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CALLUS INITIATION AND PLANT REGENERATION OF *CALADIUM BICOLOR* (AITON) VENT. BY *IN VITRO* CULTURE

A.M.A. SAKPERE+ and A.C. ADEBONA
Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

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Abstract

A method for the direct plant regeneration of *Caladium bicolor* (Aiton) Vent. is described. Callus was induced from corm and leaf explants of *C. bicolor* on Murashige and Skoog (MS) medium supplemented with 0.8 mg/L 2,4-Dichlorophenoxyacetic acid (2,4-D) in combination with 1 mg/l kinetin. The callus which was white and compact was scanty and shortlived. Rootlets and shootlets were generated on corm explants inoculated on medium supplemented with kinetin and NAA as well as various concentrations of 2,4-D. Corm and leaf explants had a 50 % response each to all the concentrations of 2,4-D used. More callus was induced from leaf explants than from petiole or corm explants.

Keywords: *Caladium bicolor*, 2,4-D, corm, plantlets, callus, kinetin

1. Introduction

Caladium bicolor (Aiton) Vent. is an ornamental plant of the Araceae family and is tropical American in origin. It is a succulent perennial monocotyledon of the order Arales. It is a tuber forming species with a cocoyam habit (Olorode, 1984). *Caladium bicolor* is characterized by its terrestrial habit, yellow corm, petiole 30-95 cm long, and its blades (18-46 cm long, 12-25 cm broad) with rounded, divergent posterior lobes.

Apart from being an ornamental plant, *Caladium bicolor* is used in healing poisonous bites and possibly neutralizing toxins. It has medicinal uses in tropical America, as well as being eaten as a vegetable and its' dried powdered leaves make a dressing for wounds (Bown, 2000). In Brazil the stem juice of *C. bicolor* is used as an enema to expel roundworms and destroys maggots when applied to the skin. Peasants use leaf decoctions to get rid of external cattle festers caused by worms (Balbach, 1980). In the West Indies, Hedrick (1919) observed that the corms are eaten roasted or boiled and the leaves are eaten, boiled as a vegetable.

In view of the multipurpose use of this species, developing an efficient protocol for the rapid propagation of *C. bicolor* is in order. Reddy *et al.* (2001) reported that *in vitro* propagation methods offer powerful tools for plant germplasm conservation and multiplication. The first and, to date, the most extensive practical application of tissue culture techniques to horticultural crops involves the multiplication of ornamental plant species (Torres, 1989). Plant tissue culture and micropropagation techniques allow a far greater number of plants to be produced in a given time than can be achieved by conventional propagation methods. These techniques can also play an important role in biotechnology since regeneration from cells or tissues cultured *in vitro* is a fundamental requirement for most applications of plant biotechnology.

This study describes the basic procedures for the generation of callus and plant regeneration of *C. bicolor*, as part of a study aimed at developing a procedure for efficient plant regeneration and multiplication of the species.

2. Materials and Methods

Corms of *Caladium bicolor* were collected from the Senior Staff Quarters of the Obafemi Awolowo University (OAU), Ile-Ife, and grown in the Screen House of the Department of Botany, OAU, Ile-Ife. Leaf, petiole, corm and root segments from these plants were washed under running tap water to remove dirt and reduce microbial population. Corms were dehusked and selected for a healthy appearance (i.e. without malformations or presence of necrotic spots). The explants were surface disinfested in 0.7 % (w/v) sodium hypochlorite solution with 2 drops of Tween 20 for 10 mins. and then thoroughly rinsed three times in sterile distilled water. Leaf explants (1 cm *x* 1 cm), petiole cylinders (about 1 cm long), small cubes of corm (about 1 cm *x* 1 cm *x* 1 cm) and root explants (about 1 cm long) were cultured aseptically on full strength MS medium and incubated at 25±2 °C in the dark. The culture medium

+ *corresponding author* (email: asakpere@oauife.edu.ng **or** asakpere@yahoo.com)

consisted of Murashige and Skoog's (1962) medium (MS medium) supplemented with 3 % (w/v) sucrose, 0.8 % (w/v) agar (Oxoid Agar No. 1, Code L11) and several combinations of auxins and cytokinins. The pH of the medium was adjusted to 5.7±0.1, dispensed into 50 ml. Erlenmeyer flasks which were stoppered with non-absorbent cotton wool wrapped with aluminum foil and then autoclaved at 121 °C and 15 lb/in^2 pressure for 15 minutes.

Four different concentrations of kinetin (0.25, 0.5, 1.0 and 2.0 mg/l) in combination with 1-naphthalene acetic acid (NAA) (1, 3, 5, and 10 mg/l) and two concentrations of 6-benzyladenine (BA) (1 mg/l and 5 mg/l) in combination with NAA (1 mg/l and 5 mg/l) were investigated for their effects on direct regeneration and callus formation from corm and root explants. Also, three different concentrations of 2,4-D (0.4, 0.8 and 1.6 mgs/l) were combined with 1.0 mg/l kinetin to investigate the callogenic and regenerative capacities of corm, leaf and petiole explants. MS medium lacking growth regulators served as control.

The explants were evaluated visually for the presence and type of callus (Remotti and Loffler, 1995) and the responses scored (Mencuccini and Rugini, 1993). Also, the number of shoots produced per explant and the time required for generation of shoot were observed.

3. Results

(a) Callus Studies

Callus induction was observed on corm explants of *C. bicolor* after 3 weeks on MS medium supplemented with 1.0 mg/l BA and 1.0 mg/l NAA (Table 1). The callus did not, however, survive for more than two weeks. On the medium supplemented with 0.5 mg/l kinetin and 3.0 mg/l NAA (K0.5N3), roots were generated on one side of the corm explant and callus on the other side after 5 weeks in culture. However, by the 7th week, root growth had overshadowed the growth of callus and shoots were initiated on the explant. That was the only incidence of callus initiation using medium supplemented with kinetin and NAA. Callus was initiated on corm explant cultured on MS medium supplemented with 0.8 mg/l 2,4-D in combination with 1.0 mg/l kinetin. There was 17 % incidence of generation of roots from the explant and 17 % incidence of a combination of callus and roots (Fig 1B), however, growth of roots was more rapid and the callus was soon choked. The leaf of *C. bicolor* cultured on MS medium supplemented with 0.8 mg/l 2,4-D in combination with 1.0 mg/l kinetin showed various responses. There was 17 % incidence of generation of plantlets and 34 % initiation of callus (Fig 1A). Of the callus initiated, 50 % did not grow beyond the stage they were on transfer to a fresh medium of the same composition. The size of the callus remained constant but after 4 weeks, root, shoot and leaves developed from the explant. Root explants showed no response at all.

(b) Direct Regeneration

Regeneration of root was observed on corm explant on MS hormone-free medium. Rootlets and shootlets were generated on corm explants inoculated on medium supplemented with 0.5 mg/l kinetin and 3.0 mg/l NAA (Fig 1C) as well as 1.0 mg/l kinetin and 5.0 mg/l NAA though shootlets developed earlier on medium supplemented with 0.5 mg/l kinetin and 3.0 mg/l NAA (Table 2). Root explants did not respond to any of the auxin and cytokinin combinations. The

Table 1: Effect of BA and NAA on Callus Induction and Plant Regeneration

	Control		5.0mg/l BA & 1.0mg/l NAA		1.0mg/l BA & 1.0mg/l NAA		5.0mg/l BA & 5.0mg/l NAA	
Explant	Corm	Root	Corm	Root	Corm	Root	Corm	Root
TOR	-	-	-	-	C	-	RL	-
% Response ± SE	0 ± 0	0 ± 0	0 ± 0	0 ± 0	33 ± 0.24	0 ± 0	33 ± 0.24	0 ± 0
NOS ± SE	-	-	-	-	-	-	-	-
TOI in wks	-	-	-	-	3	-	-	-
SOC	-	-	-	-	< 1 cm	-	-	-

KEY

TOR - Type of Response
TOI - Time of Initiation In wks
SE – Standard Error
NOR - No of Roots
SOC - Size of Callus (diameter)
RL – Root-like structure
NOS - No of Shoots
C - Callus

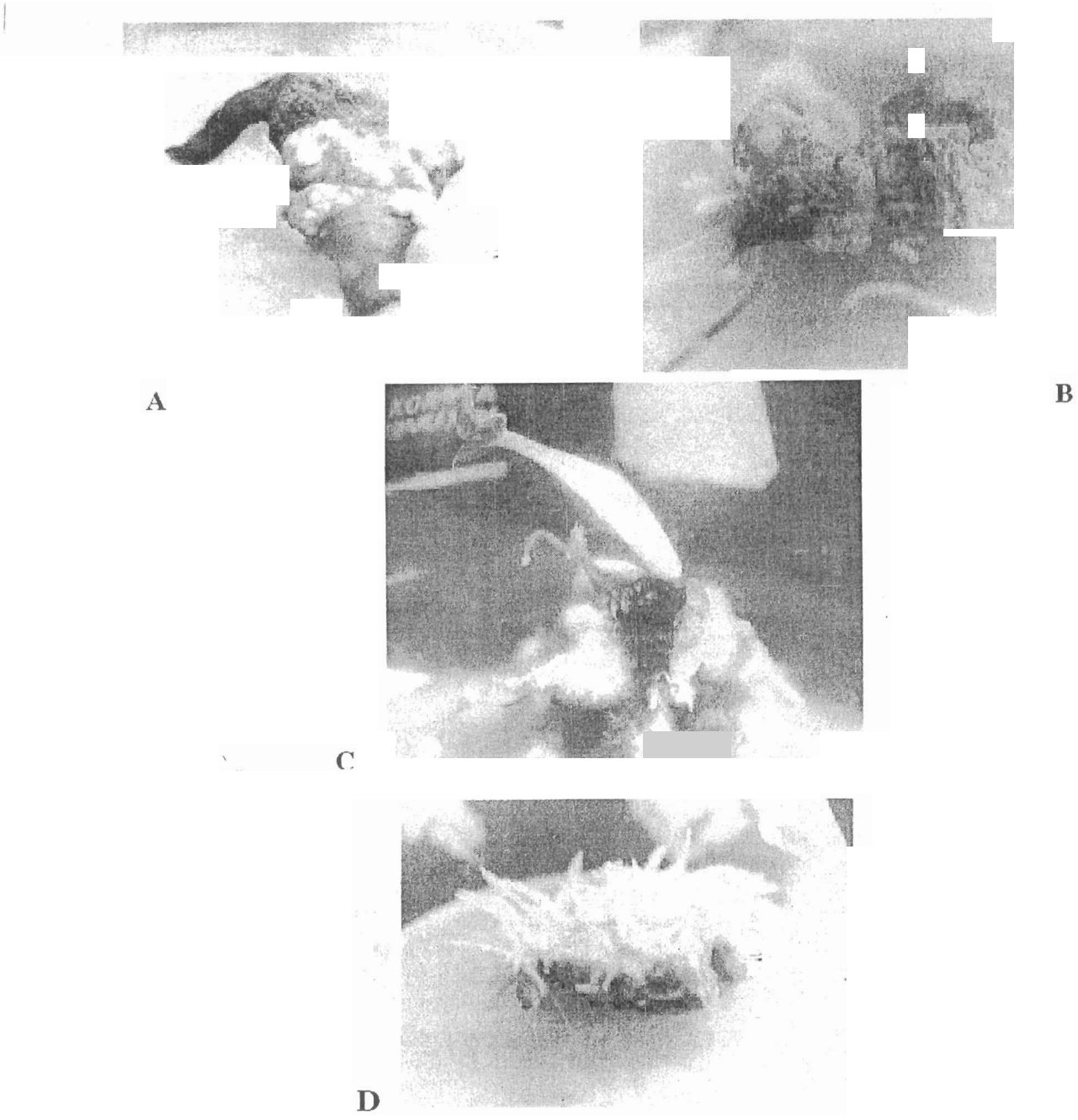

Fig. 1: (a) Callus on leaf explant of *C. bicolor* on 0.8mg/l 2,4-D, (b) Callus and root on tuber explant of *C. bicolor* on 0.8mg/l 2,4D, (c) *C. bicolor* tuber on K0.5N3 - roots and shoots developed, and (d) roots on tuber explant of *C. bicolor* on 1.6mg/l 2,4D.

leaf of *C. bicolor* cultured on medium supplemented with 0.4 mg/l 2,4-D in combination with 1 mg/l kinetin generated roots and shoots after an average of 19 weeks. Corm explants developed plantlets after 9 weeks on medium supplemented with 0.4 mg/l 2,4-D in combination with 1 mg/l kinetin much earlier than leaf explants. Roots and shoots were also generated on leaf explants cultured on medium supplemented with 0.8 mg/l 2,4-D in combination with 1 mg/l kinetin after 13 weeks. Thirty three percent rooting was observed on corm explants on the same concentration of supplements out of which 17 % was in combination with callus. On 1.6 mg/l 2,4-D in combination with 1mg/l kinetin, *C. bicolor* corm generated a lot of roots (Fig. 1D). No response was observed on petiole explants apart from swelling of the explants.

In general, more callus was induced from leaf explants than from petiole or corm explants (Table 3). Only 0.8 mg/l 2,4-D in combination with 1 mg/l kinetin induced callus in corm and leaf explants of *C. bicolor* and the callus which was white and compact was scanty.

Table 2: Effect of Kinetin and NAA on Callus Initiation and Plant Regeneration

	EXPLANT	TOR	%RESP. ± SE	NOS ± SE	TOI In wks (Average)
K_0N_0	CORM	R	33 ± 0.24	0 ± 0	4
	ROOT	-	-	-	-
K_0N_3	CORM	-	-	-	-
	ROOT	-	-	-	-
$K_{.25}N_1$	CORM	-	-	-	-
	ROOT	-	-	-	-
$K_{.5}N_3$	CORM	R/S	33 ± 0.24	2 ± 0.25	5/12
	ROOT	-	-	-	-
K_1N_3	CORM	-	-	-	-
	ROOT	-	-	-	-
K_1N_5	CORM	R/S	67 ± 0.33	2.5 ± 0.69	11.5/26
	ROOT	-	-	-	-
K_2N_{10}	CORM	R	33 ± 0.24	0 ± 0	4
	ROOT	-	-	-	-

KEY
TOR - Type of Response S - Shoot NOS - No of Shoots
TOI - Time of Initiation In wks SOC - Size of Callus (diameter) C - Callus
N – Naphthalene Acetic acid SE – Standard Error R – Roots
No. Subscript - concentration of growth regulator used in mg/l K – Kinetin

Table 3: Effect of 2,4-D on callus initiation and plant regeneration

1mg/l kinetin + 2,4-D >	0.4mg/l			0.8mg/l			1.6mg/l		
Explant	Corm	Petiole	Leaf	Corm	Petiole	Leaf	Corm	Petiole	Leaf
TOR	R/S		R/S	R/C		C/R&S	R		
%Response ± SE	17 ± 0.03	0 ± 0	33 ± 0.06	33±0.06/ 17± 0.03	0 ± 0	25±0.04/ 25± 0.04	17 ± 0.03	0 ± 0	0 ± 0
NOS ± SE	1 ± 0.17	0 ± 0	4.5±1.07	0 ± 0	0 ± 0	2 ± 0.33	0 ± 0	0 ± 0	0 ± 0
TOI in wks	9		19	6		9/13	6	-	
SOC				> 1cm		< 1cm			

KEY
TOR - Type of Response S - Shoot NOS - No of Shoots
TOI - Time of Initiation In wks SOC - Size of Callus (diameter) C - Callus
R - Roots SE - Standard Error

4. Discussion

Among the wide range of auxins and cytokinins which have been used to induce callus, the auxins 2,4-D and NAA were always indicated as the best auxins for callus induction (Remotti and Loffler, 1995). Using 3 combinations of BA and NAA as supplements, callus was only induced on corm explant of *C. bicolor* cultured on MS medium supplemented with 1 mg/l BA and 1 mg/l NAA. This is in contrast to the work on *C. bicolor* by Ahmed *et al.* (2002) who found cultures of explants on 1 mg/l BA and 1 mg/l NAA yielded plantlets directly. Combinations of kinetin and NAA used did not induce callus except on medium supplemented with 0.5 mg/l kinetin and 3.0 mg/l NAA in which there was initiation of callus which was quickly overgrown by roots.

Among the combinations of 2,4-D used, MS medium supplemented with 0.8 mg/l 2,4-D in combination with 1.0 mg/l kinetin was the only one that induced callus (both on corm and leaf explants). There was induction of exclusively callus only on 17 % leaf explants. All other incidence of callus initiation was in association with root and shoot growth which soon overcrowded it. Satish *et al.* (2003) also reported

that in *Fritillaria hupehensis* (Lilaceae), protocormlike bodies (PLB) cultured on MS basal medium supplemented with either 0.8 mg/l 2,4-D or NAA in combination with 0.5 mg/l kinetin proliferated and produced bulblets and callus. The use of 1.6 mg/l 2,4-D in combination with 1 mg/l kinetin as supplement on the MS medium did not induce callus on the explant neither was shoot produced. Only roots were generated on the corm explants. This is in contrast to the statement of Carter *et al.* (1967) that a higher concentration of 2,4-D is required for callus induction in monocotyledons.

Combinations of BA and NAA used did not yield direct organogenesis but most of the combinations of kinetin and NAA used generated roots and shoots. *C. bicolor* appears to be more capable of direct organogenesis since callus induction formed only 27.27 % of the responses. Roots were developed even in the control which had no growth regulator. The incidence of root formation on auxin free medium may be due to the availability of high quantity of endogenous auxin in explant (Minocha, 1987). The induction of callus on MS hormone-free medium has also been reported on internode and leaf explants of *Holostemma ada-kodien* (Martin, 2002). In explants that generated roots and shoots, roots were usually generated first and shoots later developed on the same medium without need for transfer into any special medium for either root or shoot initiation. Combinations of growth regulators that formed only roots had a high concentration of auxin. This indicates that the high auxin probably suppressed the growth of shoots since there was a higher auxin to cytokinin ratio.

Corm and leaf explants had a 50 % response each to all the concentrations of 2,4-D used although corm explants responded across board while leaf explants responded to only two of the concentrations. This is in agreement with Satish *et al.* (2003) who worked on *Pinellia ternate* (Araceae) a perennial medicinal herb that grows wildly in Japan and China. They observed that maximum response from tissue culture occurred in bulbils followed by leaf blades, and petiole explants. The root explants used had 0 % response. This could be due to a number of factors since root explants of other monocots generated callus and plantlets in culture, for example barley (Malepszy and Gay, 2003) and *Cymbidium ensifolium* (Chang and Chang, 1998) which even regenerated plantlets from root induced calli. The fact that the root explant used was not the root tip could be a deciding factor. Ahmed *et al.*, (2002) regenerated plants from root tips of *C. bicolor*, though Malepszy and Gay (2003) generated callus sporadically from non meristematic root explants of Barley.

In conclusion, the present communication presents a procedure for the micropopagation of *C. bicolor* through direct organogenesis in the dark. In future work, an attempt will be made to develop an efficient procedure for callus induction of the species.

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Planted fallow reduces weed seedbank in southwestern Nigeria

FRIDAY EKELEME,*

International Institute of Tropical Agriculture, Ibadan, Nigeria, Correspondence address: C/O L.W. Lambourn and Company, Carolyn House, 26 Dingwall Road, Croydon, CR9 3EE, UK E-mail: E-mail: F.Ekeleme@cgiar.org

I. OKEZIE AKOBUNDU,

139 Lincoln Avenue, #1, Albany NY 12206-1402, USA. E-mail: akobundu@albany.net

AUGUSTINE O. ISICHEI,

Department of Botany, Obafemi Awolowo University Ile-Ife, Oyo State, Nigeria

DAVID CHIKOYE,

International Institute of Tropical Agriculture, Ibadan, Nigeria

Abstract

Weeds are a major constraint to crop production in smallholder farms in tropical Africa. The weed seedbank and annual recruitment are the main sources of weed infestation in crops. This study in Ibadan, Nigeria, evaluated the effect on the seedbank of two types of planted fallow (alley cropping with *Leucaena leucocephala* and live mulch with *Pueraria phaseoloides*) and a natural fallow under four land-use intensities. Type of fallow was the main plot. Land-use intensities, consisting of continuous cropping, 1 cropping year followed by 1, 2 and 3 years of fallow, were the subplots. After 4 years, the seedbank was 50% lower in plots seeded to *Pueraria* and 44% lower in *Leucaena* plots, compared to natural bush. The difference in seedbank between *Leucaena* and natural fallow was marginal (11%). Seed density of annual broadleaf weeds was high (38%-85%) and dominated the seedbank of both planted and natural fallow. Weed seeds of grasses and sedges occurred in very low populations in all plots cultivated after 2-3 years of fallow. Seed density of perennial broadleaf weeds increased as land-use intensity decreased. Major annual broadleaf weed seeds were *Ageratum conyzoides*, *Spermacoce ocymoides* and *Spigelia anthelmia*. Major perennial weed seeds were *Chromolaena odorata* and *Talinum triangulare* while *Mariscus alternifolius* was the dominant sedge. Major grass weeds were *Cynodon dactylon* and *Eleusine indica*. Live mulch with *Pueraria*, especially when combined with 2-3 years of fallow, lowered the seedbank measurably.

Subject index: alley cropping, live mulch, planted fallow, weed seedbank, *Leucaena leucocephala, Pueraria phaseoloides.*

Introduction

Weeds are a major constraint to crop production in smallholder farms in tropical Africa. Parker and Fryer (1975) have estimated that weed competition in developing countries results in 125 million tonnes of loss in food production annually. In addition, over 40% of farm labour are expended in removing weeds in smallholder agriculture in developing countries (Labrada and Parker, 1994). In southwestern Nigeria, smallholder farmers are faced with increasingly high weed pressure as a result of continuous cultivation of available arable land. Crop production is carried out on land that is highly fragmented (average farm size 1.5 ha), marginal in fertility, and with hardly any use of fertilisers or herbicides (Raji *et al.*, 1995). These conditions favour the proliferation of weeds. The weed seedbank and annual recruitment from weeds that escape management practices are the main source of weed infestation in crops. Weed seedbank characteristics can influence both weed population and the success of weed management in cropped fields (Douglas *et al.*, 1997). High seedbank populations can lead to substantial weed densities and several years of intensive management may be required to minimise the problem associated with these densities (Kegode *et al.*, 1999).

Various weed management strategies have been reported to affect the seedbank of arable fields. For example, crop rotation and tillage systems have been reported to influence weed seed population and composition (Barberi *et al.*, 1998). Herbicides have been reported to reduce the weed seedbank of many weed species in smallholder agriculture (Akinola and Egunjobi, 1991). However, studies have shown that herbicides could cause shifts in species composition in favour of species that are less susceptible to applied herbicides (Ball and Miller, 1990). Although herbicides have been shown to reduce the population of weed seeds in the soil, their potential impact on the agroecosystem is not fully understood. Edward *et al.*, (1998) have pointed out that weed control strategies based on herbicide technology should balance herbicide use, crop productivity, food safety, profit, and water quality. In low input agriculture where environments are fragile and the resource base is low, there is a pressing need for alternatives to herbicides.

Smallholder farmers faced with high weed pressure need an efficient weed management system that would reduce the weed seedbank. Such a system should require little use of external inputs because the majority of the farmers are resource-poor. An example of such a system is the planted fallow system. This system has been shown to be an effective substitute for the natural fallow in smothering weeds and depleting the weed seedbank (Akobundu *et al.*, 1999). Planted fallows have been advocated as a better alternative to natural bush fallow systems because of their efficiency in protecting the soil from erosion, restoring of nutrients, and controlling weed (Mulongoy and Akobundu, 1990). The use of planted fallow to reduce the weed seedbank therefore seems to be an attractive option. Information is scanty on the use of planted fallow for this purpose in the humid and subhumid tropics. The objective of this study was to evaluate the effect of planted fallow on the weed seedbank in experiments simulating smallholder farms in the subhumid tropics.

Materials and Methods

This study was established in 1989 as a long-term fallow management experiment and was continued through 1994 at the research farm of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (7^{O} 30' N, 3^{O} 54' E). The experimental site is located in the humid forest/savanna transition zone with a mean annual temperature of 26 ^{O}C. The site receives an average annual rainfall of 1250 mm which has a bimodal distribution trend. Most of the rainfall occurs in July and September. The period November through February/March constitutes the dry season. The soil type was an alfisol with approximately 68% sand, 13% silt, and 17% clay, organic matter < 2%, and pH 6.2 in the top soil.

The experiment was set up as a split plot in a randomised block design with three replications. Main plot treatment consisted of traditional bush fallow and two improved fallow types (live mulch with *Pueraria phaseoloides*, alley cropping with *Leucaena leucocephala*). Subplot treatments consisted of land-use intensities (continuously cropped, one year of cropping followed by one, two and three years of uncropped fallow). Each subplot was 12 by 20 m in size. In 1989, *L. leucocephala* was planted at 3 kg ha^{-1} in rows spaced 4 m apart. In subsequent years, the vegetation in each plot was manually cut and burnt at the beginning of each cropping season. Before crops were sown hedgerows of *L. leucocephala* were cut (50 cm) above ground in plots that were due for cultivation in any given year. Hedgerows were pruned again during the cropping season at 4 and 8 weeks after planting (WAP). During the cropping phase, each subplot was sown to corn (*Zea mays* L.) at a population of 40,000 plants ha^{-1} and to cassava (*Manihot esculenta* Crantz) at 10,000 plants ha^{-1}. *P. phaseoloides* was sown at a seed rate of 15 kg ha^{-1} in the maize/cassava inter-row space at 4 WAP every year in plots that were designated for this main plot treatment. Plots were not tilled during land preparation and were weeded three times during the cropping phase. Plots were free of fertilisers, herbicides, and insecticides during the entire study period.

Soil samples were collected in early March from each subplot before the rains and before the vegetation was cleared for cropping in 1994. Thirteen soil cores were taken to a depth of 10 cm with a precision bucket auger from each subplot. The soil cores were bulked to form one sample per plot. The soil samples were passed through a 2-mm sieve to remove litter, large stones and root fragments. Seeds larger than 2-mm diameter retained on the sieve were removed and then returned to the sieved soil. Direct germination in the screen house was used to estimate the seedbank for a period of 3 months.

Data was analysed using the PROC GLM procedure in SAS (Statistical Analysis Systems, SAS Institute, Cary, NC 27512-8000). Means were separated using the standard error of the mean. For all variables main effects of treatments are presented because interactions were not significant. Species composition was assessed by Redundancy Analysis (RDA) using the software CANOCO (ter Braak, 1990).

Results and Discussion

Seedbank Size

Type of fallow and land-use intensity affected the seed bank size ($P < 0.05$) (Fig. 1). Total seed density in plots cultivated after *P. phaseoloides* fallow was

significantly lower (8565 seeds m^{-2}) compared to that in plots after natural bush (17051 seeds m^{-2}) and *L. leucocephala* fallow (15258 seeds m^{-2}). After 4 years, the seedbank was 50% and 44% lower in *P. phaseoloides* plots compared to natural bush and *L. leucocephala* plots. The difference in seedbank between *L. leucocephala* and natural bush was marginal (11%). The low seed densities observed in *P. phaseoloides* plots may be attributed to good canopy cover, high biomass and litter production which covered the ground better compared with *L. leucocephala* and natural bush. *P. phaseoloides* is a prostrate cover crop that attains complete ground cover within one year after planting (F. Ekeleme, IITA, unpublished data). The closed canopy of *P. phaseoloides* reduces light quality reaching the ground and this leads to suppression of weed growth. Seed production and seed rain are prevented and this may lead to the depletion of the weed seedbank over time. Seed rain has been reported as the main source of enrichment of the weed seedbank (Forcella *et al.*, 1996).

Within each fallow type, the total weed seed number was higher in continuously cultivated plots than in the other land-use intensities ($P < 0.05$). Overall, weed seed population was 3-4 times higher in continuously cultivated plots than in plots cultivated after 3 years of fallow (Fig. 1). The high seedbank in continuously cultivated plots may be attributed to high weed densities.

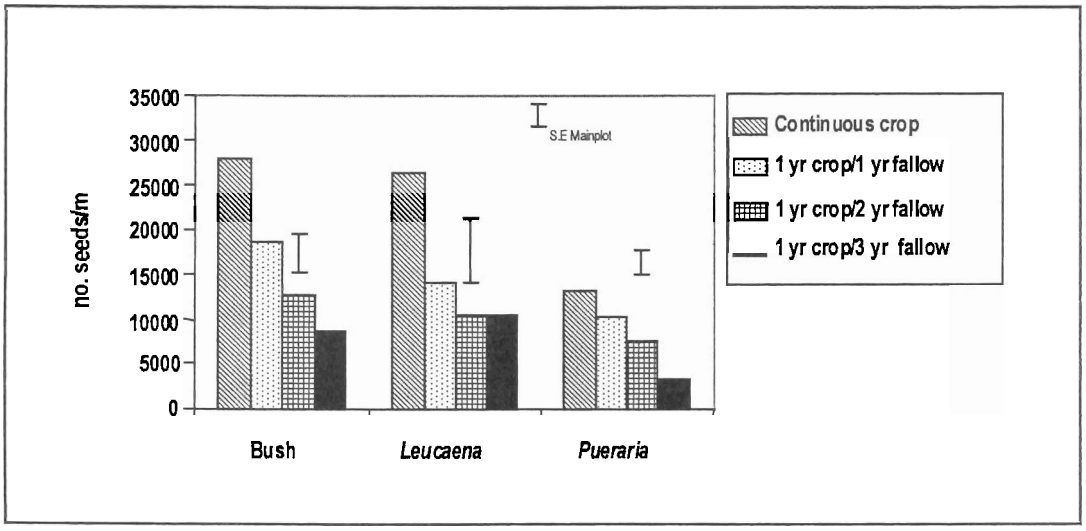

Figure 1. Effect of type of fallow and land-use intensity on total weed seedbank.

High weed densities could lead to high seed rain and subsequently to an increase in the seedbank. Akobundu *et al.* (1999) reported higher weed densities in continuously cultivated plots compared to plots that were in fallow before cultivation.

Weed Seedbank Composition

Fallow type and land-use intensity affected the composition of the seedbank. Seed density of annual broadleaf weeds was higher and dominated the seedbank of both planted and natural fallow contributing between 35%-85% to the total seedbank. Akinola and Egunjobi (1991) have also reported the prevalence of annual species in the seedbank of cultivated fields in western Nigeria. In general, there were more grass weed seeds in plots cultivated after natural bush and *L. leucocephala* than

after *P. phaseoloides* (Fig. 2). Seeds of broadleaf weeds dominated the seedbank of subplot treatments.

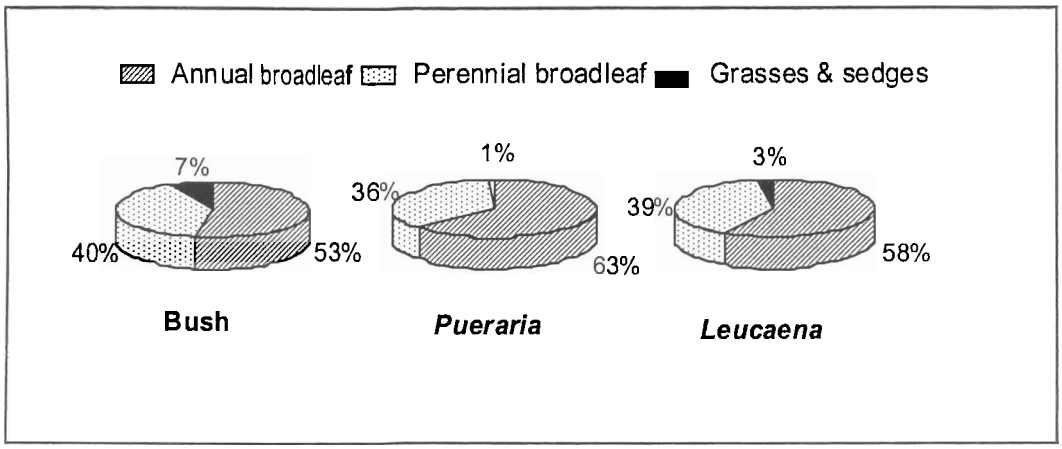

Figure 2. Effect of type of fallow on seed population of broadleaf weeds, grasses and sedges.

The population of grasses and sedges was highest in continuously cultivated plots irrespective of fallow type, but these weeds occurred in low populations in all plots cultivated after 2-3 years of fallow (Fig. 3). Overall seed density of perennial broadleaf weeds increased as land-use intensity decreased. This trend agrees with the work of Thomas *et al.*, (1996) and Akobundu *et al.*, (1999).

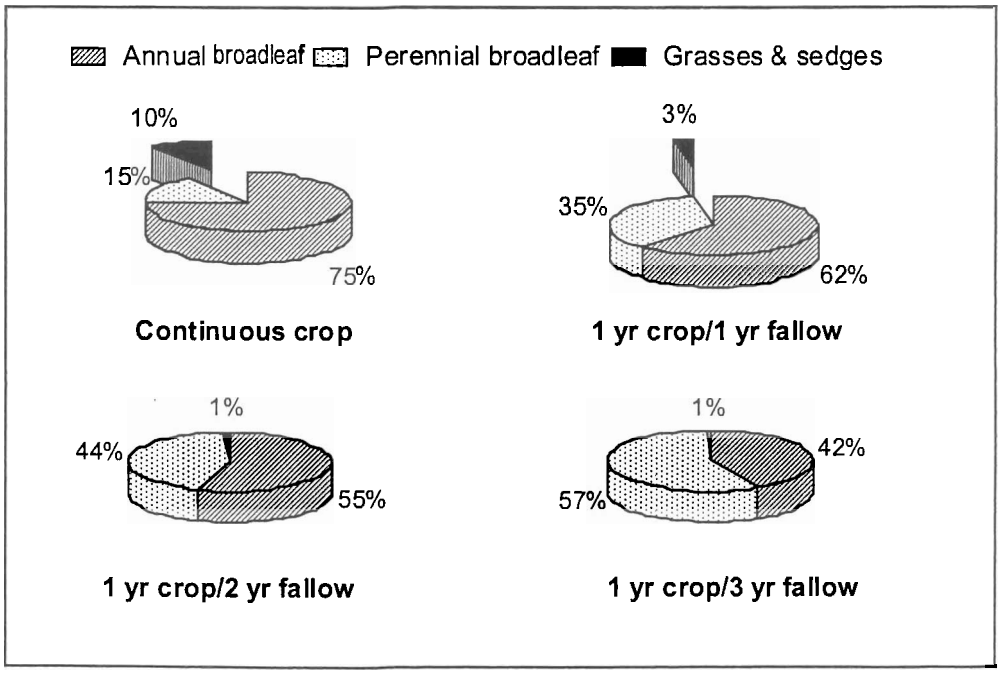

Figure 3. Effect of land-use intensity on seed population of broadleaf weeds, grasses and sedges.

Their reports showed that weed communities under continuous cropping differed in composition to those in rotations that included fallow.

one, two and three years of fallow; B1, B2, B3 = natural bush plots cropped after one, two and three years of fallow.

These treatments were associated with *Ageratum conyzoides* L., *Pouzolzia guineensis* Benth., *Spermacoce ocymoides* Burm f. (annual broadleaf weeds), *Digitaria horizontalis* Willd. and *Cynodon dactylon* [L.] Pers. (grasses). The planted fallow plots cropped after 2 and 3 years of fallow were similar in species composition and differed from natural bush plots fallowed for the same length of time before cultivation. Natural bush fallow plots were dominated by *Chromolaena odorata* L. (perennial broadleaf weed), *Boerhavia erecta* L., *Amaranthus viridis* L. (annual broadleaf weeds), and *Eleusine indica* Gaertn. (grass weed). The dominant weeds in the planted fallow plots were *Talinum triangulare* (perennial broadleaf), *Synedrella nodiflora* Gaertn., *Spigelia anthelmia* L., *Celosia trigyna* L. (annual broadleaf), and *Mariscus alternifolius* Vahl. (sedge).

Our study shows that the use of *Pueraria* as live mulch, especially when it is combined with 2-3 years of fallow, lowered the seedbank and was superior to *L. leucocephala* and the traditional bush fallow system in depleting the weed seedbank and in maintaining better weed management.

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