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Assessing the Residual Effects of Fluridone in Humid Tropical Soil Using Sequentially Planted Peanut (*Arachis hypogea* L.) and Maize (*Zea mays* L.) Seedlings

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Abstract: Fluridone is recommended for glyphosate-resistant weeds in crop fields, but its persistence in soil and its residual effects are clearly discerned. This study was to test residual effects of Fluridone on the germination, growth and performance of groundnut (*Arachis hypogea* L.) seedlings, followed by a further bioassay using maize (to test if the effects persisted after harvest of the groundnut seedlings). The results show that groundnut seedlings did not exhibit any adverse effects of residual Fluridone in the test soil, following protracted wet and dry cycles, except for reduction in shoot-to-root ratio. Also, no effect of Fluridone was observed in maize after 10-days of growth after harvesting the groundnut seedlings. The differences in growth rate was not significant and no whitening of leaves, which is typical Fluridone effect, was not observed in the maize seedlings. The findings of this study suggest that the adverse soil residual effects of Fluridone on crops planted in the subsequent season may be avoided if tolerant species such as groundnut are planted instead of susceptible ones like maize (or cereals). The findings suggest a mix crop of groundnut and maize, with groundnut planted at least three weeks ahead of maize, may also withstand the residual effects of Fluridone. Nevertheless, it is suggested that further studies would be necessary, to discern the how to neutralise the residual effects of Fluridone in soil.
Keywords: Groundnut, Residual Fluridone, Bioassay, Maize, Tropical Soils.

INTRODUCTION

Although Fluridone is famous as an aquatic herbicide, it was indeed originally formulated for use in the control of weeds on arable lands; but trials showed it was not cost-effective and rather unsuitable for use as terrestrial herbicide due to its persistence and non-intended carryover effects non-target crops [1].

Fluridone is increasingly been used to control glyphosate-resistant weeds in crop fields, but the persistence of Fluridone in soil and the response of crops to Fluridone vary widely and, not fully understood. In the aquatic system, Fluridone mostly remains in the liquid-phase and is decomposed by light (photolysis), into non-toxic products hence it is often non-lethal to other life forms in the aquatic environment [2]. In the terrestrial ecosystem, however, Fluridone being a weak base gets adsorbed to soil colloidal matter (both mineral and organic), thereby persisting in the soil with the potential to control weeds longer, but also capable of destroying young seedlings of crop plants, in the subsequent planting season [3].

As reported by [4], the effect of Fluridone manifests in the bleaching of the leaves and stems of crops as a result of its mode of action, i.e., inhibition of the enzyme phytoene desaturase, a key enzyme in the biosynthesis of carotenoids (and abscicic acid, ABA). Reduced carotene in the cells accelerates the photolysis of chlorophyll, while reduced ABA reduces the ability of the affected plants to withstand stress, leading to the necrosis of the affected plant [4, 5]. However, some crops are reported to be resistant to Fluridone, while others are said to be quite vulnerable, and the effect of Fluridone has been observed on maize planted a few weeks after Fluridone application [1, 3], or following protracted wet and dry periods, akin to the dry season [4]. The effects of Fluridone on physiological processes are said to be reversible, hence some plants are able to withstand the damaging effects of Fluridone, especially the case with mature plants [1, 6]. Also, some plants, especially groundnut, are reported to be resistant to Fluridone, hence do not exhibit any adverse effects of the herbicide [7]. Therefore, since Fluridone carryover effects persists across dry seasons, it is imperative that crops to be planted in the subsequent wet season are carefully chosen to avoid damage by the residual soil Fluridone.

Groundnut (*Arachis hypogaea*) is an plant of global importance because of its oil content, which has served as food and industrial raw material across different human civilisations [8]. It is cultivated in a wide swath between latitudes 40° N and 40° S of the equator. Most of groundnut is cultivated during the rainy season. In Nigeria, especially in the extreme northern parts, groundnuts are usually planted at the onset of the rainy season periods of 3-4 months. This means that the residues of chemical compounds applied during the previous rainy season may affect groundnuts seedlings planted in the subsequent season, if the said chemical compound is capable of persisting through the long dry season. The reports of [4] showed that Fluridone persisted through the dry season and exhibited deleterious residual effects on maize seedling. However, since maize is a cereal (grass family) and obtained their nutrients from only the medium in which they grow, this is not the case with groundnut, which is a legume that obtains nitrogen from nitrogen fixation. More so, their growth habits and morphologies are remarkably different. Therefore, the observed effect of residual Fluridone on maize might not be applicable to groundnut, hence, the need to use groundnut as test crops. This will enable the choice of crops whose seedlings can withstand the residual effects of Fluridone to be included in the cropping system that involve the application of Fluridone to soil. In its report on peanut outlook 2017, the University of Georgia Extension Peanut Team indicated that Fluridone is being evaluated for use as herbicide for controlling weeds in peanuts [9].

This study was conducted to test whether Fluridone applied to soil in one cropping cycle has any effect on the germination, growth and performance of groundnut seedlings in the subsequent cropping cycle after prolonged soil desiccation in the dry season. Groundnut (*Arachis hypogea* L.), also called peanut, is an annual legume that is grown for its high oil and protein containing seeds. Originally from South America, it is presently grown across a wide range of climatic conditions (between latitudes 40°N and 40°S of the equator [8]. Groundnut is an economically important food crop that usually planted at the start of the rains.

MATERIALS AND METHODS

Experimental Site and Test Soil

This characteristics of the soil used in this study has been reported by [3]. The soil was obtained, during the rainy season of month of June 2017, from a vegetated land within the University of Port Harcourt campus, which is located in the coastal plains of the humid Niger Delta region. In general, the soil is slightly acidic, with pH 5.6, and loamy in nature; with moderate levels of soil organic matter characterised of soil under bush regrowth in the area [3]. The soil was sieved free of plant debris and used in a potted screen-house 13-week experiment where 30 µmol Fluridone solutions was added directly to the soil, or indirectly through spraying of yam plants.

At the end of that experiment, the soil was air-dried and stored for 30 days, under ambient conditions. Thereafter, 250 g was placed in plastic pots and maize grown in it for 21 days in a screen-house (10 - 31 Oct. 2017). Following the 21-day maize experiment, the soil was subjected to wet and dry cycle for 30 days (31 Oct. - 30 Nov. 2017). The soil was then left to dry in the screen-house (during dry season) until the start of the rains (Feb.) and used for this experiment.

Experimental design and treatment

The soil used in this experiment modelled the natural cycle in rain-fed agriculture: the soil was planted to yams for 13 weeks, followed by 30 days of maize seedling growth, and then left to undergo wet and dry cycles as normally happens under field conditions, and finally a 77-day dry period to mimic the dry season. The experiment was laid in a completely randomised design with three treatments and three replications. The treatments have been described by [10] and they included No Fluridone (NF) Previous Soil-Applied (SA), and Previous Foliar-Applied (FA) Fluridone solution (30 µmol). A previous experiment [4] showed that Fluridone persisted even after prolong drying through the dry season; however, some researchers reported that some non-cereal crops are resistant to Fluridone. Hence, we proposed a hypothesis to suggest that planting a non-cereal or leguminous crop could neutralise the effect of the residual Fluridone and diminish its persistence.

Therefore, rewetted and planted to peanuts (*Arachis hypogea* L.) for 21 days, followed by maize for 10 days. Prior to planting the maize, the peanut was harvested and the roots extracted. To remove the roots, the shoot was first harvested, then the soil was left to air-dry to below field capacity, then plastic pot was squeezed to crush the soil, thereby freeing the roots, which was then emptied into a bigger container and the roots removed by gently shaking off the soil. The soil was then recovered and then planted to maize (var. Oba Super 6) and grown for 10 days.

Test plants

The groundnut used for this trial is Samnut-24, which is adapted to Nigeria, while Oba Super 6 variety of maize used. The seeds of the groundnut were sourced from northern parts of Nigeria. Five seeds were planted per pot, which was pruned to three after seedling emergence. Samnut-24 Samnut-24 an early-maturing (80-90 days) variety with vigorous plant growth, good yield and high oil content [8]. The Oba Super 6 maize variety is a single cross maize hybrid produced in Nigeria and adapted to the study environment [11]. Ten *(*10 nos.) seeds of Oba-Super 6 were planted per pot to observe germination (seedling emergence) and later pruned to 3 seedlings per pot at 3 DAP.

Plant sampling/Data Collection

Seed germination and establishment were assessed by planting five seeds of groundnut seeds into each pot and monitored for germination. At 5 days after planting (DAP), when >95% of the seeds had germinated, the seedlings in each pot were pruned to 3 per port for the experiment. Similarly, 3 maize seeds were planted per pot per treatment per rep and pruned to 2 plants per pot on 3 DAP after emergence.

Seedling measurements were measured, using a graduated rule, from the base of the stem to the tip of the longest leaf (by raising the leaves upwards against the rule). The heights of the seedlings were measured each day from 7 DAP through to 14 DAP and then the last day (at 21 DAP), prior to seedlings harvested. Height measurements were taken, from the point of attachment of seed cotyledons to 1) the tip of the shoot, 2) to the first node, and then 3) the total height (i.e., the longest point of the foliage). For the maize, the heights of the seedlings were taken at 3 DAP (sprouts), 5 DAP and 10 DAP.

Chlorophyll contents of the groundnut leaves were measured using a rapid and non-destruction method of chlorophyll measurements. The method utilises a handheld device, atLEAF® chlorophyll meter, introduced by a technology firm in the USA (FT Green LLC), and described in details Dey et al. (2016). The atLEAF® meter estimates chlorophyll based on algorithms derived fromthe transmissions of red light (at 660 nm wavelength) when chlorophyll absorbs all light and infrared light (at 940 nm wavelength) when no light is absorbed by chlorophyll.

At the end of the experiment (21 DAP), the shoots were harvested by neatly cutting the seedling at the base (soil surface) and then the roots were washed gently to separated the roots from the soil medium. The shoots and the roots were oven-dried to constant weight at 70° C to estimate dry matter content of seedlings.

STATISTICAL ANALYSIS OF DATA

The data sets collected in the course of this trial were subjected to statistical analysis using Genstat® Computer software. Analysis variance and regression were carried out.

RESULTS AND DISCUSSION

Germination and Growth of Groundnut Seedlings

The groundnut seeds had started germinating by the third day after planting (DAP) and by 5DAP, >95% of the seedlings had emerged. The height of the seedlings, measured from the seed leaf point to the seedling growth tip, from 7 DAP ‒ 21 DAP is shown in Fig. 1. The figure shows consistent linear growth of groundnut seedlings irrespective of the treatment.

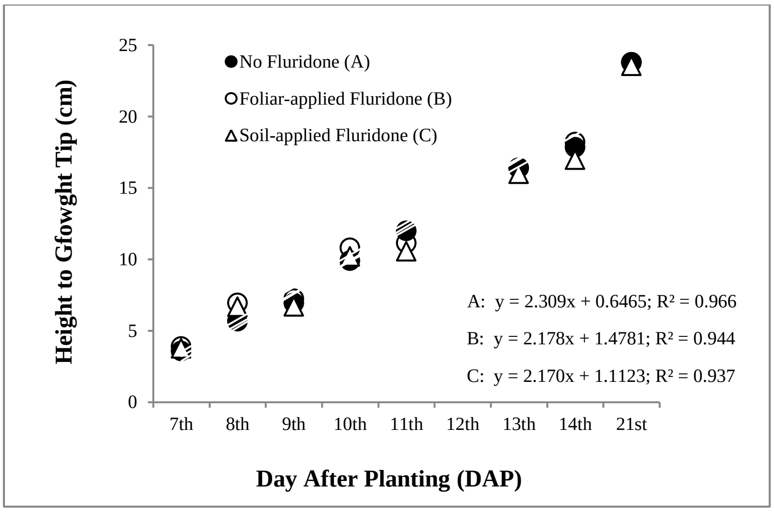

Fig-1: Effect of Fluridone on height to growth tip of groundnut seedlings

There were not drastic differences between the different treatments, however, the linear fit of the growth pattern, as shown by the slope, suggest that that pattern of growth was Control > Foliar-applied fluridone treatment > Soil-applied fluridone treatment (Fig. 1).

The total groundnut seedling height, measured from the seed-leaf to the highest point when the leaves are folded upwards is shown Fig. 2. Although the pattern of growth indicate linear growth up to the end of the study, which is similar to the height of growth tip shown in Fig. 1, the trend differed in the sense that the Control had the least rate of increase in total seedling height.

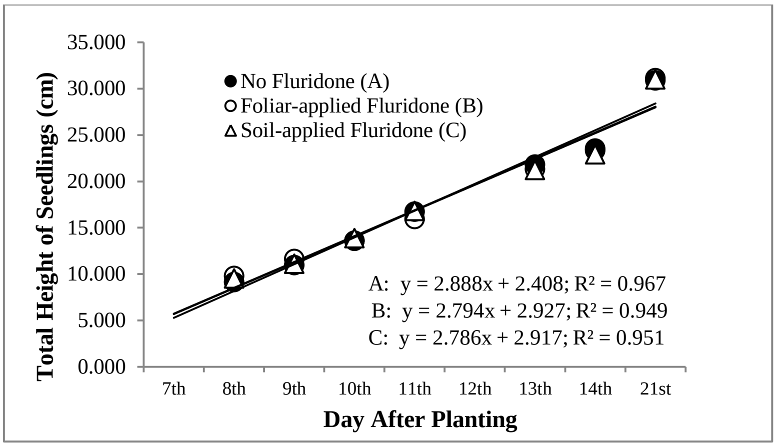

Fig-3: Effect of fluridone on total height of groundnut seedlings

The height of groundnut seedling measured from the seed-leaf to the first node is shown in Fig. 3. The as shown in the figure, the first showed fast growth in the first 9 DAP and the growth stabilised thereafter, with the formation of new nodes [12]. There were no discernible differences between the different treatments in terms of the elongation of the first node. It was obvious that the active growth is at the apex of the plant, not at the base where the first node is located and this observation indicates that growth and elongation in the first node slowed down drastically by the first week after planting [12].

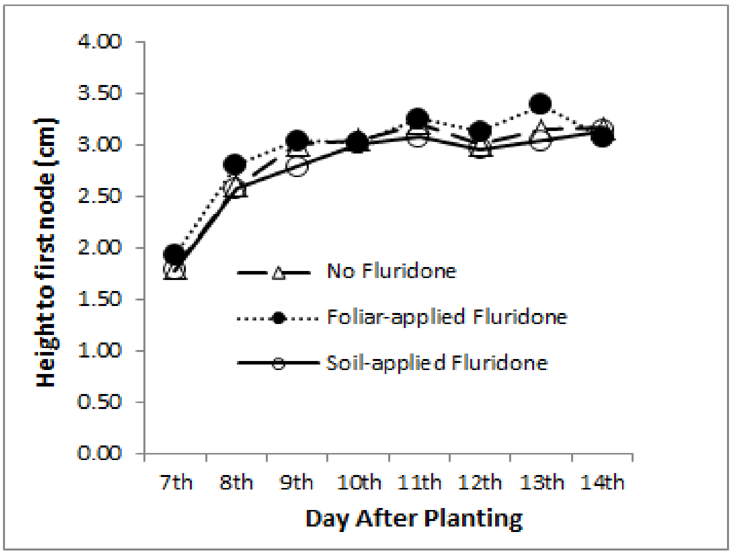

Fig-3: Effect of fluridone on hseedling height to first node

Chlorophyll Contents of Groundnut Seedling Leaves

The results of measurements of chlorophyll contents in the leaves of groundnut seedlings, carried out on the 14th and 21st DAP are shown in Fig. 4. There were no significant differences in the chlorophyll contents in the leaves of groundnut seedlings on both sampling dates. This indicates that groundnut is resistant to residual Fluridone carried over a dry season period. This is in contradiction to the response of maize which showed significant chlorosis and eventual necrosis of the leaves within 2 weeks when plant either soon after the harvest of the main crop, or after a prolonged drying and wetting cycle [3, 4].

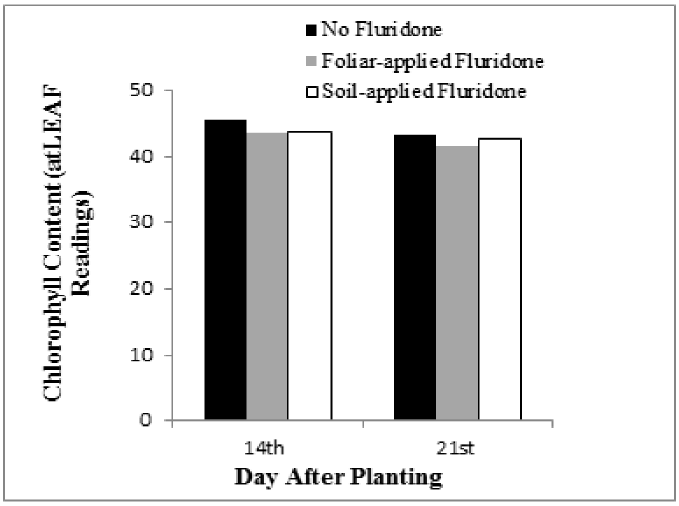

Fig-4: Effect of Fluridone on chlorophyll content of groundnut seedlings

The absence of the diagnostic bleaching effects of fluridone [4, 13] suggests that groundnut should be considered in the event of the occurrence of any residual fluridone in soil. In trials conducted over three years (2015-2017) in the United States of America, to test the tolerance of groundnut to Fluridone applied pre-emergence in the first year only, Fluridone affected peanuts only in 2015 and not in 2016 or 2017 [6]. This suggests that Fluridone either did not persist in the soil or the residual Fluridone was not potent enough to affect groundnuts.

Shoot Dry Matter and Shoot to Root Ratio

The dry weight data (Table 1) for groundnuts indicate to drastic differences between the various treatment, although it appeared that the dry weight of shoots reduced slightly, while there was a slight increase in root dry weight. The patterns of shoot and dry weights manifested in wider shoot-to-root ratio in the Control than the Fluridone treatments.

Table-1: Shoots and roots dry weights of groundnut seedlings at 21 DAP

	Shoot (St) Dry Weight	Shoot Dry Matter	Root (Rt) Dry Weight	St to: Rt Ratio
	mg pot^{-1}	*%*	*mg pot^{-1}*	-
No Fluridone	1.60	11.6	0.557	3.06
Foliar-applied	1.57	11.7	0.677	2.36
Soil-applied	1.56	11.7	0.590	2.64
S.E.D	ns	ns	ns	0.467

Germination and Growth of Maize Seedlings

After harvesting groundnut at 21 DAP, maize was grown for 10 days to test for residual fluridone, since maize had proved effective as a bioassay in a similar experiment [3, 4]. The growth of the maize seedlings from emergence to 10 DAP is shown in Fig. 5. Although trend was No Fluridone > Foliar-applied Fluridone > Soil-applied Fluridone, the differences in the growth of maize seedlings were not significant ($P \leq 0.05$).

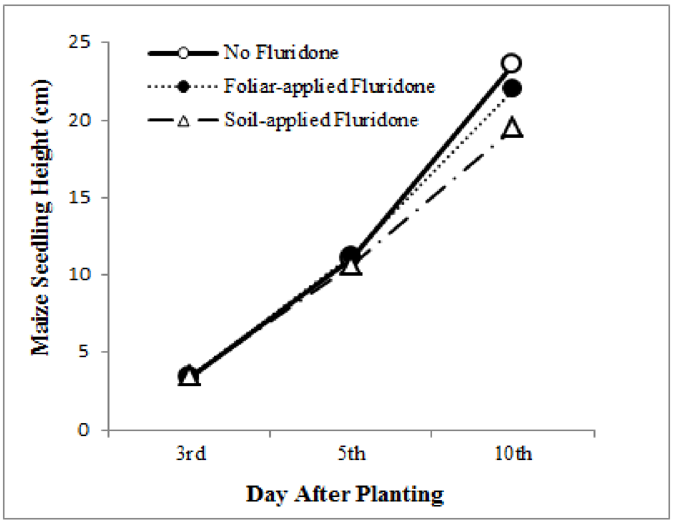

Fig-5: Growth of maize seedlings planted after groundnut harvest

Chlorosis of leaves is a diagnostic system of the effects of Fluridone reported for yams [10, 13], maize [4] and many other crops [1]. Perhaps due to its rapid growth rate, the chlorotic effect of fluridone manifests within five days to one week of planting maize, which makes it a test crop for bioassay of fluridone in soil. The effects of has have been reported for maize planted within a month after soil application of Fluridone [3] or in the subsequent season following a dry season [4].

No chlorosis of maize seedling leaves was observed throughout the 10-day growth of the maize seedlings, which is in contrast with the observations of [4] that observed clear whitening of maize leaves by 6th DAP in Fluridone-treated soils subjected to prolong wet and dry cycle. This suggests that when growing on soils treated with Fluridone in the previous season, could neutralise the residual adverse effects of fluridone in that soil.

CONCLUSION

The findings of this study show that, groundnut seedlings resist the residual effects of Fluridone following protracted wet and dry cycles. The effects of Fluridone were minor and did not manifest in drastic or lethal outcome in groundnut seedlings at 21 DAP. Interesting, a 10-day maize bioassay trial conducted on the soil after harvesting the groundnut seedlings did not manifest any obvious signs of Fluridone effects, especially the whitening of leaves. The implication of these findings is that if Fluridone is applied to the soil in one season, there is need to ensure that only crops that can withstand the effects of Fluridone are planted in the subsequent season, in order to avoid losses; and groundnut is a good candidate. Since cereals (maize) are known to be susceptible to the effects of Fluridone, if follows that if they must be planted, they should be preceded by groundnuts. However, further studies are recommended so as to elucidate further, the best way to neutralise the residual effects of Fluridone in soil. It is pertinent that trials using mix cropping involving cereals and legumes (especially groundnuts) are undertaken in this regard.

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