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ANNUAL PROGRESS REPORT

DEVELOPMENT OF STRESS TOLERANT SEASHORE PASPALUM FOR GOLF COURSE USAGE

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1994 Funding

USGA-Greens Research Section	$20,486
Georgia Seed Development Comm.	8,500
Total	$28,986

SUMMARY - 1994 ANNUAL PROGRESS REPORT

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A 274-ecotype collection of Paspalum vaginatum Swartz has been assembled. During 1994, collection trips were made to Hawaii (34 ecotypes), Sea Island, GA (133 ecotypes), Jekyll Island, GA (2 ecotypes), Fort Pulaski, GA near Savannah (30 ecotypes), and Tybee Island, GA (6 ecotypes). Four accessions from Brazil and two from Israel cleared quarantine. The oldest known native seashore paspalums located in the U.S., thus far, are those from Sea Island, GA. The golf course on the island was initially built in 1925 and the grass was already established at that time. Possible collection sites for 1995 may include coastal regions in Florida, Alabama, Mississippi, Louisiana, Texas, and southern California.

By 1 October 1994, over 3000 plots of seashore paspalum have been planted in Georgia, with about 2800 plots located in the Griffin area and 350 plots at Blairsville in North Georgia. Over 2200 tissue-culture-regenerated paspalums from six diverse ecotypes were planted in the field. We have collected data on a number of the somaclonal variants and will leave them exposed to winter cold temperatures in order to assess and hopefully select improved winterhardy genotypes.

The winterhardiness (field survival) of seashore paspalum is apparently much higher that is published in the literature, at least for some ecotypes. Adalayd supposedly is killed at 17°F, but I had Adalayd derivatives (Excalibre, Adalayd, Fidalayel), SIPV-2 (from Sea Island), and three accessions from Argentina (PI509018-1, PI509020, PI509022) to survive -3°F at Blairsville during 1993-1994. In general, the finer-textured turf types survived better than the coarse-textured types at both Griffin (+3°F) and Blairsville. An additional 124 ecotypes are being evaluated at Blairsville (1500 feet elevation) during 1994-1995. Additional winterhardiness evaluation and improvement has been initiated with calli and tissue-culture-regenerated plants using a cold chamber and modified bermudagrass (Taliaferro, Oklahoma State) cold shock-recovery protocol.

Eighteen paspalum ecotypes are being evaluated on a USGA-specification green. The grass was mowed at ½-inch during the 1994 establishment (from sprigs) year and will be maintained at 5/32-inch with a greens mower during 1995. Additional plots have been established with B. J. Johnson for herbicide management studies to begin during 1995 and with Bob Carrow for fairway moisture utilization studies.

Several genetic analyses techniques have been employed on seashore paspalum, including flow cytometry, random amplified polymorphic DNAs (RAPDs), restriction fragment length polymorphisms (RFLPs), and microsatellites. The techniques will be useful in assessing diversity/relatedness within the collection, in definitively fingerprinting ecotypes, and in genome mapping. Seashore paspalum is a sexual diploid (2N=20), but sexual incompatibility reduces viable seed production to 5% or less with most ecotypes. Some ecotypes have photoperiod or cool temperature ($\leq$60°F) requirements to initiate flowering. Several breeding techniques have been employed to enhance the production of viable seed.

PROJECT: Development of stress tolerant seashore paspalum for golf course usage

Principal investigator:	**R. R. Duncan**	**1994 USGA Funding**	**$20,486**
	University of Georgia	**1994 Georgia Seed Develp. Cmsn.**	**8,500**
	Griffin, GA	**Total**	**$28,986**

Objective 1. Establish an extensive collection of genetic material

I have a total of 274 Paspalum vaginatum, 1 P. hieronymii ('Lalo' paspalum, 20 chromosomes), and 20 P. distichum (20, 30, 40, and 60 chromosomes) ecotypes in the collection (Table 1). Collection trips during 1994 provided 34 ecotypes from Hawaii; 133 from Sea Island, GA; 2 from Jekyll Island, GA; 30 from Fort Pulaski, GA, and; 6 from Tybee Island, GA. Four accessions from Brazil (PI 29192, 29193, 29194, and 29195) and 2 from Israel (PI 28959, 28960) cleared quarantine at Glenn Dale, MD and were planted in the field for the first time this year in Georgia. One additional accession from Israel that was collected from an oasis in the desert was submitted for quarantine growout. The breakdown by origin for the current P. vaginatum collection is also included in Table 1. These include 15 ecotypes from Alden Pines, 15 from D. Kopec (Hawaii, Caribbean, Arizona ecotypes), 10 from Australia (via Adalayd derivatives collected in California, Florida, or Georgia), 13 from Argentina, 4 from Africa, 6 from miscellaneous sites in Florida or Texas (mainly very coarse indigenous types), and two additional species: P. hieronymii is a diploid line with the potential for supplying wear tolerance genes to P. vaginatum. P. distichum involves diploid, triploid, tetraploid, and hexaploid (with possible apomixis) lines as a possible source for cold tolerance genes.

Possible collection areas during 1995 may include coastal sites in Florida, Alabama, Mississippi, Louisiana, Texas, and southern California. Future possible collection trips will need to be made to the Caribbean islands, Brazil and Argentina, Australia, and the Pacific rim islands/coastal areas.

As a result of the collection trips thus far, a common set of immediate needs has emerged concerning seashore paspalum.

1) Individualized management protocol tailored specifically for paspalum usage on greens, tees, fairways, roughs, home and residential lawns.

2) Judicious herbicide pre- and post-emergence application options. Only legally labelled herbicide for paspalum right now is Ronstar. Trimec can be used under it's broad label.

Center(s) of Origin

I have found documentation that Australia introduced Paspalum vaginatum Swartz from South Africa during 1935. Apparently, (and I am still digging through obscure forage/herbage publications) seashore paspalums are native to southern Africa and the Americas between 35°N and 35°S latitudes. Future genetic analyses may help to definitively pinpoint the origin. I have found indications that Sea Island, GA may have been the first (on or before 1925) continental U.S. location to introduce the fine-textured paspalums, although native (indigenous) coarse types have been in the states much longer. Interestingly, the native types in Hawaii were the most predominant on the windward side of each island where salt spray and high tides are common occurrences.

Table 1. Origin or collection site for seashore paspalum in the Georgia collection

Site	Number of ecotypes	Date/Collector
Sea Island, GA	133	May 1994 Duncan
Jekyll Island, GA	2	May 1994 Duncan
Fort Pulaski, GA	30	June 1994 Duncan
Tybee Island, GA	6	June 1994 Duncan
Hawaii	34	January 1994 Duncan
Alden Pines, Pine Island, FL (near Ft. Myers)	15	July 1993 Duncan
Hawaii, Arizona, Caribbean	15	____ Kopec
Israel	2	____ Gad Ron
Brazil	4	____ Vargas (Cenargen-Brasilia)
Australia (via Florida and California)	10	____ various people
Argentina	13	Burson, PGRC Unit
Africa	4	PGRC Unit
Miscellaneous	6	1993,1994 Duncan
P. hieronymii (Lalo)	1	Plant Materials Center, Molokai, Hawaii
P. distichum	20	PGRC Unit
Total	295	

PGRC = Plant Genetic Resources Conservation Unit, Griffin, GA

Seashore paspalum - Collection Trip 16-29 January 1994

Hawaii
R. R. Duncan

General

Hawaii has a 16-17-year history with S.P. as a grass for golf course, resort, and home lawn turf situations. It was smuggled into the islands about 1976 and planted on the salt lake beds of the Hawaiian International Country Club. The first planting site was a fairway next to a salt pond emanating from the ocean. The grass predominated and began spreading from that initial site. It was subsequently transplanted to other fairways and roughs as the bermuda died from salt stress. The source for the initial sprigs is thought to be Sea Island, Georgia. Over the past 3-5 years, the grass has seen a resurgence onto golf course and resort usage, especially on ocean-side courses and several resorts where brackish water must be used for irrigation, and where high-tides and salt spray predominate.

Sites where S.P. is being grown or found naturally

1) Oahu

Ewa Beach International Country Club - all S.P. except for bermuda greens. Well managed.
West Lock Golf Course - encroachment onto greens, in fairways
Honolulu International Country Club - oldest S.P. on the islands
Fairways, especially salt-affected lake-bed areas. This original source of S.P. came from Sea Island, Georgia.

Other sites
Kailua beach/Lanakai beach on windward side
Wild, indigenous type predominates right on the beach

Suppliers of S. Paspalum on Oahu
Quality turf - Waimanalo
Southern turf nurseries - Kahuku
Hawaiian Sugar Plantation Association

2) Molokai

Supplier
Plant Materials Center - near airport (not turf types)

Collections made near Waialua, Moku, Pukoo, Kamalo
- mainly wild types

3) Lanai

The Challenge at Manele Golf Club and Resort - contaminated on tees
Found on Manele Bay sod farm near the airport
Manele Bay beach park - indigenous and planted types
Most extensive indigenous source of coarse type found was on the windward side of the island (facing Maui).

4) Maui

Original planting in Kihei beach area

Other sites with S.P.

Maui Setset condos - excellent management
Grand Hyatt Wailea Resort - best managed S.P., excellent

Sugar Cove Resort - Struckleville	Four Seasons Resort
Several condos near Lahana	Kea Lani Hotel
Marriot	Stoffer Wailea Beach Resort
Kana Poly Beach Hotel	Wailea Golf Course

Supplier - James Tavares, Kahului

5) Kauai

Prince Course at Princeville - encroachment onto greens,tees
Kiahuna Golf Club - Koloa (fairways)
Lawai Beach resort (condos) - Lawai valley near Poipu, entire frontal area facing street, and ocean, around pool
Kiahuma Village Resorts

6) Hawaii (Big Island)

Ritz Carlton - Mauna Lani (Kona side)
Mauna Lani Bay Resort and Golf Course - entrance way, hole nearest ocean

Supplier

Kirk T. Eubank - E. Scape, Kapaau
Hawaiian Turf - Kapaau (in bad shape)

Countries Having S.P. - Pacific Rim

Malaysia	Indonesia
Thailand	Hinan Island - South China
Okinawa	Guam

Problem Areas

1) Pre-emergence/post-emergent herbicide management program for greens, tees, fairways, roughs
2) Fertilizer management package for greens, tees, fairways

Current limitations

1) Insufficient wear/traffic tolerance for tees - divots do not recover as fast as bermuda. May have to rotate tee-off areas similar to how the holes are moved on greens.

2) Too-aggressive growth on greens (mowed in morning - too tall in afternoon). Positive attribute on tees, fairways and roughs. May need to reduce fertilizer/water on greens.

3) Encroachment onto bermuda greens. Must be able to control S.P. Cut back on fertilizer.

Objective 2. Improve and assess the adaptability of seashore paspalum

As of 1 October, a total of 3140 plots of seashore paspalum had been planted in field plots in Georgia (Table 2). At the Georgia Station in Griffin, 2514 plots are planted; while 276 can be found at Bledsoe farm 12 miles west of Griffin, and 350 plots at the Blairsville location (1500 feet elevation, major field screen for cold tolerance).

Table 2. Plots of seashore paspalum in the field as of 1 October 1994 - R. R. Duncan breeding program

Blairsville[†]		Georgia Station in Griffin		Bledsoe farm (Pike County)	
Primary -	280	Primary[‡] -	267	Primary[‡] -	209
Tissue culture[‡‡] -	70	Checks in other tests -	8	Checks in other tests -	61
Total	350	Polycross blocks -	49	Polycross blocks -	6
		P. distichum[‡] -	20	Total	276
		TCR[§] -	2148		
		Golf Green -	18		
		Overseeded[++] -	4		
		Total	2514		

Grand total = 3140

[†]small plots = 2'x2' centers, North Georgia, 1500 feet elevation

[‡]Mainly 10'x10' mother nursery plots

[§]3'x3' plots from five different ecotypes = tissue culture regenerants

[++]various sizes from 30'x50' to 10'x10'

[‡‡]only fine-textured ecotypes

Tissue culture regeneration

A Ph.D. student (Cesar Cardona) has developed and streamlined the regeneration protocol for seashore paspalum. He worked initially with 6 diverse ecotypes (HI-1 from Hawaii, PI 509021 from

Argentina, K3 - a D. Kopec selection from Hawaii, PI 299042 - a coarse type from Zimbabwe, Mauna Key from Hawaii, and Adalayd - an intermediate type originally from Australia, Georgia selection). Three additional ecotypes have recently been subjected to callus induction: AP-6 from Alden Pines, FL; K-7 from Hawaii; and SIPV-1 from Sea Island, GA. These latter three ecotypes were added due to their winter survivability at Blairsville during 1993-1994 or other traits. We hope to identify additional somaclones of each ecotype with better turf quality traits and improved winterhardiness during 1995 field plantings.

Field evaluation: A total of 2148 tissue-culture-regenerated plants were planted in the field at the Georgia station during 1994. An additional 70 fine-textured regenerants were planted at Blairsville. The number of plants regenerated by ecotype is presented in Table 3.

Table 3. Number of tissue-culture-regenerated plants of seashore paspalum planted in the field during 1994

Identification		Number of regenerated plants	Location
§PI 509021		378	Georgia station in Griffin
§K-3		113	
§HI-1		453	
§Mauna Key		30	
§Adalayd (normal)		126	
§Adalayd (cold treated)†		81	
PI 299042 (normal)		913	
PI 299042 (cold treated)‡		54	
	Subtotal	2148	
HI-1		60	Blairsville
Adalayd (cold treated)†		10	
	Subtotal	70	
	Grand total	2218	

†Plantlets subjected to prehardening and 2 hour exposure to -19°C in a cold chamber followed by slow thawing out

‡Callus stored at 42°F in the dark for 4 months and then plantlets regenerated

§Internode length (near crown) and spread diameter data collected during September 1994 (1181 plants total)

PI 299042, a coarse accession from Zimbabwe, was the most prolific producer of callus and regenerated plants. Mauna Key and HI-1 were among the best producers of regenerated plants; however, less callus was subcultured for these two ecotypes, as compared to PI299042. The poorest producers of calli and regenerated plants were Adalayd and PI509021. Internode length (near the crown) and spread diameter have been collected on the 1181 fine-textured regenerants (from PI 509021, K-3, HI-1, Mauna Key, and Adalayd). Additional data will be collected on tuff quality traits and mutational tendency during November 1994. The data collection did not include 967 regenerants from PI 299042 because of their extreme rapid growth, difficulty in properly measuring diameter, and their non-turf-quality characteristics. All regenerants will be left in the field at Griffin and Blairsville during the winter months 1994-1995 and cold temperature survivability (winterhardiness) will be assessed during the Spring 1994.

Tissue culture protocol

Immature spike inflorescences of 6 P. vaginatum cultivars were used as explants for the initiation of calli. After surface sterilization, the explants were cultured in Murashige and Skoog media modified with 3% sucrose, Gamborg's B5 vitamins (1 ml L^{-1}), 5% coconut water (c.w.), and a combination of 2,4-D (levels 0.0, 1.0, 2.0, 6.0 mg L^{-1}) with BA (levels 0.0, 1.0, 2.0 mg L^{-1}) in a factorial experiment with 3 replications. The cultures were kept in the dark at a room temperature of 26-28°C and inspected frequently to remove pathogen contamination or document callus formation. The callus was graded by quality and quantity into three categories; good (friable, compact and abundant), intermediate (friable, compact and moderately abundant); poor (friable clear colored, watery or compact but in very low quantity). All callus was subcultured after initiation in the above media with 2 mg L^{-1} of 2,4-D. Subculturing has continued every 30 days for more than one year.

For induction of somatic embryos, ten clumps (3-5 mm ea.) of friable compact or semi-compact calli were planted in half strength MS media described above, without c.w. and modified with a combination of NAA (levels 0.0, 0.5, 1.0, 1.5, 2.0 mg L^{-1}) and BA (levels 0.0, 1.0, 2.0) in a factorial experiment with 3 replications. Calli were cultured under a 16-hour photoperiod (Sylvania White F40 tubes 46-50 μmol s^{-1}).

The number of plantlets was recorded after 6 weeks. Selected plants were transferred to MS media without hormones or c.w., and with vitamins and 8% sucrose for root induction and development.

The production of calli was highly influenced by the plant genotype and 2,4-D level (Table 4). Treatments without 2,4-D (00, 01, 02 mg L^{-1}) did not produce calli, while treatments without BA (20, 60 mg L^{-1}) did, indicating that additional BA is not necessary for calli induction. PI 509021 and 299042 responded to all treatments with 2,4-D, while the other cultivars had an insignificant response to some treatments, indicating that calli production is a heritable trait under genetic control.

Table 4. Effect of genotype and treatment on calli production in explants (immature spikes) of P. vaginatum

Treatment	PI 509021	HI-1	Mauna Key	Adalayd	PI 299042	K3
10	9	0	0	4	13	1
11	10	0	0	2	11	0
12	9	0	0	1	3	1
20	17	0	0	0	12	5
21	9	5	0	0	11	0
22	10	0	2	1	7	0
60	5	4	3	1	10	2
61	11	4	3	4	8	2
62	3	2	0	0	11	0
Efficiency‡ (%)	22	5	3	4	24	10

†Treatment codes: first digit = 2,4-D level; second digit = BA level

‡Efficiency of calli initiation = number of explants with callus / total number of explants plated minus losses due to pathogen contamination X 100

When c.w. content was increased from 5 to 10% concentration in the media, calli initiation was significantly increased in Adalayd (from 6 to 24% efficiency) and HI-1 (from 9 to 14% efficiency). PI509021 was not significantly affected by an increased level of coconut water (22 vs. 26% efficiency). Evidently, hormones and associated cofactors in the c.w. interact with 2,4-D treatments and, depending on the genotype, affect calli initiation in seashore paspalum.

Somatic embryos were obtained from spontaneous origin when the calli were approximately 90 days old, and they were also produced by treatment with a combination of BA and NAA in half-strength MS media (Table 5). Genotype and hormone interactions were involved in the response. Cultivars Hawaii-1, Mauna Key and PI 299042 produced a good embryogenic response in the absence of added BA, while PI 509021 and Glenn Oaks Adalayd were highly dependent on BA level for embryogenic response. BA at 1.0 mg L^{-1} with either level of NAA was the best treatment across cultivars for the induction of somatic embryos.

Table 5. Number of somatic embryos produced by spontaneous origin or by treatment with BA/NAA combinations (induced embryogenesis)

	Genotype						
	PI509021	Hawaii-1	Mauna Key	G.O. Adalayd	PI299042	K3	Total
Spontaneous embryos	145	30	0	0	223	96	494
Induced embryos	1370	7515	8868	3934	6543	†	28,230

†Not included in study due to low volume of calli

In summary, the supply of an additional auxin was essential for induction of callus formation in P. vaginatum, while an additional cytokinin was not necessary. Additional cytokinin, although not essential for somatic embryo formation, greatly enhances embryogenesis when combined with an auxin at very low levels (between 0.5 and 2.0 mg L^{-1}). Genotypical differences were found between cultivars of P. vaginatum in their response to auxins and cytokinins for calli and embryo formation.

During late 1994 and early 1995, C. Cardona will attempt to streamline the protocol for callus-suspension cell - protoplast - plantlet regeneration in seashore paspalum. We have been successful in regenerating two plants from suspension cell culture. This protocol will be needed for future transformation research involving seashore paspalum.

Winterhardiness

In general, the survivability of fine-textured seashore paspalum to low winter temperatures is much better than is found in the literature. Most of the published research, which concentrated on the California derivatives of Adalayd that was originally introduced from Australia, has stated that this grass dies at 17°F. During the winter 1993-1994, the Blairsville location reached a low temperature of -3°F, with five days below 0°F and another 10 days below 10°F. At Griffin, the lows reached 6°F on station and 9°F at the Bledsoe farm. The two weeks preceding the blast of cold air from which the lowest temperatures were recorded averaged 50°F. So, the plants were not properly hardened off and most hybrid bermudas in the Atlanta area and north were killed. All coarse-type paspalums were killed at Blairsville, but some survived at Griffin.

Spring green-up for seashore paspalum lags behind all other warm-season grasses. Paspalum was about 2 weeks later at Griffin, and 3 weeks later at Blairsville than the hybrid bermudas. Among the 33 ecotypes evaluated at Blairsville, PI 509018-2, 509020, 509022, (all from Argentina), SIPV-2 (from Sea Island), and Excalibre, Adalayd, Fidalayel (all tracing back to the original introduction from Australia) survived in both reps. Single rep survivors included PI 509018-1, 509021, 509023, Temple I, and 561-79 (all from Argentina), SIPV-1 (from Sea Island), and Mauna Key, K-3, K4, and K-8 (all from Hawaii). In general, only fine-textured ecotypes survived at Blairsville.

These survivors plus 12 additional Sea Island, 6 Alden Pine, 12 Fort Pulaski, 2 Israeli, 3 Brazilian, 4 Utah (FuTurf types), 3 Kopec, and 22 HI (Hawaii) selections are being evaluated at Blairsville during the winter 1994-1995. In addition, 60 tissue-culture-regenerated HI-1 and Adalayd types are also being evaluated.

Initial cold chamber screenings of both callus and regenerated plantlets offered some hope of using these techniques to improve cold hardiness in seashore paspalum. Adalayd-regenerated seedlings were subjected to a prehardening temperature ramp-down to -19°C and slow (1°-2° increments) ramp-up to room temperature. Several plants survived and were increased in the greenhouse prior to planting 10 plots at Blairsville and 81 at Griffin. In addition, PI 299042 callus was maintained at 42°F in a dark cold chamber for 4 months, and then plants were regenerated and increased in the greenhouse. Fifty-four plants were planted in the field at Griffin. Several petri dishes with calli from different ecotypes have been subjected to -5°C cold shock and -19°C cold shock. All -19°C cold-shocked calli died, but some -5°C calli survived. Some of the -5°C survivor calli has been coerced to re-initiate growth, a portion of the expanded calli have been used to develop suspension cultures, and will be used in future protoplast experiments. Needless to say, we are moving cautiously and slowly on this.

Additionally, C. Cardona is now streamlining the protocol for cone-tainer cold hardiness evaluation of paspalum ecotypes, following the techniques initially developed by C. Taliaferro and associates for bermudagrass. Results are too preliminary to report at this time. The temperature ramp-down/ram-up cycle in 1°C increments from 5°C to -5°C is critical for survival of plants in the cone-tainers. Thermal sensors have been installed in the chamber to monitor both external and internal cone-tainer temperatures. All monitoring is via a computer tie-in. Insulated boxes to hold the cone-tainers have been developed that simulate the root placement in the soil and hopefully will closely mimic the normal freezing/thawing cycle under field conditions.

USGA GREEN EVALUATION

Eighteen paspalum ecotypes (K-3, K-6, K-7, K-8 from Hawaii and D. Kopec; Excalibre from California selection of Adalayd and Australia; PI 28959 and 28960 from Israel; AP10 and AP14 from greens at Alden Pines on Pine Island, FL; Mauna Key from Hawaii; PI 29193 from Brazil; HI 14, 25, and 39 from greens in Hawaii; SIPV-2-1 from Sea Island; Temple 2, 310-79 and PI 509018-1 from Argentina) were planted in

May on a USGA-spec green in 10' x 10' cubicles. Tifgreen was planted as a check. K-6 was planted on two cubicles. The ecotypes chosen for this initial evaluation included the intermediate-texture types like Excalibre, PI28960, and Mauna Key, as well as the super-fine, slowing growing types like AP10 and AP14. By late September, the bermudagrass had completely covered the cubicle, while HI 14 (98%), HI 25 (95%), K6 (85%), and K3 (85%) were close behind. The intermediate group as far as % spread included PI509018-1 (75), Mauna Key (65), PI29193 (65), Temple 2 (60), AP10 (55), Excalibre (50), SIPV-2-1 (50), and 310-79 (50). The slowest growing ecotype was AP14 (45% spread). The green this summer has been mowed at about ½-inch with a rotary mower, and has been on a 10-day cycle of either 7-14-21 or 14-14-14 (½-lb/1000 ft^2 per application), alternated with 34-0-0 (½-lb/1000 ft^2) since July, and irrigation 1-3 times per week depending on the rainfall pattern. The green will be mowed at 5/32" during the summer 1995 with a walk-behind greens mower. Fertilizer and irrigation will be substantially reduced from the 1994 establishment program to one irrigation per week and bi-monthly fertility for management.

Cooperative Evaluations

B. J. Johnson

During August 1993, 3' x 24' plots (4 reps) were planted with 4 ecotypes (Mauna Key, Adalayd, PI 509022, PI 299042), representing intermediate, fine, and coarse-textured types. We had problems with bermudagrass contamination and encroachment during 1994, and spent the summer cleaning up the plots. A herbicide evaluation program involving Illoxan, Drive, Banvel, Image, DMC, and Trimec will be initiated during 1995. A second area 3' x 50' plots (4 reps) were planted in June 1994 with 4 ecotypes (AP10, HI 25, PI 28960, and K7). Additional herbicide studies will be initiated on these plots during 1995.

Bob Carrow

Forty-eight (15' x 15') plots of Adalayd, SIPV-1, Mauna Key, and HI-1 were planted in June 1993 under native soil (fairway) conditions and two N-programs. Checks included Tifway and Tifgreen (Sea Island selection). These plots were plagued with bermudagrass contamination, which was sprayed with Roundup and resprigged with paspalums where plots were thin. We had so much rainfall during 1994 that water-extraction data was delayed until 1995 when, hopefully, water-stress conditions and full canopy cover will provide better data on root volume.

Kris Braman

The second repeat of the tawny/southern mole cricket greenhouse evaluation of 21 paspalum ecotypes was conducted during the summer 1994. A range in susceptibility to tawny mole cricket (*Scapteriscus vicinus*), southern mole cricket (*Scapteriscus borellii*), and armyworm (*Pseudaletia unipunctata*) was demonstrated among those accessions during 1993 and 1994.

The two selections that were most tolerant of mole cricket activity during both years were PI509023 and HI-1. Those that were consistently least tolerant of mole cricket-induced injury were PI509021, PI364985, and Glenn Oaks Adalayd. High susceptibility to armyworms in free choice tests also identified PI509021 and PI364985. Paradoxically HI-1, which was tolerant of mole cricket infestation, was highly susceptible to armyworm during 1993. Similarly, Glenn Oaks Adalayd, which was susceptible to mole cricket induced injury, was one of the six least preferred by armyworms during 1993.

B. R. Wiseman

Eighty-one Paspalum spp. were evaluated in a fall armyworm bioassay, using 9-day weight of neonate larvae fed on media containing 10-grams of oven-dry grass biomass. A second study was conducted during late summer 1994 and results have not been analyzed. A follow-up study has been initiated in which a couple of the most tolerant and most susceptible species will undergo investigation to pinpoint the biochemical deterrent to fall armyworm feeding. Additional cooperators will include the USDA lab in Athens, GA.

Genetic Analyses (in cooperation with R. L. Jarret and Steve Kresovich)

The flow cytometry paper evaluating 35 species and 81 accessions of Paspalum has been approved for publication in Genetic Resources and Crop Evaluation (copy enclosed). Nuclear DNA was extracted from 35 species (81 accessions) of Paspalum and examined by laser flow cytometry. P. vaginatum was not included in this initial study, but P. distichum was evaluated. DNA contents varied 4X among the species/accessions. Eight diploid (2N=20) species (of which P. vaginatum is a member) were analyzed. Significant differences in DNA contents both within and among species emphasized the complexity and diversity of the genus. An initial study using flow cytometry to evaluate DNA contents with P. vaginatum P. distichum, P. dilatatum, P. notatum, and bermudagrass as checks) revealed several of the coarse and dallisgrass types were 2X higher and P. distichum was 3X higher in DNA contents than fine-textured P. vaginatums. Bahiagrass was similar to the fine-textured vaginatums. The RAPD-marker paper evaluating 46 ecotypes of seashore paspalum has also been approved for publication in Genome (copy enclosed). Random amplified polymorphic DNA was used to assess genetic relationships and variation within seashore paspalum. A total of 46 ecotypes were screened with 34 oligonucleotide (10-mer) primers. We observed 195 RAPD fragments and 87% were polymorphic, indicating a high level of genetic diversity among accessions. A phenogram with distinct clustering has been developed. An RFLP paper for Crop Science and microsatellite paper for Theoretical and Applied Genetics have also been submitted for consideration of publication. Restriction fragment length polymorphisms were used to analyze 51 accessions of Paspalum representing 29 species. A total of 204 polymorphisms were scored, revealing some phylogeny relationships that we hope to incorporate into an interspecific hybridization program. Microsatellites are segments of DNA with very short sequence motifs (1-6 bases long) repeated in tandem; their numerous alleles differ in the number of these repeat units, such that they become the marker choice for high-density genome mapping i.e. DNA fingerprinting use for specific clone identification. This work has just begun on P. vaginatum, but the species in preliminary work appears to be ideally suitable for analyses using this technology. A post-doc student, (Stuart Brown), is continuing the microsatellite work involving seashore paspalum. Five microsatellite primers have been developed for this species and are currently undergoing evaluation. We may also try AFLP technology on the paspalums during 1995.

BREEDING

Since seashore paspalum is a sexual diploid (2N=20), but self-incompatibility (self-sterility) creates problems in attaining viable seed set, polycross blocks involving diverse genetic background ecotypes were established during 1994. Six blocks at the Bledsoe farm, and 49 blocks on the Georgia Station at Griffin, should provide sufficient information on whether this technique can successfully be used for intraspecific hybridization and attainment at high levels of viable seed. Seed harvest should occur during early November and germination studies will be conducted to check on seed viability. No successful crosses were achieved during 1994 involving P. vaginatum x P. distichum crosses. Efforts are still underway to find some technique to aid in synchronization of flowering, as well as to break through the self-incompatibility barriers limiting viable seed development.

I am now working with Image (imazaquin), which inhibits the acetohydroxy acid synthase enzyme responsible for production of the amino acids valine, leucine, and isoleucine. When this herbicide was applied to paspalum in Hawaii, the reaction was a burn-back, similar to paraquat, followed by recovery and a proliferation of spikes. We hope to learn how to manage the synchronization of spike-initiation better using this herbicide. This will have ramifications later concerning field seed production protocol.

We know that some paspalum accessions have these requirements for initiation of spikes: 1) thermal requirement of $<50^{o}F$; 2) thermal requirement $>70^{o}$ at high ($>75\%$) humidity; 3) photoperiod requirement <11 hrs; 4) photoperiod requirement >13 hrs.; 5) severe drought stress; 6) N-P-K starvation; 7) combinations of the above. I am still trying to get a handle on these responses on an accession-basis. This species is quite complex and it is not surprising, due to its stressful native habitat, that very specific environmental conditions are necessary to trigger flowering. Finding the right combinations will be a definite challenge.

SEED PRODUCTION CAPABILITY

Field-collected spikes containing possible viable seed of 18 ecotypes at Blairsville and 13 ecotypes at Griffin were germinated in rolled towels. Six ecotypes were the same at both locations (Adalayd, PI509020, PI509021, PI509018-1, HI1, and 561-79). The Blairsville average germination for these common ecotypes was 10.1% (range 2.3-20.5), while the average for Griffin was 2.9% (range 0.5-7.4%). Overall, germination at Blairsville averaged 11.9% (range 0% for K-2 to 45% for Temple 2). Griffin averaged 4.9% (range 0% for Parrish to 12.2% for PI299042). The dual-location mean was 8.4%. Obviously, the ecotypes vary tremendously in viable seed production. We are checking further to see if K-2 and Parrish are steriles.

OVERSEEDING

Since seashore paspalum follows a similar pattern of winter dormancy and spring green-up as hybrid bermudas, two large areas (one 50' x 60' - K7, and one 30' x 30' - PI 28960), and two small areas (10' x 10', PI 29193, and K17) were planted in late July for overseeding at Griffin. Various combinations of Fults alkaligrass, perennial ryegrass, tall fescue, creeping bentgrass, and Poa trivialis have been planted to initially evaluate cool- and warm-season grass transitioning involving the paspalums.

Publications

1. R. R. Duncan. 1993. Paspalum vaginatum Swartz -- diversity in collected cultivars. (Abstr.) American Society of Agronomy 85:188.

2. R. R. Duncan. 1994. Seashore paspalum may be grass for the year 2000. Southern Turf Management 5(1):31-32.

3. R. E. Wilkinson and R. R. Duncan. 1994. Seashore paspalum (Paspalum vaginatum Sw.) seminal root response to calcium ($^{45}Ca^{2+}$) absorption modifiers. Journal of Plant Nutrition 17:1385-1392.

4. Z. Liu, R. L. Jarret, and R. R. Duncan. 1994. RAPD-based polymorphism in seashore paspalum. (Abstr.) American Society of Agronomy 86:186.

5. C. Cardona and R. R. Duncan. 1994. Tissue culture regeneration of seashore paspalum. (Abstr.) American Society of Agronomy 86:186.

6. Z. Liu, R. L. Jarret, and R. R. Duncan. 1994. Genetic relationships and variation of ecotypes of Paspalum vaginatum as detected by random amplified polymorphic DNA (RAPDs). (Abstr.) 4th Int'l Congress of Plant Molecular Biology, 19-24 June 1994, Amsterdam, The Netherlands.

7. Z. Liu, R. L. Jarret, and R. R. Duncan. 1994. Assessment of taxonomic relationships among Paspalum spp. using restriction fragment length polymorphisms (RFLPs). (Abstr.) 4th Int'l Congress of Plant Molecular Biology, 19-24 June 1994, Amsterdam, The Netherlands.

Additional publications (in press) and (submitted) for review.

1. Z. Liu, R. L. Jarret, R. R. Duncan, and S. Kresovich. 1994. Genetic relationships and variation of ecotypes of seashore paspalum (Paspalum vaginatum Swartz) determined by random amplified polymorphic DNA (RAPD) markers. Genome **(in press)**.

2. R. L. Jarret, P. Ozias-Akins, S. Phatak, R. Nadimpalli, R. R. Duncan, and S. Hiliard. 1994. DNA contents in Paspalum spp. determined by flow cytometry. Genetic Resources and Crop Evolution **(in press)**.

3. Z.-W. Liu, R. L. Jarret, S. Kresovich, and R. R. Duncan. ____. Characterization and analysis of simple sequence repeat (SSR) loci in seashore paspalum (Paspalum vaginatum Swartz). Theoretical and Applied Genetics **(submitted)**.

4. Z.-W. Liu, R. L. Jarret, R. D. Webster, and R. R. Duncan. ____. Phenetic analysis of restriction fragment length polymorphisms (RFLPs) among Paspalum spp. Crop Science **(submitted)**.