EFFECT OF HERBICIDE APPLICATION ON LEGUME *Rhizobium* SYMBIOSIS WITH AND WITHOUT STARTER NITROGEN[1] /

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Resumen

Se estudia el efecto de tres formulaciones de herbicidas, Preforan (2-4-dimitro-4-trifluormetil-difenil eter), Dacthol (dimetril 2,3,5,6- Tetracloroteriftalato) y Diral (2-etil-6 metil- 2-metoxi-1-etil)-a-cloroacetinilida) sobre la simbiosis leguminosa Rhizobium *con y sin nitrógeno como iniciador. El estudio se llevó a cabo en medio arenoso bajo condiciones de invernadero.*

Los resultados indican que en el caupi tratado con Dual presentó un menor peso de nódulos secos (1.56 mg/planta) que el Preforam o el Dacthol cuyos nódulos pearon 3.50 y 3.75 mg/planta respectivamente. El herbicida Dual también causó una disminución del N fijado pues las plantas así tratadas contenían 0.27% N mientras que plantas tratadas con Preforam y Dacthol contenían 0.35% N y 0.42% N, respectivamente.

La adición de nitrógeno como iniciador produjo 117 mg/planta de sustancia seca, en contraposición con 66.2 mg/planta obtenida en plantas inoculadas sin aplicación de N.

Introduction

It has become well-established that the legume *Rhizobium* symbiosis contributes highest to the N reserve of the ordinary soil compared with other biological contributors (2). This contribution becomes particularly important in tropical farm management practices where not much fertilization is done. However, in recent times, the problem of biological N accumulation in soils has been compounded by the indiscriminate introduction and use of herbicides in many tropical countries. The use of herbicides is being recommended without proper evaluation of their effect on the microbiological balance, especially on such an important natural process as symbiotic nitrogen fixation.

In a recent study, Parker and Dowler (12) showed that the application of Trifuralin to soybean resulted in reduction of nodule number. However, it was not clear whether this reduction was due to herbicidal injury on the host plant or on the *Rhizobium* component. There is no doubt from the above cited studies that any herbicide being introduced into the soil may exert deleterious effects on legume-*Rhizobium* symbiosis, either as a whole or on the micro-or macrosymbiotic component.

There is a need therefore for a proper evaluation of the herbicide effect on the symbiotic process in tropical soils. For instance, the work of Newman and Downing (11) showed that 2,4-dichlorophenoxy-acetic acid inhibited nodulation through inhibition of the growth and functions of the symbionts, that is, the legume host and the *Rhizobium* sp.

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Hence, this report is part of a study designed to evaluate the effects of some of the commonly used herbicides in Nigeria on *Rhizobium* legume symbiosis.

Materials and methods

Rhizobium strains

Two *Rhizobium* strains were used for inoculation in this study. The first strain, TAL 385, was a cowpea *Rhizobium* from Hawaii. The second was isolated from nodules of cowpea [*Vigna unguiculata* (L.) Walp. c.v. Ife Brown] at the University of Ife, and is referred to as the IFB isolate. The laboratory infectivity tests on both of them showed very positive results. The organisms were grown on Mannitol Yeast Extract Agar slants which were made up of mannitol (10 g), K_2HPO_4 (0.5 g), $MgSO_4 \cdot 7H_2O$(0.2 g), NaCl (0.1 g), $CaCO_3$ (3 g), yeast extract (10 g), agar (15 g) distilled H_2O (1 liter). Before use, the organisms were harvested with 10% sucrose and grown on Mannitol Yeast Extract broth on a rotatory shaker.

Herbicide formulations

The three herbicides used in this study, namely Dacthal (WP 75% active ingredients), Dual in form of an emulsifiable concentrate (500 g active ingredients/liter) and Preforan, an emulsifiable concentrate (30% active ingredients), were obtained from National Cereals Research Institute, Ibadan, where they are being constantly used for weed control in plantations of legume and legume-maize crop mixtures. For this investigation Preforan was applied at the rates of 0.4 and 8 kg a i/ha which were denoted as H_0, H_1 and H_2, respectively. Similarly, Dacthal was applied at the rates of 0, 5 and 10 kg a i/ha while Dual was used at the rates of 0, 0.5 and 1.0 kg a i/ha. Earlier in the study, the recommended rates of 2 and 4 kg a i/ha for Dual killed cowpea seedlings before 14 days after planting and therefore had to be scaled down to the rates of 0.5 and 1.0 kg a i/ha.

The sand used in these experiments was white sand obtained from the Bar Beach, Lagos. To remove organic materials and other foreign matter, the sand was washed, using several changes of 0.5 N H_2SO_4 and then neutralized by washing with NaOH solution. Thereafter, the bulk sand sample was rinsed in several changes of distilled water and then spread to dry after which it was sterilized at 1.1 kg/cm^2 pressure for 24 hours. Five kilograms of the sterile sand was then used to fill 5-liter plastic buckets which had been surface sterilized with ethanol. The basally perforated buckets were plugged with cotton wool to allow for adequate drainage.

The cowpea variety used in this investigation was *Vigna unguiculata* (L.) Walp. c.v. Ife brown which is an upright cowpea variety bred at the Faculty of Agriculture, University of Ife, Ile – Ife, Nigeria. A thick slurry of a 3-day old culture (TAL 385) mixed with bagasse and some quantity of gum arabic was used to inoculate the seeds at planting.

Starter nitrogen at the rate of 18 ppm N as ammonium sulfate was added to those treatments requiring the addition with or without inoculation. These treatment combinations resulted in a $3^2 \times 2^2$ factorial experimental design. The pots were arranged on the greenhouse bench using a split plot design with 5 replications. The different herbicide formulations were then applied with the aid of a pipette to the surface of the sand immediately after the seeds were planted.

Ten days after seed planting, a second inoculation prepared as before, was carried out to ensure a high population of the organism in the rhizosphere of the germinating legume. Watering was done with Jensen's (9) N-free nutrient solution. However, about the third week after planting, the plants appeared stunted in growth. Thereafter, the watering was reduced to twice weekly with the nutrient solution and deionized, distilled water was used in between.

The average night and day temperatures of the greenhouse during the period of the investigation were 26°C and 31°C respectively.

At 68 days after planting which coincided with initiation of flowering, each plant was carefully uprooted from the sand and the roots were rinsed in running tap water. The nodules were counted. The whole plants were packed into paper bags and dried in a forced air oven at 80°C for 48 hours. After cooling, the dry matter weights were determined. The nitrogen content of weighed samples was determined by an autoanalyser after digestion of the plant tissues with concentrated sulfuric acid.

Results and discussion

A slight reduction in rootlet development was observed in the Ife Brown cowpea variety whose seeds were inoculated with TAL 385 impregnated into bagasse which was used as the *Rhizobium* carrier. This reduction in root growth was suspected to be due to charring by bagasse and the gum arabic. The root crown was also partly burnt. The significance

of this observation is that the plants would probably be deprived of some infection sites for the *Rhizobium* (7, 11).

However, the stimulatory role of starter N (18 ppm) was evident in the generally more luxuriant growth of those plants that received the starter N compared to those that did not. A similar trend was also observed in the degree of nodulation and dry matter yield of plants that were inoculated and received starter N compared to those inoculated but without starter N, irrespective of the type and rate of herbicide application (Figure 1). The mean dry matter weight of plants to which starter N with inoculation was applied was 117 mg/plant compared to 66.2 mg with inoculation but no starter N.

When the herbicide rates and types are considered, results of analysis indicate that it is the herbicide type rather than the rate that has a significant effect ($P \leq 0.01$) on nodulation.

The plants that were treated with either Dacthal or Preforan had significantly higher nodule dry matter weight per plant compared to those that were treated with Dual (Table 1). This significant reduction could be due to the fact that Dual inhibited the root growth and further reduced the root size of the legume plants which had already been slightly charred by the *Rhizobium* carrier used for inoculation. The degree of nodulation is known to correlate closely with the number of infection sites available on the root system (5, 7, 11). This significant reduction in nodule dry weight of plants treated with Dual relative to the other two herbicides agrees with results using agar culture where it was found that Dual inhibited significantly the growth of this *Rhizobium* sp. compared to the other herbicides (3).

As shown in Figure 2, starter N boosted very significantly ($P \leq 0.01$) the total N accumulation in the cowpea plants which were inoculated. This is consistent and shows a very high correlation ($r = 0.96$) between total N and dry matter accumulation when starter N was applied, irrespective of the type and rate of herbicide applied. This is not a peculiar observation because Agboola (1) amongst others, had demonstrated a straight linear relationship between dry matter weight and total N of legumes. However, the herbicide type influenced very significantly the total N of the inoculated cowpeas (Table 1). The significantly higher total N ($P \leq 0.01$) under Dacthal and Preforan treatment compared to Dual is again probably explained by the phytotoxicity of Dual to the legume roots. This trend in total N was also found to be consistent with N fixed. Application of Dual significantly ($P \leq 0.01$) reduced the amount of nitrogen fixed, when compared to either Dacthal or Preforan. This behaviour of Dual with regard to the nitrogen fixed

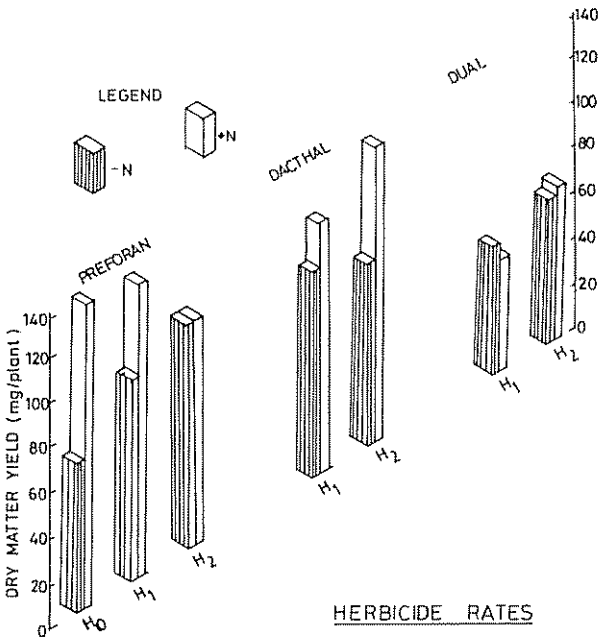

Fig. 1 Effect of herbicide type and rate on dry matter yield of Ife Brown cowpea (*Vigna unguiculata* L. Walp.) inoculated with *Rhizobium* sp. and with or without stater N.

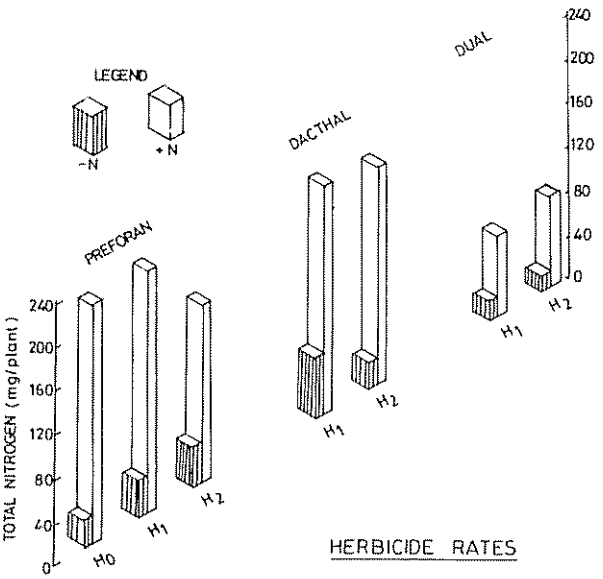

Fig. 2. Effect of herbicide type and rate on total nitrogen of cowpea (*Vigna unguiculata* L. Walp.) var. Ife Brown inoculated with *Rhizobium* sp. with or without starter N.

Table 1. Effect of herbicide type on nitrogen fixation by Ife brown inoculated with TAL 385 *Rhizobium* sp. and with added stater N.

Variable	Preforan	Dacthal	Dual	DMR+
Nodule dry wt mg/plant	3.50[b]	3.75[b]	1.56[a]	1.94*
Nitrogen fixed (%)	0.35[b]	0.42[b]	0.27[a]	0.13*
Total Nitrogen mg/plant	114.86[b]	113.08[b]	67.75[a]	40.68**

\+ Duncan Multiple Range test. Numbers followed by the same letter are not significantly different from each other.

* Significant at $P \leq 0.05$

** Significant at $P \leq 0.01$

would agree with the reductions in plant vegetative growth and nodulation earlier referred to.

Because of the charring of roots suspected to be caused by the gum arabic and bagasse used as the inoculant carrier, another experiment was set up as before but with a few modifications. Instead of TAL 385 strain of *Rhizobium* sp, IFB isolate was used as a thick slurry instead of being impregnated into a carrier. All the treatments received 18 ppm N as starter nitrogen since it was found to boost plant growth. Apart from these few modifications, the experimental details were as before.

Results indicate theat Dual treatment of plants significantly ($P \leq 0.01$) depressed nodule formation when compared to Dacthal and Preforan (Table 2). This depression of nodulation might be a manifestation of inhibition of root development as known to be common with the amide group of herbicides (6). The inhibition by Dual is probably more of a phytotoxic effect than the effect of the herbicide on the *Rhizobium per se*, since in agar culture there was no significant inhibition of this strain of *Rhizobium* Daethal or Dual. As noted earlier, it is possible that the reduction in total N fixed in plants treated with Preforan is a manifestation of Preforan inhibition

Table 2. Effect of herbicide type and rate on dry matter yield and nitrogen fixation in Ife brown inoculated with IFB isolate.

Herbicide type	Herbicide rate	Dry matter[1] g/plant	Nitrogen fixed[2] g/100 g plant
None	H_0	0.68[a]	0.76[a]
Preforan	H_1	0.49[b]	0.15[bc]
	H_2	0.46[b]	0.11[cd]
Dacthal	H_1	0.54[ab]	0.22[b]
	H_2	0.69[a]	0.77[a]
Dual	H_1	0.27[c]	0.07[d]
	H_2	0.18[c]	0.08[d]

1 LSD = 0.17, numbers followed by same letter are not significantly different from one another.

2 LSD = 0.07, numbers followed by same letter are not significantly different from one another.

by Dual (3). In the case of Preforan, it is possible that the microbial toxicity exhibited in agar culture is also exhibited in the sand culture, although to a reduced degree. However, in all the treatments, the application of the highest rate (H_2) of the herbicides significantly depressed ($P \leq 0.01$) nodulation when compared to the H_0 treatment, whereas the H_1 depression rate was only significant at 5 percent.

In terms of dry matter yield, the herbicide type also becomes important (Table 2). Plants treated with Dacthal had significantly ($P \leq 0.01$) higher dry matter accumulation when compared to either Preforan or Dual treatments. A similar stimulatory action of Dacthal on dry matter accumulation in horticultural crops had earlier been reported by Iyer (8). With respect to the total amount of N fixed, Table 2 also indicates that nitrogen fixation was significantly impaired when the plants treated with Dual were compared with those treated with Dacthal, but there was no difference when the effect of Preforan was compared with either of agar culture of this *Rhizobium* sp. (3). The higher dry matter accumulation induced by Dacthal and Preforan is also reflected in the higher amount of N fixed by plant under these herbicide treatments.

On the basis of these results, it is recommended that the use of Dual as a herbicide for weed control in legume production should be discouraged. The other two herbicides, namely Dacthal and Preforan, should be used only at the recommended rates of 5 and 4 kg a i/ha for effective weed control in legume crop production. Also efforts should be geared towards finding a suitable *Rhizobium* carrier for use in the tropics.

Summary

In a first experiment, application of Dual to the seeds of Ife Brown in sand culture inoculated with TAL 385 strain of *Rhizobium* depressed nodulation, and reduced the amount of nitrogen fixed significantly when compared with either Dacthal or Preforan. Addition of starter N to the inoculated seeds boosted dry matter yield and percent nitrogen when compared to those without starter N.

In a second trial, application of Dual to Ife Brown inoculated with IFB isolate also significantly depressed nodulation, reduced dry matter yield and nitrogen fixation of the legume when compared with Dacthal treatment and non-significantly when compared with Preforan.

Based on these findings, it is suggested that in legume production either Dacthal or Preforan should be used preferentially to Dual. Also applying the intermediate rates (H_1) of the herbicides effectively controlled weeds and did not have the deleterious effects on the vital parameters of nitrogen fixation as the H_2 rates.

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