

Seed germination and growth of *Eleusine indica* and *Euphorbia heterophylla* as influenced by depth of planting and glyphosate

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SUMMARY

Three experiments were conducted in a glasshouse and a laboratory to provide information on the effect of glyphosate (N-(phosphonomethyl) glycine) on seed germination, seedling emergence and growth of goosegrass (*Eleusine indica* (L.) Gaertn) and wild poinsettia (*Euphorbia heterophylla* Linn). Glyphosate sprayed on top of potted soil did not affect the emergence of seeds planted 2 cm deep. However, glyphosate at 1.5 kg ha⁻¹ stimulated the emergence of *E. heterophylla* seeds planted on the soil surface. Stimulatory effect of direct contact with glyphosate on germination of *E. heterophylla* seeds was confirmed in laboratory tests. The concentrations of glyphosate solutions that stimulated seed germination in *E. heterophylla* sown on filter paper in 9 cm petri-dishes were in the range 10-30 ppm at quantities equivalent to 1.3-3.8 × 10⁻¹ kg a.i. ha⁻¹. The herbicide did not affect seed germination in *E. indica*. Glyphosate-treated seeds transferred to untreated soil emerged. Some of the emerged seedlings died eventually. Others had their growth adversely affected only 2 weeks after planting. At 4 weeks after planting, those seedlings which survived the adverse effect of the herbicide, in the first 2 weeks, were as vigorous as the control plants.

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Introduction

Goosegrass (*Eleusine indica* (L.) (Gaertn) is an ubiquitous weed in Nigeria. Holm (1969) listed it as one of the world's worst weeds. Njoku (1959) described wild poinsettia (*Euphorbia heterophylla* Linn) as a quick-growing weed of waste places, but

RÉSUMÉ

EKE, A. O. & OKEREKE, O. U.: *L'effet de la profondeur de semis et la glyphosate sur la germination et la croissance d' Eleusine indica et d' Euphorbia heterophylla*. Trois essais ont été faits dans une serre et un laboratoire pour fournir des informations sur l'effet de la glyphosate (N-(phosphonomethyl) glycine) sur la germination, l'émergence des plantules et la croissance d'*Eleusine indica* (L.) Gaertn et d'*Euphorbia heterophylla* Linn. La glyphosate, pulvérisé sur un sol mis en pot n'a aucune effet sur la germination du grain semée à 2 cm de profondeur. Pourtant, la glyphosate à 1.5 kg/ha a stimulée la germination de la graine de *Euphorbia heterophylla* semée dans la surface du sol. L'effet stimuloire du contact direct de la glyphosate sur la germination de la graine d' *Euphorbia heterophylla* a été confirmé dans des essais laboratoires. Les concentrations des solutions de la glyphosate qui ont stimulée la germination de la graine d'*Euphorbia heterophylla* semée sur le papier-filtre dans des boites de petris de 9 cm du diamètre s'écartent entre 10 à 30 ppm à des quantités équivalents de 1.3 - 3.8 × 10⁻¹ kg a.i. ha⁻¹ des ingrédients actifs. L'herbicide n'a pas influencé la germination de la graine d' *Eleusine indica*. La graine traité avec de la glyphosate et semée dans le sol non-traité ont germées. Quelques plantules sont affaiblis finalement. D'autres ont leurs croissances affecté défavorablement juste après deux semaines de la semence. A quatre semaines après la semence, les plantules qui ont survivaient l'effet défavorable de l'herbicide dans les deux premières semaines étaient tant vigoureux que les temoins.

it has gradually become a troublesome weed of cultivated lands. It resisted the common herbicides used to control weeds in cowpea in Nigeria (Akobundu, 1979, 1982). Both *E. indica* and *E. heterophylla* are also important pests of maize in Ghana (Carson, 1978; Ampong-Nyarko, 1985).

Glyphosate (N-(phosphonomethyl) glycine) is one of the herbicides used in seed-bed preparation (Lee, 1965). The effect of the glyphosate on ungerminated seeds lying in the soil at the time of application has not been investigated in Nigeria.

Studies elsewhere have shown that at 0.46-4.48 kg ha⁻¹, glyphosate had little effect on germination of corn, soybean and wheat (Sprinkle, Meggit & Penner, 1975) as well as three turfgrasses (Klingman & Murray, 1976) if applied to the soil before seeding or directly on seeds lying on the soil surface. However, Weidman & Appleby (1972) showed that sub-lethal rates of many herbicides stimulate plant growth. Moshier, Turgeon & Penner (1976) observed an increase in the germination of red fescue (*Festucarubra* L.) and Kentucky bluegrass (*Poa pratensis* L.) when low levels of glyphosate were sprayed on seeds lying on the soil surface.

Stimulation by glyphosate of seed germination in redroot pigweed (*Amaranthus retroflexus* L.) lying on the soil has also been reported (Egley & Williams, 1978). Emergence of this weed from a soil depth of 0.5 cm was, however, reduced by glyphosate at 2.2 kg ha⁻¹. Segura, Bingman & Foy (1978) also reported reduction in seedling numbers when glyphosate was applied directly over seeds of red clover (*Trifolium pratense*) and Italian ryegrass (*Lolium multiflorum*). Salazar & Appleby (1982) found that the effect of glyphosate was more severe on legumes than grasses. These reports suggest that the effect of glyphosate on seeds lying in the soil, among other factors, depends on the plant species, depth of seed burial and rate of application.

The objective of this study was to determine the effect of glyphosate on seed germination, seedling emergence and growth of goosegrass and wild poinsettia.

Materials and methods

Seed collection

Panicles of *E. indica* and fruits of *E. heterophylla* were collected from farmlands in Nsukka, Nigeria (latitude 06° 52' N, longitude 07° 24' E, altitude 400 m). The panicles and fruits were sun-dried. After all the fruits of *E. heterophylla* had exploded, the

seeds were separated from their pericarps by winnowing. Panicles of *E. indica* were threshed and sieved with 0.15 mm mesh. The seeds retained on the sieve were collected. Seeds of both species were stored in separate containers and removed as required for the conduct of the experiments.

Experiment 1: Seedling emergence of *E. indica* and *E. heterophylla* as influenced by depth of planting and glyphosate

While seeds of *E. heterophylla* used in this experiment were counted manually, the number of *E. indica* seeds was estimated by weight. Six lots (400 seeds/lot) of *E. indica* seeds counted manually were weighed and the mean weight was estimated as 0.14 g per 400 seeds. Subsequently, the number of seeds of *E. indica* required for the experiments was obtained by weighing. Seeds for each experimental plot were kept in separate seed envelopes - 200 seeds/envelope for *E. heterophylla* and 0.14 g/envelope for *E. indica*.

Sub-soil was collected from the farm, sun-dried and sieved with a 1.7 mm mesh. Twenty-four 2.5 l plastic pots of diameter 17.6 cm were filled with 1.60 l (1.80 kg) of sieved soil. The seeds in these pots were not covered with soil after planting. Another 24 pots were filled with 1.40 l (1.55 kg) of soil. Seeds in these latter pots were covered with soil after planting.

Half of the pots containing either 1.60 or 1.40 l of soil were each planted with 200 seeds of *E. heterophylla*. The remaining half were each planted with 0.14 g seeds of *E. indica*. The seeds were spread thinly and uniformly on the soil surface leaving a small margin round the edge. Seeds planted in pots initially filled with 1.40 l of soil were covered with 0.20 l (0.25 kg) of soil to give a planting depth of 2 cm. Thus, all seeds were either exposed on 1.60 l of soil or planted 2 cm deep in 1.60 l of soil.

A commercial formulation of glyphosate (Roundup) was applied to the soil containing both exposed and covered seeds at the rates of 0, 0.75, 1.5 and 3.0 kg a.i. ha⁻¹. Thus, it was a 2 × 4 factorial experiment with three replications. Spraying was done with an Allman APT 20 Knapsack sprayer

fitted with three nozzles in a boom arrangement. The force applied was 2×10^6 dyne/cm² and the Knapsack was calibrated to deliver 300 l of spray liquid per hectare. After spraying, the pots were arranged inside a glasshouse, in a completely randomized design.

Each pot was placed on a plastic tray to retain any herbicide material that drained through the bottom of the pots. Soil in the pots was kept moist in the first week following herbicide application by sub-irrigation through filling the trays with water as required. Subsequently, the soil was kept moist as necessary by over-head irrigation. Mean maximum and minimum air temperatures during the experiment were 33.6 and 25.0 °C, respectively.

Experiment 2: Germination of glyphosate-treated seeds of E. indica and E. heterophylla

In this experiment, 50 seeds of *E. heterophylla* were planted per plot. The weight of 50 seeds of *E. indica* (0.03 g) was estimated as described in Experiment 1.

Glyphosate solutions at concentrations of 0, 10, 20, 30 and 40 ppm, respectively were prepared using a commercial formulation. Fifty seeds of each weed species were spread on a double layer of filter paper in 9-cm petri-dishes. Paper in each petri-dish was saturated with 8 ml of a herbicide solution or distilled water (equivalent to 0, 1.3, 2.5, 3.8 and 5.0 $\times 10^{-1}$ kg a.i. ha⁻¹). Petri-dishes were then covered with lids and placed on top of laboratory benches. Ambient temperature in the laboratory at 14.00 h was about 22 °C. Each species was set up as a separate experiment arranged in a completely randomized design with three replications. Germination count was taken daily for 2 weeks starting 3 days after sowing the seeds. Germination was defined as the protrusion of the radicle from the seed coat. Length of the radicle was measured at the end of 2 weeks.

Experiment 3: Germination and growth of glyphosate-treated seeds of E. indica and E. heterophylla

Germination tests of glyphosate-treated seeds were set up as described in Experiment 2 except that replication was increased to four. Top soil was collected from the farm and mixed thoroughly with

well-rotted poultry manure in the ratio 2:1. A 10-l (11.5 kg) of soil/manure mixture was placed in each of fifteen 24-cm plastic pots. Soil in each pot was heavily watered by over-head irrigation and left to drain overnight.

As seeds germinated in the petri-dishes, they were counted, removed and scattered evenly on soil contained in the plastic pots. The germinated seeds planted in the pots were covered lightly with 0.5 l of soil. Germinated seeds from one herbicide treatment were distributed among three pots which constituted three replicates.

The seedlings were counted after emergence in the potted soil. At 2 weeks after planting, plant height was estimated by measuring the height of 10 plants/pot in both species. Thereafter, seedlings of *E. heterophylla* were reduced to 40 plants/pot. Two weeks later (4 weeks after planting), percent mortality of emerged seedlings was estimated and the surviving plants harvested. The following growth parameters were estimated at harvest:

- i) plant height (cm) from soil surface to the tip of the last fully expanded leaf for *E. indica* or to the growing point for *E. heterophylla*, and
- ii) fresh weight of above-soil parts per plant (g).

Results and discussion

Emergence of E. indica and E. heterophylla seedlings as influenced by depth of seed burial and glyphosate

Planting at a depth of 2 cm significantly increased the emergence of *E. heterophylla* from 23.0 where seeds were planted on top of the soil to 73.3 per cent (Table 1). *E. indica* responded differently to seed burial.

Emergence of exposed seeds of *E. indica* was significantly better than emergence of covered seeds. Covering seeds lightly with soil is known to provide optimum germination conditions for many plant species (Egley & Williams, 1978). This was true for *E. heterophylla* seeds covered with 2 cm of soil. However, the result with *E. indica* is also in agreement with the observation that germination of grasses decreased as the depth of planting was

TABLE 1

Main Effect of Depth of Seed Burial on Emergence of *E. indica* and *E. heterophylla* Seedlings Four Weeks after Planting

Depth of seed buried (cm)	Seedling emergence (%)	
	<i>E. indica</i>	<i>E. heterophylla</i>
0	28.7	23.9
2	22.2	73.3

SE for comparing the means of the two species and two depths of seed burial = ± 1.3 .

increased (Ezeigwe & Olunuga, 1974). Similarly, Okereke, Blair & Caseley (1981) showed that for some grasses, the time from planting to seedling emergence increased with depth of planting. They attributed the delay in emergence to dormancy induced by low oxygen concentration, high levels of carbon dioxide or ethylene in soil atmosphere.

Emergence of *E. heterophylla* seedlings increased from 19.4 in the absence of glyphosate to 38.5 per cent with glyphosate applied at the rate of 1.5 kg ha⁻¹ (Table 2), where the seeds were planted on top of the soil. Glyphosate had no significant effect where the seeds were covered with soil. The increase in percent emergence from exposed *E. heterophylla* seeds sprayed with glyphosate occurred only at application rate of 1.5 kg ha⁻¹ but not at 0.75 or 3.0 kg ha⁻¹. The concentration of the herbicide in the soil water at

TABLE 2

Effect of Glyphosate on Emergence of *E. heterophylla* from Seeds Planted at Different Depths

Rate of glyphosate (kg a.i. ha ⁻¹) (cm)	Seedling emergence (%) ¹	
	Depth of burial (cm)	
	0	2
0.00	19.4a	75.0a
0.75	21.1a	75.1a
1.50	38.5b	69.3a
3.00	18.3a	73.7a

¹Means followed by the same letter within each column were not statistically different at $P=0.05$ as determined by Duncan's Multiple Range Test.

0.75 kg ha⁻¹ was, therefore, below that required to cause stimulation of seed germination and emergence. At the rate of 3 kg ha⁻¹, the stimulatory range was exceeded and the effect of the herbicide did not differ significantly from the control.

It is true that there could have been seeds of different ages which would not germinate at the same time, because a single plant of *E. heterophylla* produces several generations of seed before it dies (Egunjobi & Kuporuyi, 1973). However, the seeds for all the treatments were taken from the same seed stock; therefore, the characteristic of different ages of seed from the same plant of this annual weed species does not explain the differences in emergence between treatments. The result agrees with some reports. Moshier *et al.* (1976) reported inhibition of shoot elongation but not germination or emergence of some grasses placed in direct contact with glyphosate. Salazer & Appleby (1982) showed that the major inhibitory effect of glyphosate on legumes was as a result of direct contact with the seeds.

The herbicide did not affect buried seeds because of its adsorption onto soil colloids. Sprankle *et al.* (1975) had suggested that since glyphosate was rapidly inactivated in soil, a thin soil barrier may protect seeds and seedlings from the herbicide. Soil adsorption capacity for the herbicide has to be exceeded before substantial quantity becomes available for absorption by buried seeds. It follows also that the effect of glyphosate on buried seeds will vary with soil type.

Germination of *E. indica* and *E. heterophylla* seeds treated with glyphosate

Glyphosate at 10-30 ppm ($1.3-3.8 \times 10^{-1}$ kg a.i. ha⁻¹) stimulated seed germination in *E. heterophylla* but had no significant effect on seed germination in *E. indica* (Table 3). This result confirmed the stimulatory effect observed on direct contact of *E. heterophylla* seeds with glyphosate in an earlier experiment which involved spraying glyphosate after planting seeds at different soil depths. It agrees also with the observation that glyphosate had no significant effect on seed germination of

TABLE 3

Effect of Glyphosate on Seed Germination and Radicle Length of *E. indica* and *E. heterophylla* Seedlings Two Weeks after Sowing

Concentration of solution (ppm)	Glyphosate rate Quantity per unit area ($\times 10^{-1}$ kg a.i. ha ⁻¹)	Germination (%) ¹		Length of radicle (cm)	
		<i>E. indica</i>	<i>E. heterophylla</i>	<i>E. indica</i>	<i>E. heterophylla</i>
0	0.0	12.9a	48.0bcd	3.0a	12.7a
10	1.3	12.8a	59.3a	2.8ab	8.8b
20	2.5	12.0a	58.1ab	2.2abc	6.5bc
30	3.8	11.0a	50.7abc	1.7bcd	4.2cd
40	5.0	12.5a	44.7cd	1.3cd	3.3d

¹Means followed by the same letter within each column were not statistically different at $P=0.05$ as determined by Duncan's Multiple Range Test.

three grass species, even when applied directly on seeds (Klingman & Murray, 1976). However, at a very high rate (17.9 kg ha⁻¹), Moshier *et al.* (1976) observed inhibition of seed germination in red fescue (*Festuca rubra* L.), which is a grass species. As reported in this experiment, Egle & Williams (1978) had observed stimulation of seed germination in a broadleaved species (*Amaranthus retroflexus* L.) at 30, 120 and 125 mg l⁻¹ (equivalent to ppm).

Glyphosate reduced radicle length significantly in both species (Table 3). The length of radicle was reduced from 12.7 cm in *E. heterophylla* seedlings in the absence of glyphosate to 3.3 cm at 40 ppm of the herbicide (a rate equivalent to 5.0×10^{-1} kg a.i. ha⁻¹). Similarly, the length of radicle in *E. indica* was correspondingly reduced from 3 to 1.3 cm. The reduction of radicle length in *E. indica* occurred despite the fact that the chemical did not influence seed germination in this species. This observation is similar to that of Moshier *et al.* (1976). They observed inhibition of shoot elongation in some grasses by glyphosate although seed germination and emergence were not affected.

Growth of *E. indica* and *E. heterophylla* from glyphosate-treated seeds

The germination of glyphosate-treated seeds in the petri-dishes was similar to the result of the second experiment.

When the germinated seeds were transferred to

untreated potted soil, glyphosate did not influence the number of leaves produced per plant, but it significantly reduced plant height in *E. heterophylla* at 2 weeks after planting (Table 4). The height of *E. indica* was not affected.

Results of this experiment agree with those of other works on the broadleaved species but differ on the grasses. Moshier *et al.* (1976) observed reduction in plant height of four varieties of bluegrass treated with 10⁻⁴ M glyphosate. Similarly, Egle & Williams (1978) and Salazar & Appleby (1982) reported reduced seedling growth of some grasses and legumes when exposed seeds were treated with glyphosate at 1.0 and 3.0 kg ha⁻¹. Although these reports indicated that glyphosate affected plant growth in both legumes and grasses, they also pointed out that the effect was more severe on legumes than grasses.

Mortality of emerged seedlings at 4 weeks after planting (Table 4) ranged from 7.5 among *E. heterophylla* plants treated with 10 ppm glyphosate (1.3×10^{-1} kg a.i. ha⁻¹) to 60 per cent among those plants treated with 40 ppm glyphosate (5.0×10^{-1} kg a.i. ha⁻¹). Mortality of emerged seedlings was less in *E. indica* than *E. heterophylla*.

Surviving plants recovered from the effects of the herbicide and by the fourth week of growth in potted soil, they were as tall as the control plants and produced similar fresh weight per plant (Table 5).

TABLE 4

Effect of Glyphosate-treated Seed on Plant Height of E. indica and E. heterophylla Seedlings at Two Weeks and Seedling Mortality at Four Weeks after Planting in Untreated Soil

Concentration of solution (ppm)	Glyphosate rate		Plant height (cm) ¹		Seedling mortality (%) ²	
	Quantity per unit area ($\times 10^4$ kg a.i. ha ⁻¹)		E. indica	E. heterophylla	E. indica	E. heterophylla
0	0.0		3.5a	7.8a	0.0	0.0
10	1.3		4.0a	4.1b	0.0	7.5
20	2.5		4.1a	3.8b	0.0	22.5
30	3.8		3.5a	3.8b	4.5	50.0
40	5.0		3.2a	4.1b	7.4	60.0

¹Means followed by the same letter within each column were not statistically different at $P=0.05$ as determined by Duncan's Multiple Range Test.

²SE for comparing the means of the two glyphosate rates in *E. indica* and *E. heterophylla* = ± 2.1 and ± 6.9 , respectively.

The seedlings recovered from the effect of glyphosate because of soil dilution factor. Sprankle *et al.* (1975) found that glyphosate was readily bound to clay and organic matter through its phosphoric acid moiety. Once in the soil, some quantity of the herbicide may move from the treated seedlings into the soil medium (Rodrigues, 1979; Rodrigues, Worsham & Corbin, 1982). The work by Moshier *et al.* (1976) also illustrated the influence of soil on the activity of glyphosate. When glyphosate was applied directly over seeds of red clover at 17.9 kg ha⁻¹, they observed inhibition of seed germination. However, this high rate applied pre-plant and soil incorporated, resulted in non-

significant reduction in the growth of the seedlings. With the addition of new plant tissues during growth in this experiment, the concentration of glyphosate per unit of plant tissue was further reduced. At the reduced concentration, the herbicide could become stimulatory to growth. If this happened, the surviving plants made up for the initial growth inhibition.

Results of these experiments indicate that although appropriate rates of glyphosate kill existing vegetation in a prepared seed-bed, some seeds of *E. indica* and *E. heterophylla* which do not germinate at the time of herbicide application will not be prevented from germinating and

TABLE 5

Effect of Seed Treatment with Glyphosate on Height and Fresh Weight per Plant of E. indica and E. heterophylla at Four Weeks after Planting in Untreated Soil

Concentration of solution (ppm)	Glyphosate rate		Plant height (cm) ¹		Fresh weight per plant (g) ¹	
	Quantity per unit area ($\times 10^4$ kg a.i. ha ⁻¹)		E. indica	E. heterophylla	E. indica	E. heterophylla
0	0.0		8.8a	15.0a	1.8a	0.95a
10	1.3		9.0a	16.1a	1.7a	0.99a
20	2.5		10.1a	17.0a	1.7a	0.95a
30	3.8		9.6a	15.5a	1.5a	0.97a
40	5.0		9.6a	15.8a	1.6a	0.89a

¹Means followed by the same letter within each column were not statistically different at $P=0.05$ as determined by Duncan's Multiple Range Test.

establishment. More seeds of *E. heterophylla* may be stimulated to germinate and establish than in the absence of glyphosate, if the seeds absorbed sublethal quantities of the herbicide not intercepted by the target vegetation. Once established, surviving seedlings will overcome the initial adverse effect of the herbicide and become active. Some precautions should, therefore, be taken to prevent germination, emergence and establishment of ungerminated seeds in the soil when glyphosate is used to destroy unwanted vegetation prior to planting crop seeds.

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