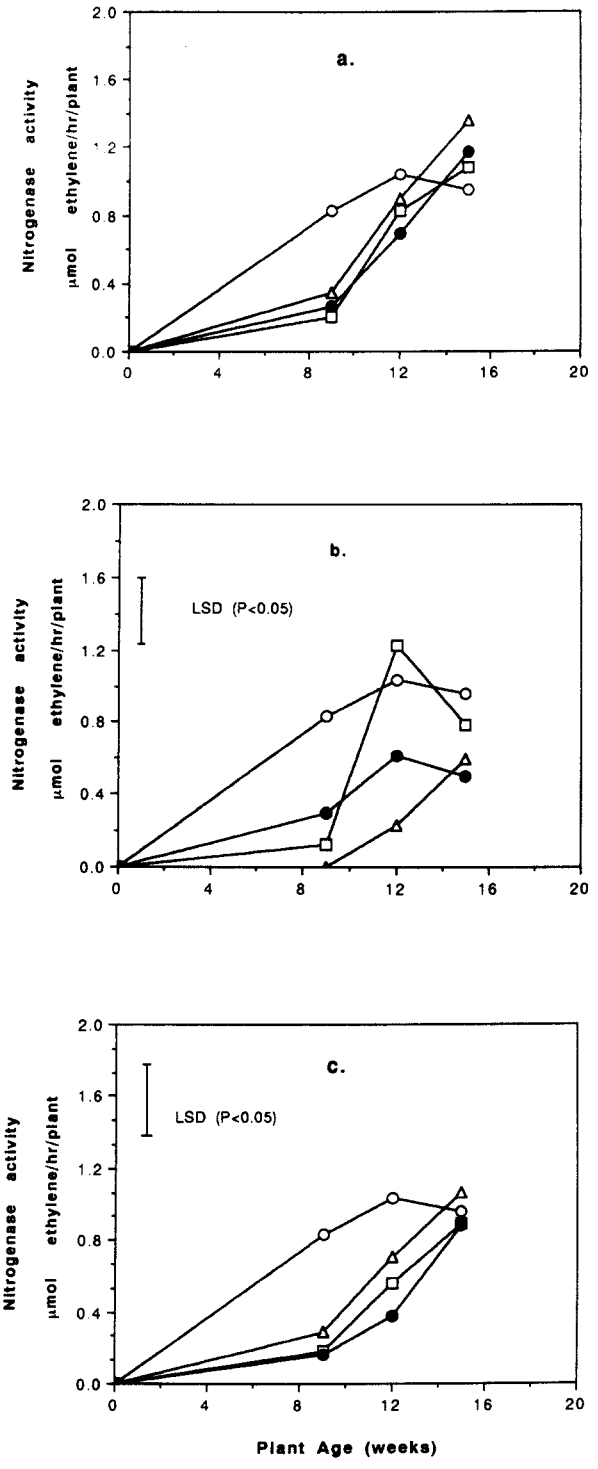

Fig. 2. The effect of the herbicides 2,4-D (**a**), diquat (**b**) and trifluralin (**c**) on the nitrogenase activity of subterranean clover plants at 9, 12 and 15 weeks of age. The initial herbicide application rates are denoted as 0(○), 2(△), 5(□) and 10(●) μg ai g^{-1} soil.

tion in nodules formed per plant may do this by restricting root growth and, hence, the number of root sites available for infection. In this study, root weight and the number of nodules formed per plant were both reduced by residues of 2,4-D. However the lack of correlation between these two parameters (Table 5a) indicates that residues of this herbicide interfere with the process of nodule initiation directly and that the observed reduction in nodulation was not directly related to the reduction in the size of the root system. Additionally, growth and nodulation potential of *R. trifolii* TA1 has been shown to tolerate concentrations of 2,4-D in nutrient culture of up to 20 mg ai L^{-1} (Eberbach and Douglas, 1989). Hence, the reduction in nodules formed in the present study is unlikely to be due to a specific effect of the herbicide on the rhizobia but instead on the process of nodule establishment. The lack of a relationship between nodulation and nitrogenase activity in these plants at 15 weeks of age (Table 5b) suggested that although the herbicide reduced nodulation, the nitrogenase system remained unaffected and that presumably the plants could still produce adequate amounts of inorganic nitrogen.

Diquat

Residues of diquat, unlike those of paraquat, reduced root growth, nodulation ($P < 0.05$) and nitrogenase activity ($P < 0.01$) (Tables 1, 3 and 4; Fig. 2b). This result suggested that either the degradation rate of paraquat was faster than that of diquat in this soil, or that deactivation of paraquat and its residues by adsorption mechanisms (Knight and Tomlinson, 1967) was faster than that of diquat under these conditions. Results obtained by Weber and Weed (1968) indicate that the latter mechanism was probably the dominant factor as the rate of adsorption of paraquat by montmorillonite and kaolinite exceeds that of diquat.

The significant trend ($P < 0.001$) between root weight and nodulation in diquat-affected plants (Table 5a) suggests that in reducing the growth of the root system, the diquat residues reduced plant nodulation presumably by reducing the number of sites on the root system available for

Table 5a. Linear regression and levels of significance of root weight (independent variable) *versus* nodulation (dependent variable)

Herbicide	Equation[a]	R^2(adj)	F-value	SE[b] of y	SE of b
2,4-D	$y = 82.78 - 13.85\times$	−0.082	0.84	19.6	127.4
Diquat	$y = 23.16 + 200.36\times$	0.537	19.58***[c]	19.7	45.3
Trifluralin	$y = 33.54 + 119.53\times$	0.023	5.78*	123.9	49.1

Table 5b. Linear regression equations and levels of significance of nodulation (independent variable) *versus* nitrogenase activity[d] (dependent variable)

Herbicide	Equation[e]	R^2(adj)	F-value	SE of y	SE of b
2,4-D	$\sqrt{y} = 1.373 - 0.003\times$	−0.013	0.84	0.21	0.003
Diquat	$\sqrt{y} = 0.210 + 0.009\times$	0.234	5.88*	0.41	0.004
Trifluralin	$\sqrt{y} = 0.546 + 0.006\times$	0.098	2.75	0.43	0.004

[a] Equations are in the form of $y = a + bx$ where y = number of nodules; a = intercept; b = gradient of regression line; x = root weight.
[b] SE, standard error.
[c] * Significant at the probability of less than 5%.
[d] Square root of nitrogenase activity of clover plants at 15 weeks of age.
[e] Equations are in the form of $\sqrt{y} = a + bx$ where y = nitrogenase activity; a = intercept; b = gradient of regression line; x = number of nodules.

infection by Rhizobium. Similarly, the significant ($P < 0.05$) relationship between nodule number and nitrogenase activity of the plants at 15 weeks of age (Table 5b) indicated that the observed reduction in nitrogenase activity was due to fewer nodules produced and not as a direct effect of the herbicide on nodule function. It is noteworthy that diquat residues affected only roots and the root associated activities of nodulation and nitrogenase activity while shoot growth remained unaffected (Table 2), which is in agreement with other observations (Ashton and Crafts, 1981).

Trifluralin

Plants growing in soil treated with trifluralin suffered no decrease in root weight or shoot weight (Tables 1 and 2). However, the number of nodules formed per plant and plant nitrogenase activity was reduced (Table 3 and 4; Fig. 2c). Similarly, several authors have reported that the residues of trifluralin have only affected nodulation of soybean plants (Behran *et al.*, 1979; Brock, 1972; Mallik and Tesfai, 1985; Parker and Dowler, 1976). De Rosa (1978) reported that trifluralin may affect nodule establishment in white clover plants by the induction of abnormal deformation of root hairs, thereby affecting the primary sites of nodule initiation. Results of the present study further suggest that the trifluralin residues delayed the establishment of nitrogenase activity in the clover plants (Fig. 2c). This is in contrast with results of Rennie and Dubetz (1984) who noted a slight trifluralin-induced stimulation in nitrogenase activity of soybean plants in the first year of a two-year trial. Lack of correlation between nodulation and nitrogenase activity in trifluralin-treated clover plants (Table 5b) suggests that this herbicide may affect legume-Rhizobium symbiosis by interfering with nodule establishment but is unlikely to affect the process of nitrogen fixation in these plants.

Conclusions

While the soil incubation conditions in the present study allowed sufficient deactivation of paraquat and diclofop-methyl to allow plants to grow normally, residues of atrazine, chlorsulfuron and amitrole had a lethal effect on the clover seedlings. Residues of 2,4-D, diquat and trifluralin were not lethal to the seedlings but they remained in sufficient concentrations in

available forms to affect growth and or nodulation and nitrogenase activity of plants.

Residues of diquat affected root growth and nodulation of the clover and the reduction in nodules formed per plant was closely related to the reduction in root growth. It was considered that the effect of this herbicide was primarily on growth of the plant and not directly on the process of nodule initiation. Conversely, residues of 2,4-D reduced root growth and the number of nodules formed, but the reduction in nodulation was not correlated with the reduction in growth of roots. Hence, the effect of 2,4-D on the process of nodulation of the clover was considered to be independent of the effect of this herbicide on growth of the plant. Similarly, residues of trifluralin were shown to affect the number of nodules formed while root growth was not affected.

Although some herbicides may affect legume nodulation by reducing growth of the root system, others may interfere with the process of nodule initiation. However, while the present study has made some attempt to discriminate as to which way a herbicide may interfere with the establishment of nodulation, it is not possible using the available data to speculate as to which mechanisms affect the nodulation process.

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