Nodulation Effectivity, N-Accumulation and Yield of Soybean *(Glycine max)* in a Clayloam Soil Treated with Pre- and Post-Emergence Herbicides

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Summary

Introduction of exogenous micro-organisms in the rhizosphere of crop plants for plant growth enhancement requires a careful study of factors affecting their performance. We studied the effect of pre-and post-emergence herbicides on nodulation, N-accumulation and yield of soybean inoculated with exotic Rhizobium *strains in a Dystric Leptosol. The treatments comprising a control with no herbicide treatment, a pre-emergence herbicide treatment and a combination of pre- and post-emergence herbicide treatment were set out in the field using a randomized complete block design. The results show that both post-emergence and a combination of pre- and post-emergence herbicide treatments applied at 3 weeks after planting reduced nodulation, shoot dry weight, N-accumulation in the biomass and seed yield. Pre-emergence herbicide application reduced weed density and sparingly affected nodule dry weight, N-accumulation and seed yield. The results of the work suggest that selection and timing of herbicide application in soybean plots affect weed competition, nodulation, N-accumulation and overall performance of the crop in the field.*

Résumé

Etude de la nodulation, de la fixation de l'azote et du rendement du soja (*Glycine max*) cultivé sur un sol limono- argileux traité avec des herbicides en pré- et post-levée

L'utilisation des microorganismes exogènes dans le rhizosphère pour accroître la croissance des plantes requiert une étude préalable des facteurs affectant leurs performances. L'effet des herbicides appliqués en pré-levée et post-levée sur la nodulation, la fixation de l'azote et le rendement du soja cultivé dans un leptosol dystrique, inoculé avec des souches exotiques de Rhizobium *a été étudié dans un essai réalisé en blocs aléatoires (un témoin sans herbicides, un traitement d'herbicides en pré-levée et une combinaison d'herbicides appliqués en pré-levée et en post-levée). Les résultats obtenus montrent que les deux applications (application d'herbicides en pré-levée et la combinaison d'herbicides appliqués en pré-levée et en post-levée) réalisées trois semaines après le semis réduisent d'une manière significative la nodulation, le poids sec des tiges, la fixation de l'azote dans la biomasse ainsi que le rendement en graines. L' application d'herbicide en pré-émergence réduit la densité des adventices et affecte modérément le poids sec des nodules, l'accumulation d'azote et le rendement en graine. Cet essai montre également que le temps d'application des herbicides a un effet significatif sur la compétition du soja avec des adventices, un effet sur sa nodulation ainsi que sur sa capacité de fixation de l'azote et également sur toutes les performances de la plante.*

Introduction

In the tropics, weeds constitute a major impediment in the production of improved crop cultivars, particularly food legumes such as soybean (2). They can reduce yield by over 80 percent, depending on the density and species of weed (9). Those commonly associated with soybean are Barnyard grass (*Echinochloa* sp.), *Amaranthus spinosus,* volunteer cereals, nightshade (*Solanum* sp.), and nutsedge (*Cyperus* sp.) (12). Volunteer cereals are cereal crops, maize (*Zea mays* L.), sorghum (*Sorghum bicolor*), millet (*Pennisetum* sp.) etc. from previous years cropping whose seeds germinate and grow in the field in the concurrent season. They were not planted by the farmer and are technically weeds.

Weeds interfere with crops by competing for nutrients, water and light. They may also introduce allelochemicals into the habitat they share with crops. The effects of weed interference may at first be reduced plant vigour, delayed development, or suppression of specific growth characters such as flowering and fruit formation. But the ultimate effect is reduction of crop yield.

Some yield components are good indicators of weed competition in soybean (13). These indicators are: leaf

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area index (LAI), shoot dry weight and plant height. All of these yield indicators are reduced when weeds adversely affect grain yield (9).

The data available for weed infestation of soybean in tropical environments show that weeds reduce crop quality and yield by as much as 40-80% (9, 13). They also indicate that soybeans in weed-infested fields are more liable to lodging than those in fields where weeds are adequately controlled because weeds remove some nutrients that would otherwise have been used by the plants, thus leaving the plants spindly, etiolated, and liable to lodging.

Control of weeds in soybean production can be done through cultural methods, biological control, and chemical control using herbicides. Herbicides are the key products in sustaining large-scale agricultural production but in order to minimize agro-environmental concerns regarding their use, continued assessment of their behaviour under different management practices is required. Information on the effect of applied herbicide on nodulation and overall performance of soybean is important to agricultural extension workers and farmers who grow soybean.

The objective of this work is to study the effect of pre- and post-emergence herbicide on nodulation, N-accumulation, crop characteristics and yield of soybean grown under field conditions.

Material and methods

The experiment was conducted in the experimental farm of the Faculty of Agriculture, Ebonyi State University, Abakaliki, Nigeria. Abakaliki is located between latitude 06° 04'N and longitude 08° 65'E in the derived Savanna zone of the southeast agro-ecological zone of Nigeria. The climate is typical of the humid tropical zone. High temperature and high precipitation characterize the area. Annual rainfall is between 1800 – 2000 mm concentrated between April and November. Mean relative humidity is 65-80 percent during the rainy months in the study site. The soil of the study site is classified as Dystric Leptosol (4).

A composite soil sample (Table 1) was taken at the depth of 0-20 cm at the beginning of the experiment and was analyzed for pH in 1:2.5 soil-water suspension (11).

Particle size distribution was analyzed by the hydrometer method (8). Total nitrogen was determined using the modified Kjeldahl Method (5). Available phosphorus was determined by Bray-2 method as outlined in Page *et al.* (16) while exchangeable calcium, magnesium and total acidity were determined by the procedure described by Tel and Rao (18). The rhizobial population per gram of soil was estimated with cowpea, using the most probable number method (3). Organic carbon was analyzed according to the procedure set out by Nelson and Sommers (14).

The experiment was laid out in a Randomized Complete Block Design (RCBD) with four replications and four treatments on a soil with no history of soybean cultivation. The treatments consisted of a control plot with no herbicide application, a pre-emergence herbicide – Gramaxone (Paraquat) applied immediately after planting at the rate of 1.5 kg active ingredient (a.i.).ha^{-1}, a post-emergence herbicide (Fluazifop-butyl) at the rate of 1.5 kg (a.i.).ha^{-1} applied 21 days after planting (DAP) and a mixture of the pre-and post-emergence herbicide at the rate of 1.5 kg (a.i.).ha^{-1} applied at 21 DAP. Active ingredients were supplied to the plots with water acting as the carrier. Herbicides were applied manually using a Knapsack sprayer.

Table 1
Some physicochemical properties of the study soil

Sand (%)	48.2
Salt (%)	32.0
Clay (%)	17.8
pH (H_20)	4.9
C (%)	0.87
N (%)	0.08
P (mg.kg^{-1})	3.10
Ca (cmol (+).kg^{-1})	1.51
Mg (cmol (+).kg^{-1})	0.06
K (cmol (+).kg^{-1})	0.20
CEC (cmol (+).kg^{-1})	6.34
Total acidity (cmol (+).kg^{-1})	3.13

Experimental plots were 3 m x 3 m prepared into a sunken bed with raised ridges around each plot to minimize treatment contamination. Planting was done with early maturing soybean variety TGX 1485-E which is an erect variety with no lodging obtained from the International Institute for Tropical Agriculture (IITA), Ibadan. Soybean was inoculated with a mixture of exotic strains of *Rhizobium japonicum* (R25B + IRJ 2180a) prepared from peat and gum arabic solution as recommended by Vincent (19). Inoculated seeds were planted using an intra-and inter-row spacing of 15 cm x 25 cm. Two seeds were planted per hill and thinned down to one plant per hill at 7 DAP, thus given a plant population of 57,600 stands.ha^{-1}. Weeding was done manually at 42 and 63 DAP in all plots and weed density obtained by collecting all weeds from each plot and measuring fresh weed weight in the field at 42 and 63 DAP, respectively.

At 42 DAP and 63 DAP, 5 plants were sampled per plot. The plants were separated into shoot and root and the shoot was dried at 65 °C, weighed and the dried shoot ground to pass through a 0.4 mm sieve. Nitrogen content of the tissues was determined by the method outlined by Ferrari (7). Nodule number, taproot nodule and dry weight were also determined. Leaf area index was estimated at 42 and 63 DAP using the formula:

Number of plants per m^2 x number of leaves.plant^{-1} x leaf area (m^2)/ Area of ground covered (m^2) (20). At

Table 2
Effect of pre-and post-emergence herbicide application on crop growth characteristics

Treatment	Plant height ($cm.plant^{-1}$)		Shoot dry weight		LAI[a] (cm^2)		Weed density ($kg.ha^{-1}$)	
	Days after planting (DAP)							
	42	63	42	63	42	63	42	63
Control	42.58	98.08	18.30	28.94	2.90	3.07	112	311
Pre-Emergence	34.99	63.22	24.80	52.64	3.67	4.39	24	127
Post-Emergence	36.84	4.94	16.50	23.40	1.74	2.65	54	144
Pre and Post Emergence	34.66	48.79	25.60	4.20	2.85	3.96	35	107
F- LSD (0.05)	2.48	6.20	3.70	6.90	0.96	1.04	10.8	16.2

a- Leaf area index
DAP= Days after planting
F-LSD (0.05)

maturity, seed yield was measured in an area of 2.5 m x 2.5 m.

Results and discussion

Effect of pre- and post-emergence herbicide application on crop growth characteristics

The different treatments affected the plant height, shoot dry weight, leaf area index and weed density of the plots in varying degree (Table 2).

Plants were taller (P= 0.05) in control plots (no herbicide treatment) by about 13-18% at 42 DAP. However, no significant treatment difference (P< 0.05) in plant height was observed between the pre- and post-emergence herbicide treatments used singly or in combination. At 63 DAP, plant height increased significantly (P< 0.05) in the control plots relative to plots treated with pre-emergence, post-emergence and a combination of both herbicides by about 35, 43 and 50% respectively. Similarly, plots treated with pre-emergence herbicide had taller plants than plots treated with post-emergence herbicide by 13 and 23%, respectively. However, no significant treatment difference in plant height was observed between plots treated with post-emergence herbicide and combinations of both pre- and post-emergence herbicides at 63 DAP. Prominent weed species found in the plots were *Imperata cylindrica, Tridax procumbens, Cyperus rotundus, Solanum* sp.*, Sida acuta,* and *Amaranthus spinosus.* Early weed competition may have induced plants to grow tall because of competition for light and space. This result was corroborated by the findings of Akobundu and Poku (2).

The results also indicate that shoot dry weight significantly increased (P< 0.05) in plots treated with pre-emergence herbicide relative to untreated plots at both 42 and 63 DAP by about 26 and 45%, respectively. In plots treated with a combination of pre- and post-emergence herbicides, shoot dry weight also increased significantly (P< 0.05) by between 28 and 34% at 42 and 63 DAP, respectively (Table 2). However, no significant treatment difference was observed between plots treated with post-emergence herbicide and control plots. This indicates that the critical period of weed interference (the period in which if weed is not removed, it will cause the most damage) for shoot dry weight of soybean is within the early growth stages.

Higher leaf area index was also observed in plots treated with pre-emergence herbicide relative to other treatments at 63 DAP by about 30 and 39% for control and post-emergence herbicide treatments respectively. However, no significant treatment differences in leaf area index were observed between plots treated with pre-emergence herbicide and a combination of pre- and post-emergence herbicides. It was observed that plants in control plots despite early plant height increments were spindly and etiolated, probably as a result of competition with weeds for sunlight, space and nutrients. Akobundu and Poku (2) elucidated that while competition between soybean plants and weeds for light may be of little importance, the allelopathic effect and the large consumption of nutrients and water by weeds may be responsible for soybean production losses. The results suggest that proper vegetative growth in soybean plots can only be achieved if weeds are adequately controlled. This evidence is further supported by the fact that higher (about 78 and 59%) weed density ($kg.ha^{-1}$) was found in the control plots at both 42 and 63 DAP, respectively (Table 2). Lower weed densities were also observed in plots treated with a combination of pre- and post-emergence herbicides at both 42 and 63 DAP relative to the control plots. The higher weed density observed in the control plots suggest that weeds must have interfered with plant growth resulting in reduced plant vigour and suppression of developmental characteristics of the soybean when compared to the herbicide treated plots.

Effect of pre- and post-emergence herbicide on nodulation, N-accumulation and seed yield

The effect of the treatments on nodulation, N-accumulation and seed yield of soybean is presented in table 3.

Table 3
Effect of pre- and post-emergence herbicides on nodulation, N-accumulation and seed yield

Treatment	Taproot of nodules (number.plant^{-1})		Total number of nodules (number.plant^{-1})		Nodule- dry weight (mg.plant^{-1})		N-accumulation (mg.plant^{-1})		Seed yield (kg.ha^{-1})
	Days after planting (DAP)								
	42	63	42	63	42	63	42	63	
Control	67	25	128	84	320	141	315	492	1939
Pre-emergence	59	32	102	69	592	197	462	732	2154
Post-emergence	10	6	31	15	74	35	162	201	1476
Pre- and post-emergence	12	4	38	19	86	37	198	277	1606
F-LSD (0.05)	8.2	3.2	15	9	58	22	124	151	284

Higher total number of nodules was found in control plots at both 42 and 63 DAP. These were significantly higher ($P< 0.05$) than the number of nodules found in plots treated with pre-emergence herbicide by between 18-20% at 42 and 63 DAP, respectively. The total number of nodules in the control plots also exceeded that in plots treated with post-emergence herbicide by between 76-82% at both 42 and 63 DAP. Plots treated with a combination of pre- and post-emergence herbicide also had lower total number of nodules relative to control (about 70% at both 42 and 63 DAP). The results are indicative that the herbicide treatments may have negatively affected nodulation especially in plots treated with post-emergence and a combination of pre- and post-herbicide treatments. Plants grown on these two plots may have been adversely affected because the treatments were applied later (at 21 DAP) in contrast to the pre-emergence herbicide treatments applied at planting time. These results probably suggest that the ecotoxological effect of some applied agrochemical diminish with time in the soil. Lower number of taproot nodules was observed in plots treated with post-emergence and a combination of pre- and post-emergence herbicides. These were significantly lower ($P< 0.05$) than in plots treated with pre-emergence herbicide alone by about 79-87% at both 42 and 63 DAP. However, no significant treatment difference in number of taproot nodules was found between the control plots and those treated with pre-emergence herbicide. The lower number of taproot nodules in plots treated with post-emergence herbicide probably indicates that the *Rhizobium* strain used for inoculation may have been adversely affected. Presence of nodules on the taproots show that the inoculants brought about nodulation (17).

The nodule dry weight and nitrogen concentration per plant were also affected by the treatments (Table 3). However, there was no significant relationship between nodule dry weight.plant^{-1} and number of nodules per plant in the different treatment applications. Higher nodule dry weight.plant^{-1} was found in plots treated with pre-emergence herbicide. At 42 DAP, this was significantly higher ($P< 0.05$) than for the other treatments by 45, 88 and 85% for control, combination of pre- and post-emergence herbicide and post-emergence herbicide treatments, respectively. Similar differences were recorded at 63 DAP. The results clearly indicate that plots with the highest number of nodules.plant^{-1} did not coincide with the plots with the highest nodule dry weight.plant^{-1}. Similar observations were made by Okonkwo and Nnoke (15) where soybean plants with few nodules on their roots, were shown to grow much larger and have more weight than those with more nodules.

Higher N-accumulation (mg.plant^{-1}) was found in plants samples collected from plots treated with pre-emergence herbicide relative to other treatments. This was significantly higher ($P< 0.05$) than in control plots; plots treated with post-emergence herbicide and a combination of pre- and post-emergence herbicide by about 32, 65-73 and 57-62%, respectively at, both 42 and 63 DAP. However, despite the fact that a higher nodule dry weight.plant^{-1} was found at 42 DAP, N-accumulation per plant was higher ($P< 0.05$) at 63 DAP for all plots. This result suggests that the concurrent crop use at least part of the nitrogen 'fixed' by the legume-*Rhizobium* association. This statement is supported by the fact that the plots with the highest nodule dry weight.plant^{-1} coincided with the plots with the highest N-accumulation.

The highest seed yield of soybean (2.2 ton.ha^{-1}) was observed in the plots treated with pre-emergence herbicide although this was statistically comparable to the untreated plots (control). Seed yields from plots treated with post-emergence and a combination of pre- and post-emergence herbicides were slightly lower (about 31%) relative to plots treated with pre-emergence herbicide. The results indicate that seed yield has relationship with both N-accumulation and nodule dry weight. This indicates that the root nodules of soybean in plots with pre-emergence herbicide and untreated plots were effectively nodulated and 'fixed' nitrogen more than other plots. Although the application of both pre- and post-emergence herbicides either as a single treatment or in combination in this work controlled weeds, seed yield increased by at least 25% in plots treated with pre-emergence herbicide relative to other herbicide treatments. These results are in contrast with the work of Akobundu (1), who found that herbicides control weeds effectively, reduce production cost and increase yields of soybean. However, the results corroborate the work of Eaton *et al.* (6) who found little or no reduction in yield of soybean if the crop is kept relatively weed-free for the first 28 days after planting. In this work, post-emergence and a combination of pre- and post-emergence herbicides were applied at 21 DAP hence the reduction in seed yield as a result of weed infestation and probably, interference with activities of *Rhizobium* bacteria. Martinez-Toledo *et al.* (10) studied the effect of Captan on microbial functions in four agricultural soils under aerobic conditions and found that total fungal population, nitrifying bacteria, aerobic N-fixing bacteria and nitrogenase activity were significantly decreased at dose rates of 2.0 to 10.0 kg.ha^{-1}.

The results of this study show that the timing of herbicide application is important for weed control, nodulation, N-accumulation, and overall performance of soybean. This is because the use of pre-emergence herbicide in this work controlled weeds effectively, affected the inoculants and their capability sparingly and contributed to the overall productivity of the soybean. The use of pre-emergence herbicide affected nodulation and N-accumulation sparingly probably because the nodules were not yet formed at the time of application and microbial detoxification may have reduced the herbicide concentration before the appearance of the root nodules.

Conclusion

Results from this study show that the use of pre- and post-emergence herbicides and their combination affect plant growth characteristics such as plant height, shoot dry weight, and leaf area index in varying degrees. Plants were taller at the early stages of growth (42 DAP) in control plots relative to herbicide-treated plots probably because competition for light and space may have induced plants in control plots to be taller.

Results also show that shoot dry weight and leaf area index were higher by between 26-45% in plots treated with pre-emergence herbicide when compared to control plots. However, no significant treatment differences in shoot dry weight and leaf area index were found between plots treated with post-emergence herbicide and a combination of pre- and post-emergence herbicide. Higher weed density (59-78%) was found in the control plots when compared to herbicide-treated plots. However, lower (P= 0.05) weed densities were found in plots treated with pre-emergence herbicide.

Higher numbers of root nodules were found in control plots at both 42 and 63 DAP when compared to herbicide-treated plots. This result probably indicated that herbicide application affected nodulation negatively. However, higher N- accumulation (mg.plant^{-1}) was found in plots treated with pre-emergence herbicide relative to other treatments. The highest seed yield was found in plots treated with pre-emergence herbicide but it was statistically comparable to yield in the control plots.

Although, herbicides have been used to control weeds effectively, to reduce production costs and to increase yields, their effect on nodulation, N-accumulation and seed yield as observed in this work indicates that the type of herbicide and time of application is to be put into consideration. Food legumes when grown under field conditions are particularly vulnerable to weed interference and some studies suggest that losses in yields of soybeans can be predicted from weed densities. In all, the use of pre-emergence herbicides to control weeds should be adopted if increased soybean yield is to be achieved.

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