Tissue-cultured CREEPING BLUESTEM for restoration of phosphate-mined lands

Rob Kalmbacher, Jeff Norcini, Taraknath Chakravarty, Ike Ezenwa, and Frank Martin

ABSTRACT

Tissue-cultured creeping bluestem (*Schizachyrium scoparium* var. *stoloniferum* (Nash) J. Wipff [Poaceae]), grown in pots containing sand tailings or overburden from a Florida phosphate mine, had shoot (4.6 g/plant [0.16 oz]), root and rhizome mass (5.2 g/plant [0.18 oz]), and vegetative tiller density (4.2 g /plant [0.15 oz]) similar to that obtained from plants started from rhizomes. Seeds of creeping bluestem germinated on mine soil but failed to grow. In the field, tissue-cultured bluestem survival averaged 88% over January, July, and October planting dates on sand tailings compared with 67% on overburden. One year after October planting, total tiller density and aboveground plant dry mass was 63.5 and 117 g/plant (2.24 and 4.13 oz), respectively, on sand tailings compared with 50 and 107 g/plant (1.76 and 3.77 oz), respectively, on overburden. Except in the October planting, soil did not affect bluestem plant diameter (mean 24.2 cm [9.5 in]). Because creeping bluestem produces few seeds, tissue culture can provide plants that establish and grow well on land after phosphate mining. Weed control will be essential to success.

KEY WORDS

mine reclamation, *Schizachyrium scoparium* var. *stoloniferum*, overburden, sand tailings

NOMENCLATURE

ITIS (2002)

Phosphate mine dragline at work. Photo by Robert S Kalmbacher

The US produced 30% of the world's phosphate in 1990, and 75% of that was mined in the Bone Valley formation in central Florida (Mislevy and others 2000). The standard mining procedure is to remove the topsoil and 3 to 16 m (10 to 50 ft) of overburden with a dragline and expose the 2 to 16 m (7 to 50 ft) thick matrix, which consists of phosphate pebbles, sand-sized phosphate and quartz particles, and clay in about equal amounts. As mining progresses, overburden is cast into adjacent mined-out areas, which will occupy ~ 40% to 50% of the area after mining. Overburden is variable in texture as it is made up of clay lenses (kaolinite and montmorillinite), quartz sand, and apatite. The mined matrix is pumped as a slurry to a benefication plant where phosphate is removed and clay and quartz sand are returned as residual waste products (sand tailings).

About 63 000 ha (155 600 ac) of sand tailings and overburden are in Florida, with most being overburden. Reclamation involves leveling and contouring overburden and sand tailings. Aside from wetlands, most phosphate-mined areas are planted to introduced forage grasses. Planting to pines and citrus has also been practiced but to a lesser extent than pasture. Until recently, herbaceous native plants have seldom been used in reclamation of upland areas. The most successful approach is to harvest standing vegetation containing seeds from donor sites and apply the material to phosphate-mined land in late November (Bissett 1996). One problem is that many of Florida's native plants, especially grasses, do not set seeds or are not reliable seed producers, so they are not represented in the mulch. Creeping bluestem (*Schizachyrium scoparium* var. *stoloniferum* (Nash) J. Wipff [Poaceae]) is a major component of the upland flatwoods plant community, and it should be included in phosphate-mine reclamation. Its major method of reproduction, however, is by rhizomes, not seeds (Kalmbacher and others 1991), so no practical method to produce large quantities of propagules exists. Recently, protocols have been developed that allow rapid micropropagation of creeping bluestem by shoot multiplication culture generated from somatic embryos (Chakravarty and others 2001). It is possible to produce relatively large numbers of plants; these propagules, however, have not been grown and compared to sexually or asexually produced creeping bluestem on mine soils.

MATERIALS AND METHODS

Pot Study

On 23 August 2000, ~50 kg (110 lb) each of sand tailings and overburden soils (upper 15 cm [6 in]) were collected from CF Industries Inc phosphate mine in Fort Green, Florida (areas DB3 and DB6, respectively) and taken to the Range Cattle Research and Education Center (REC) at Ona, which is approximately 20 km (12 miles) south. At the Range Cattle REC, 50 kg of the A1 horizon of a native Myakka fine sand (sandy siliceous hyperthermic aeric Alaquods) was collected from a soil characterization site (Robins and others 1984). The 3 soils were treated with methyl bromide to eliminate weed seeds and any possible mycorrhizal contributions from the native Myakka soil. On 27 September, 15 pots (15.2-cm diameter x 16.5-cm tall [6.1 x 6.4 in]) were filled with each soil. A sample of each soil was saved for texture analyses (Soil Characterization Laboratory, University of Florida). Soil was analyzed for organic matter, pH, P, K, Ca, Mg, Cu, Fe, Mn, and Zn (Mehlich-I extractable) at the University of Florida Analytical Research Laboratory (Hanlon and Devore 1989).

On 10 October 2000, 30 to 40 creeping bluestem inflorescences were collected from a native area at the Range Cattle REC. A relatively large amount of material was needed to obtain sufficient caryopses, which were separated from the lemmas and paleas with a Woodward Laboratory air-seed cleaner (Aaron's Engineering, Fargo, Oklahoma). On 6 November, 7.5-cm-diameter (3-in) Petri dishes were filled with each of the 3 soils (5 replicates of each) and moistened, and 10 caryopses were placed on the soil surface in each. Dishes were covered and placed in a germinator set at 28 °C (82 °F) dark (12 h) and 20 °C (68 °F) light (12 h). After 28 d, seedlings were counted, and the soil and rooted seedlings were lifted intact from the Petri dishes and placed in 5 pots of each of the 3 soils. Pots received 6 s mist every 5 min for about 2 wk until they were established. They then received water 3 to 4 times daily from an overhead water trolley. Seedlings did not receive fertilizer.

On 24 January 2001, creeping bluestem rhizome sections (40 to 50 cm [16 to 20 in] long), each containing some roots and several internodes, were placed in containers (98 ml [6 in^3] Ropak Multi-pots, Stuewe & Sons Inc, Corvallis, Oregon) filled with Fafard (Fafard Inc, Agawam, Massachusetts) 4-P mix (30% sphagnum moss, 30% vermiculite, 30% aged pine bark, 10% perlite). This ecotype had been collected in Polk County, Florida, in 1984 and had been growing in a nursery at the Range Cattle REC (Kalmbacher and others 1991). It was noted to be a strongly rhizomatous, robust type with no observed seed production. These plants will be referred to as division plants, and during development were unfertilized and watered on the same regime as seedlings.

On 5 March, tissue-cultured creeping bluestem plants (Chakravarty and others 2001) were received from the North Florida REC. The explant used for tissue-cultured plants was the same as that used for division plants. Tissue-cultured and division plants were transplanted in the 15-cm (6-in) pots (1/pot), and seedlings were thinned to a single plant in each pot.

The 45 pots (5 replicates of the 3 soils each with the 3 propagule types [seed, division, tissue-culture] of creeping bluestem) were randomized on a greenhouse bench. Pots received overhead irrigation until 27 July when inflorescences were tall enough to interfere with the trolley. After 27 July, pots were placed in saucers and were watered from below. They were not fertilized.

On 23 October (232 d after transplanting), reproductive and vegetative tillers were counted. Plants were cut near the soil surface (2 to 3 cm [approximately 1 in]), and top growth was dried (60 °C [140 °F] for 72 h) and weighed. Dried vegetative tillers of similar size were composited over replications because of the limited amount of dry matter. Tissue was ground and analyzed for N, S, P, K, Ca, Mg, Cu, Fe, Mn, and Zn at Waters Agricultural Laboratory, Camilla, Georgia. Two soil cores (2.5 cm wide x 15 cm deep [1 x 6 in]) were taken from each pot and analyzed for pH, P, K, Ca, Mg, Cu, Fe, Mn, and Zn as described above. Soil was washed from roots and rhizomes before they were dried and weighed.

Seed germination, tiller number, and plant mass data were analyzed with a general linear model for a completely randomized design (SAS 1999). Means for main effects and interactions were separated with the pdiff option (LSD).

Field Studies at CF Mine

Sand tailings and overburden sites were selected at CF mine in Fort Green. These were the same mining areas (DB3 and DB6), but were not the exact locations where soils were collected in August 2000. Sand tailings had not been reclaimed, and plant cover was sparse with scattered drought-tolerant plants like natalgrass (*Melinis repens* (Wild.) Zizka [Poaceae]), Mexican tea (*Chenopodium ambrosioides* L. [Chenopodiaceae]), and roughhairy indigo (*Indigofera hirsuta* L. [Fabaceae]) on higher areas, and southern crabgrass (*Digitaria ciliaris* (Retz.) Koel. [Poaceae]), broomsedge (*Andropogon virginicus* L. [Poaceae]), dogfennel (*Eupatorium capillifolium*

TABLE 1

Herbicide treatment of creeping bluestem for weed control after planting on sand tailings and overburden.

		Site and month of planting					
		Sand tailings			Overburden		
Date of treatment	**Herbicide**	Jul	Oct	Jan	Jul	Oct	Jan
2001							
3 Dec	Weedmaster [a]				X		
3 Dec	Plateau [b]		X			X	
2002							
25 Jan	Plateau		X				X
25 Jan	Weedmaster			X			
9 Apr	Weedmaster	X	X	X	X	X	X
11 Jun	Plateau		X	X	X	X	X
6 Aug	Plateau	X	X	X	X		
6 Aug	Weedmaster				X	X	X
23 Sep	Remedy [c]				X	X	X

[a] 0.12 kg/l dicamba [3, 6-Dichloro-2-methyoxybenzoic acid] + 0.34 kg/l 2,4-D [2, 4-Dichloro-phenoxyacetic acid (4 L amine)] applied at 2.3 l/ha (1 qt/ac).

[b] 2-[4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]-5 methylnicotinic acid applied at 0.3 or 0.4 l/ha (4 to 6 oz/ac).

[c] [3, 5, 6-Trichloro-2-pyridinyloxy] acetic acid applied at 2.3 l/ha (1 qt/ac).

(Lam.) Small [Compositae]), shyleaf (*Aeschynomene americana* L. [Fabaceae]), and coffeebean (*Sesbania emerus* (Aubl.) Urban [Fabaceae]) where it graded into a lower adjacent area. The overburden site had been reclaimed over a 2-y period by direct planting of container native species or by seeding from a donor site (Kiefer 2002). Aside from a few scattered native plants, most of the dense ground cover was from roughhairy indigo, bahiagrass (*Paspalum notatum* Flügge [Poaceae]), guineagrass (*Urochloa maxima* (Jacq.) R. Webster [Poaceae]), crabgrass, and so on. For the present study, both sites were prepared by mowing and spraying with glyphosate (N-[phosphonomethyl] glycine) at 9.4 l/ha (4 qt/ac). Areas were resprayed with the same rate of glyphosate on the day of planting.

Within each site, tissue-cultured creeping bluestem was planted on 27 July, and 16 October 2001, and 24 January 2002 (Figure 1). Both sites had approximately 3% slope which provided a moisture gradient. At each date, 6 rows, 50 m (164 ft) long and oriented from top to bottom of the slope with 1 m (3.2 ft) between rows, were planted. Plants were spaced 0.5, 1.0, or 1.5 m [1.6, 3.2, 4.8 ft] apart within the rows (2 rows for each spacing). The intent of this variable spacing was to provide insight on planting density through evaluation of long-term plant coverage, which is beyond the scope of this report. The July 2001 planting was fertilized on 10 August 2001 with the equivalent of 2.5 g N/plant applied as an ammonium nitrate solution to individual plants. All plantings received 2.5 g N/plant on 11 June and 6 August 2002. Weed control after planting was practiced on both sites (Table 1).

Ten soil cores were taken (2.5 cm wide x 15 cm deep [1 x 6 in]) and composited from the upper and lower portions of each site on the day each was planted. This procedure was repeated on each site on 28 October 2002. Soil was analyzed for pH, P, K, Ca, Mg, Cu, Fe, Mn, and Zn (Mehlich-I extractable) as described above. Soil organic matter contents were determined on samples taken at planting.

Plant survival for each date was determined at 100 d after planting. On 28 October 2002, plant diameter, as indicated by extent of rhizome (tiller) extension, and survival were measured on all plants. In the October planting, selected because it represented a single year's growth, vegetative and reproductive tillers (those with inflorescences) were counted at each of the 1-m spaced plants. There, every third plant was cut near the soil surface, dried (60 °C [140 °F], 120 h), and weighed.

On 25 October 2001, 5 samples of vegetative tillers of uniform size in the July planting at CF and at the Range Cattle REC on the native Myakka fine sand site were cut at the soil surface. On 28 October 2002, vegetative tillers of uniform size from the 1-m spaced plants sampled were cut at the soil surface to form 5 samples from each of sand tailings and overburden sites. Tissue samples for both years were analyzed for N, S, P, K, Ca, Mg, Cu, Fe, Mn, and Zn as described above.

On 24 April 2002, penetrometer resistance was determined at 5-cm (2-in) increments into the soil starting at the soil surface down to a 45 cm (18 in) depth at each of the 1-m spaced plants (n = 100) in the October planting on both sites. Penetrometer

TABLE 2

Particle size distribution in soils at the start (March) of the pot study.

Soil separates	Soil: Myakka fine sand	Sand tailings	Overburden
	%		
Sand			
Very coarse (1 to 2 mm)	0	1.6	1.4
Coarse (1 to 0.5 mm)	1.6	12.6	9.6
Medium (0.5 to 0.25 mm)	21.6	51.2	42.2
Fine (0.25 to 0.1 mm)	53.2	31.4	38.4
Very fine (0.1 to 0.005 mm)	5.6	0.8	2.8
Total	92.0	97.6	94.4
Silt	6.9	1.3	1.4
Clay	1.1	1.1	4.2

data were analyzed with the MIXED procedure in a model that included site, depth, and site x depth interaction (SAS 1999).

Plant survival from October 2002 was analyzed with the CATMOD procedure (SAS 1999), which models categorical data by fitting linear models to functions of response frequencies. The model for plant survival included site, date of planting, and their interaction. Plant diameter and tiller number was analyzed with the MIXED procedure in a model which included site, date of planting, and their interaction (SAS 1999). In analyses for plant survival, tiller number, and penetrometer resistance, we blocked data by grouping observations based on position from the top to bottom of the gradient.

RESULTS AND DISCUSSION

Pot Study

Soil

All 3 soils contained > 90% sand, but size of sand particles differed (Table 2). Myakka contained the most fine sand, and sand tailings contained the most medium, coarse, and very coarse sand. Myakka had more silt than sand tailings and overburden. Overburden contained more clay than Myakka or sand tailings.

Sand tailings and overburden were very high in P, Ca, and pH compared with Myakka soil (Table 3). Elemental concentrations, pH, organic matter, and soil separates from soils used in this study compare well to those reported for sand tailings in a phosphate-mine reclamation study (Mislevy and Blue 1981; 1985). Our overburden, however, was very high in P, Ca, and pH compared to the 390 and 930 ppm for P and Ca, respectively, and pH 6.0 reported by Mislevy and Blue (1985) for their overburden.

Soil did not affect 28-d germination and emergence of creeping bluestem (P = 0.57). Germination on Myakka was 49%, 54% on sand tailings, and 53% on overburden. These percentages were similar to creeping bluestem germination on blotter paper in petri dishes (Kalmbacher and others 1991). When moisture and temperature are not limiting, sand tailings and overburden are a favorable environment for germination.

Plant propagules

On 5 March, which was 119 d after transplanting seedlings from Petri dishes to pots, seedlings in Myakka soil were 11 cm (4 in) tall compared with 1- and 2-cm tall (0.4- and 0.8-in) seedlings in sand tailings and overburden, respectively. Both division and tissue-cultured plants were 15 cm (6 in) tall on this date. Seedlings and division plants consisted of a single tiller, while tissue-cultured plants averaged 1.9 tillers/plant. Seedlings in sand tailings and overburden were chlorotic, while all others were green.

Seedling plants might not have been able to develop a secondary root system in sand tailings or overburden. These soils were extremely high in P and Ca compared with Myakka, and the pH was high as well (Table 3), which may have reduced availability of Cu and perhaps Zn. Both tissue culture and division plants had well developed root systems when they were transplanted.

On 30 April, a plant propagule x soil interaction was apparent for plant height (data not shown) primarily because of the inability of seedlings to grow on sand tailings and overburden. They remained stunted (mean = 1 cm [0.4 in] tall) and chlorotic. Plants from division on sand tailings (29 cm [11 in] tall) were taller than plants from division on Myakka (21 cm [8 in] tall), with plants from division on overburden (25 cm [10 in] tall) intermediate and not different from the previous two. Heights of plants growing on Myakka were similar (mean = 21 cm [8 in] tall). Heights of tissue-cultured plants growing on the

TABLE 3

Elemental concentrations (Mehlich-I extractable), pH, and organic matter contents of a native Myakka soil and 2 soils from a reclaimed phosphate mine and elemental concentrations in creeping bluestem vegetative tillers (composited over replications) at the end of the pot study (23 October 2001).

Item[a]	Soil					
	Myakka fine sand		Sand tailings		Overburden	
	Soil	Tissue	Soil	Tissue	Soil	Tissue
Nitrogen	–[b]	0.52	–	0.58	–	0.52
Phosphorus	10.2 c[c]	0.05	2160 b	0.06	2280 a	0.08
Potassium	14.9 a	0.48	5.7 a	0.78	11.1 a	0.77
Calcium	504 c (177)	0.17	5943 b (5900)	0.21	6244 a (6300)	0.18
Magnesium	289 b (40)	0.15	291 b (176)	0.16	476 a (320)	0.13
Sulphur	–	0.08	–	0.07	–	0.09
Copper	0.13 a	1.07	0.12 a	0.97	0.13 a	1.59
Iron	22.1 c	108	43.7 a	78.5	41.1 a	88.3
Manganese	0.18 c	20.0	5.56 a	72.8	4.80 b	64.1
Zinc	3.7	12.0	0.85	14.4	2.32	13.3
pH	6.0 b (4.2)	na[d]	8.9 a (6.9)	na	8.9 a (7.6)	na
Organic matter	2.4	na	0	na	0	na

Values for calcium, magnesium and pH in parentheses are values at the start of the study.

[a] Nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, and organic matter in percent; copper, iron, manganese, zinc in ppm.

[b] Not determined in soil.

[c] Means within a line followed by the same letter are not different ($P > 0.05$).

[d] Not applicable to tissue.

3 soils were similar (mean = 20 cm [8 in] tall) but were shorter than plants from division on sand tailings (29 cm [11 in] tall).

Tiller number

A plant propagule x soil interaction occurred for tiller number on 30 April (data not shown) primarily due to a profusion of tillers (14 tillers/plant) on tissue-cultured plants grown on Myakka. This was greater than that on overburden or sand tailings (mean = 4/plant). Tiller numbers on plants from division growing on Myakka and overburden were similar (mean = 3 tillers/plant), but they were greater than division plants on sand tailings (1 tiller/plant). It seems that division plants on sand tailings concentrated growth in height rather than to expansion through tillers.

On Myakka, tissue-cultured plants had more vegetative tillers than plants from seeds, with division plants least (Table 4). Compared with tissue-cultured plants on Myakka, tissue-cultured plants on sand tailings and overburden had fewer tillers. Plants from division grown on Myakka had the same number of tillers as those on sand tailings or overburden.

Plant mass

At the termination of the study on 23 October, we noted a plant propagule x soil interaction for shoot mass, root and rhizome mass, and vegetative tiller number (Table 4). Plants from all 3 propagule types produced more shoot mass on Myakka than on sand tailings or overburden. Seedling plants died on sand tailings and overburden, but these plants on Myakka were green and healthy. On Myakka, seedling plants had less shoot mass than plants from division with tissue-culture plants intermediate. Much of the mass came from reproductive tillers (5/plant) on plants from division grown on Myakka. The only other plants with reproductive tillers were seedling plants on Myakka, which averaged 1/plant. Plant propagules were similar for shoot mass on sand tailings and overburden.

All plant propagule types produced the most root and rhizome mass on Myakka (Table 4). Tissue-cultured plants on Myakka had the greatest root and rhizome mass compared with plants from seeds or division, which were not different. Root and rhizome mass was similar for plants from tissue culture or division on sand tailings or overburden. All plants appeared to be root-bound at the end of the study, especially on Myakka.

Elements in soil and tissue

In spite of the relatively great differences in P and Ca concentrations among the 3 soils, P and Ca in tissue from plants grown on these soils were relatively low and uniform (Table 3). Most other elemental concentrations in tissue were similar in plants

TABLE 4

Creeping bluestem shoot, root and rhizome mass, vegetative tiller density from the pot study at termination on 23 October 2001. Means for plant propagule x soil interaction.

Plant propagule type	Soil: Myakka fine sand	Sand tailings	Overburden
	Shoot mass (g/pot)		
Seedling	21 c [a] A [b]	0 b B	0 b B
Division	42 a A	5 a B	4 a B
Tissue culture	30 b A	4 a B	3 a B
	Root and rhizome mass (g/pot)		
Seedling	24 b A	0 b B	0 b B
Division	23 b A	5 a B	5 a B
Tissue culture	36 a A	3 a B	3 a B
	Vegetative tillers (no./pot)		
Seedling	15 b A	0 b B	0 b B
Division	3 c A	3 a A	2 a A
Tissue culture	21 a A	4 a B	4 b B

[a] For a given response, means within a column followed by the same lower case letter are not different. $P > 0.05$.

[b] Means within a row followed by the same upper case letter are not different. $P > 0.05$.

from the 3 soils. Iron seemed to be high in tissue from Myakka compared with the other soils, while Mn in tissue grown on Myakka was comparatively low. Concentrations of elements in creeping bluestem grown in Myakka soil in pots were very similar to respective concentrations of vegetative tillers of creeping bluestem grown from March to October on a native Immokalee fine sand (arenic Alaqouds) (Kalmbacher and Martin 1981). In that study, Fe (32 ppm) and Mn (40 ppm) were a little closer to the values for these elements in plants grown on mine soils.

Calcium and Mg concentrations and pH increased in Myakka soil from the start to end of the study because our irrigation water was high in Ca and Mg carbonates (Table 3). There was little effect on the soils from the mine because these values were high at the start. Creeping bluestem did not seem to respond to differences in soil P, Ca, Mg, and pH.

Field Studies at CF Industries Mine

Survival

Survival was very good in the July 2001 planting on both sites at 100 d after planting (Figure 2; Table 5). During this post-planting period, 692 mm (27.2 in) of rain was recorded (CF Industries, south pasture location), which is typical for the rainy season in central Florida. By 28 October 2002, survival remained high on sand tailings but had declined to 55% on overburden. On the October 2001 planting , survival in the first 100 d was 81% on sand tailings and 58% on overburden. During this period, 115 mm (4.5 in) of rain was recorded, which reflects the fact that October to December are some of the driest months in central Florida. During this period, soils usually carry a reserve of moisture from summer rain. At 1 y after planting, survival on both sites was relatively unchanged. On the January 2002 planting, plant survival in the first 100 d was 95% both on sand tailings and overburden. During this post-planting period, 293 mm (11.6 in) of rain was received. During the cooler months, evapotranspiration is less than during summer or fall.

It appears that survival during the first 100 d was related to rainfall, with the poorest survival from the drier October planting. We purposely avoided the most difficult period for any plant growing on mined soil, which would be April to early June. This is historically the most severe drought period in central Florida (Kalmbacher and Linda 1994), and a period when cattlemen suspend pasture establishment. Our research showed that creeping bluestem planted from July to January survived the critical dry spring period very well.

On the mid to lower end of the sand tailings, tunneling by mole cricket (*Scapteriscus vicinus* Scudder [Orthoptera: Gryllotalpidae]) was very apparent in the spring 2002, but there were no signs, such as wilting, that crickets were eating creeping bluestem. Missing plants were scattered on sand tailings and appeared to be random. On overburden, missing plants were clustered. We found up to 14 missing plants in a row, and adjacent rows often contained additional spaces once occupied by plants. This suggested clay pockets or other soilborne problems might occur on overburden.

Weed control was a great challenge during establishment of creeping bluestem on these mined sites. Phosphate-mined land is notorious for weedy and invasive plants (Craig and Smith 1980). Weeds were a much greater problem on overburden than sand tailings, and this was a contributing factor to the difference

Figure 1. Tissue-cultured creeping bluestem plants prior to planting.

in creeping bluestem survival. On overburden, plantings were sprayed 5 to 6 times compared with 2 to 4 times on sand tailings (see Table 1). A large soil seed bank had been allowed to build up after overburden and sand tailings were deposited and leveled. Legumes (roughhairy indigo, shyleaf, coffeebean, and showy crotalaria (*Crotalaria spectabilis* Roth [Fabaceae]) (in this order) seem to be the major broadleaf problems because of their abundance and resistance to herbicide treatments. No single herbicide was effective on all weed problems. Plateau was relatively effective on most broadleaf species and grasses (except *Brachiaria* sp. (Trin.) Griseb. [Poaceae]), but Weedmaster was needed for pusley (*Richardia* spp. L. [Rubiaceae]) and most legumes. Only Remedy was effective on roughhairy indigo.

Penetrometer resistance

A site x depth interaction ($P < 0.0001$) existed for penetrometer resistance (Figure 3). On sand tailings, resistance over depth was linear, whereas it was quadratic on overburden. The low values for R^2 reflect the extreme variability especially for overburden where the sampling variance was 6X as large as the error variance compared with 2.5X on sand tailings. Resistance on overburden reached near maximum 45 kg/cm^2 (638 lb/in^2) at 20 cm (8 in) depth, then resistence leveled-off. When resistance measurements were made (24 April), soil was very dry throughout the profile on sand tailings (not quantified), whereas on overburden some moisture was associated with clay lenses. We feel that the difference in resistance between sites was due to moisture. When survival measurements were made at 100-d post-planting, we suspected that hard clay areas on overburden would be associated with plant loss. Resistance at the site of a missing plant on overburden was not associated with plant presence or absence.

Plant diameter

Month of planting and site interacted to affect diameter (Table 5). On 28 October 2002, plant diameter was similar between sites for the January 2002 and July 2001 planting dates, but overburden had smaller plants compared with sand tailings at the October 2001 planting. Within the overburden site, plants from July 2001 were larger than plants from the October planting, and plants from the January planting were smaller than the former. Within sand tailings, plant diameters from the July and October plantings were similar, but these were larger than plants from the January planting because of differences in plant age.

The mean plant diameter in the July and October plantings (27.7 cm [11 in]) was greater than creeping bluestem plant diameter at 1 y after planting (approximately 20 cm [8 in]) in an earlier study (Kalmbacher and others 1986). The smaller diameters in the January planting date were probably not due to age alone, otherwise diameter in the July planting should have exceeded that of October with little difference between October and January due to

TABLE 5

Creeping bluestem survival at 2 dates after planting on sand tailings and overburden and plant diameter least square means on 28 October 2002 for the month of planting x site interaction.

Item	Month of planting					
	July		October		January	
	sandtailings	overburden	sandtailings	overburden	sandtailings	overburden
Plant survival (%)						
1st 100 d	100	78	81	58	95	95
28 October 2002	98	55	82	58	83	88
Plant diameter (cm)	30.8 A [a]	27.9 a ns [b]	29.1 A	22.9 b **	16.9 B	18.0 c ns

[a] For comparing dates within sites. Sand tailings means followed by the same capital letter are not different over month of planting and overburden means followed by the same lower case letter are not different ($P > 0.05$).
[b] For comparing sites within dates. NS and ** indicate that sand tailings and overburden are not different from each other ($P > 0.05$) or different ($P < 0.01$) within a month of planting, respectively.

Photo by Robert S Kalmbacher

Figure 2. Tissue-cultured creeping bluestem plants on sand tailings 6 mo after outplanting.

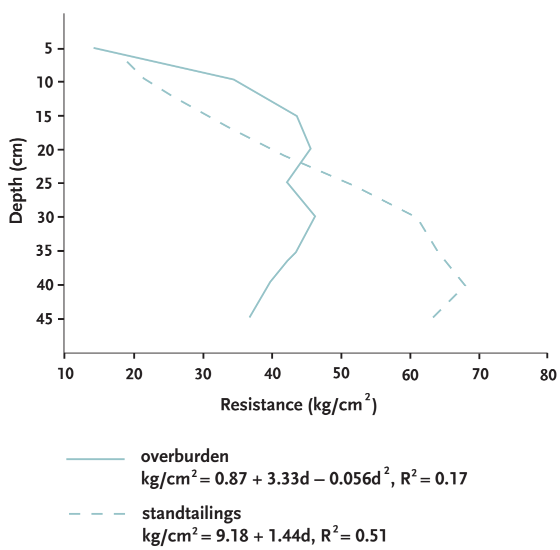

Figure 3. Penetrometer resistance over 0- to 45-cm depths on sand tailings and overburden at CF Industries phosphate mine, April 2002 (d = depth in cm). These are means over 100 observations at each site.

cooler temperatures. The January plants at the time of planting were noticeably smaller (no data) than those from previous planting dates. We attribute this to the fact that January plants were cultured and developed under short days and cooler temperatures (September to December) than earlier plants.

Tiller number and plant mass

Plants on sand tailings had more reproductive and total tillers compared with plants on overburden (Table 6). Mean tiller density (56.8) of these 1-y-old plants was similar to ungrazed creeping bluestem (63 tillers/plant) at 2 y after planting (Kalmbacher and others 1986).

Aboveground plant mass was similar between sites (Table 6). Aboveground biomass of these 1-y-old plants with abundant reproductive growth averaged 1120 kg/ha (1000 lb/ac). Annual growth of unburned creeping bluestem on a native Spodosol soil was 1350 kg dry matter/ha (1200 lb/ac) (Kalmbacher and others 1985). In another study, annual yield of unfertilized creeping bluestem was 1400 kg dry matter/ha (1250 lb/ac) (Kalmbacher and others 1993). These latter references for yields of creeping bluestem were yields of vegetative grass. Undisturbed in the native condition, creeping bluestem will flower very little unless physically disturbed, burned, or fertilized. Yields from the present study contained a great portion of reproductive growth which inflates yield.

Elements in soil and tissue

Sand tailings and overburden from the CF field study were typical in that they were relatively high in P, Ca, and Mg (Table 7). Soils from the CF field study were lower (no statistical comparison made) in P, Ca, Mg, and pH compared with these responses from respective soils used in the pot study (Tables 3 and 7). Values for elements in sand tailings at CF were similar to those obtained by Mislevy and Blue (1981; 1985), but CF overburden was higher in P and Ca compared with that of

TABLE 6

Creeping bluestem tiller number and aboveground dry matter mass on the October planting at 1 y after planting on sand tailings and overburden.

Item	Site: Sand tailings	Site: Overburden	*P* [b]
Tillers (number/plant)			
Reproductive	17	11	0.01
Total	64	50	0.02
Plant mass (g/plant)[a]	117	107	0.55

[a] Plants on 1-m centers in rows 1-m apart. kg/ha = grams/plant x 10 (lb/ac = kg/ha/1.121).

[b] Probability of a difference between sites.

TABLE 7

Elemental concentrations (Mehlich-I extractable), pH, and organic matter contents of soils at a reclaimed CF Industries phosphate mine and elemental concentrations in creeping bluestem vegetative tillers grown on a native Myakka fine sand at the Range Cattle REC. Mean of October 2001 and 2002.

Item [a]	Soil				
	Myakka fine sand [b]	Sand tailings		Overburden	
	Tissue	Soil	Tissue	Soil	Tissue
Nitrogen	0.84	–	0.93	–	1.00
Phosphorus	0.16	1871	0.44	677	0.25
Potassium	0.91	14.0	0.76	27.0	1.10
Calcium	0.17	4436	0.32	1585	0.28
Magnesium	0.15	148	0.19	71	0.14
Sulphur	0.15	–	0.09	–	0.10
Copper	2.2	0.06	2.0	0.81	4.6
Iron	67	53.1	72.4	37.5	59.3
Manganese	135	3.43	24.3	2.8	72.3
Zinc	10.6	1.37	10.9	2.25	14.5
pH	na [c]	6.7	na	5.4	na
Organic matter	na	0.48	na	0.84	na

[a] Nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, and organic matter in percent. Copper, iron, manganese, and zinc in ppm.

[b] Tissue was sampled from Myakka soil in 2001 only. Soil was not sampled.

[c] Not applicable.

Mislevy and Blue (1985). Nitrogen and P in tissue from creeping bluestem grown at CF were high compared with concentrations in tissue from plants grown on Myakka soil (Table 7) or the pot study (see Table 3). Nitrogen fertilizer was applied at CF because, with virtually no organic matter, it was recognized as a limiting element for growth.

Although mine soils are relatively fertile, which is good agriculturally, growth of creeping bluestem, which has evolved on very infertile soils, may be less on mine soil compared with native soil. This was substantiated in the pot study (see Table 4). Creeping bluestem, however, is adapted to a wide range of soil pH and concentrations of nutrient elements, and growth on these mine soils was very satisfactory.

CONCLUSIONS

Although creeping bluestem seeds germinate as well on overburden and sand tailing as on a native Myakka soil, seedlings do not develop and grow on mined soils. Mined soils are extremely high in pH and P, Ca, and Mg concentrations compared with native soil. A native Myakka soil was a better growing medium for all plant propagules compared with sand tailings and overburden. Tissue-culture plants grown in pots on Myakka soil had higher shoot, root and rhizome mass, and vegetative tiller number compared with plants from seeds or division.

In field trials on mined land, tissue-cultured plants established well when planted in January, July, or October. Survival was less on overburden than on sand tailings when the planting occurred in October, however, which was due to typical low rainfall at this time. Plant diameter as measured by rhizome expansion was good and averaged 24.2 cm over the 3 planting dates. On our sites a large soil seed bank resulted in much weed competition. We feel that weed control by preventing the build-up of weed seeds after mining, plus a diligent herbicidal weed control program after planting creeping bluestem, will be important to success.

Because it is a major component of central and south Florida native plant communities, creeping bluestem needs to be included in restoration of mined lands. Because the grass produces few seeds, tissue culture offers a practical way to produce plants that can establish and grow well on phosphate-mined land.

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AUTHOR INFORMATION

Rob Kalmbacher
rskalmbacher@mail.ifas.ufl.edu

Ike Ezenwa

Range Cattle Research and Education Center
University of Florida
3401 Experiment Station
Ona, FL 33865-9706

Jeff Norcini
North Florida Research and Education Center
University of Florida
155 Research Road
Quincy, FL 32351-5684

Taraknath Chakravarty
Chemistry and Biology/27
Institute of Paper Science and Technology
500 10th Street NW
Atlanta, GA 30318

Frank Martin
Statistics Department
University of Florida
PO Box 110339
Gainesville, FL 32611-3807