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SEASONAL MEASUREMENTS OF
CARBON, NITROGEN, ASH, IRON,
CARBOHYDRATES, AND PIGMENTS
FROM GEOGRAPHICALLY DIFFERENT
SPARTINA ALTERNIFLORA MARSHES
IN THE SOUTHEAST

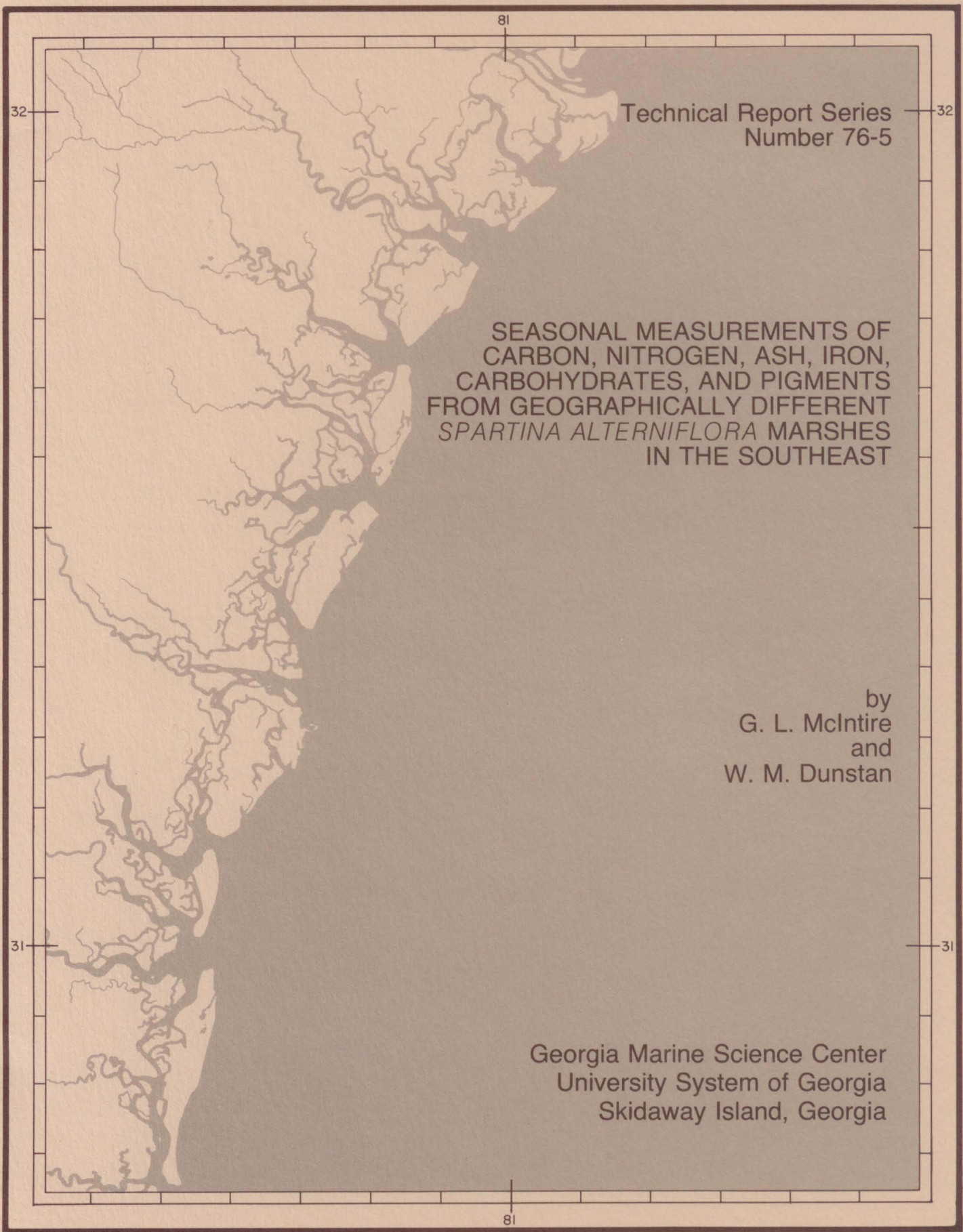
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Georgia Marine Science Center
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DIFFERENT SPARTINA ALTERNIFLORA MARSHES IN THE SOUTHEAST

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INTRODUCTION

The dominant species of marsh grass on the east coast, Spartina alterniflora is an important part of estuarine ecosystems. It is often the bulk of primary production in these systems (Teal, 1962) and provides a habitat for many species of animals. Upon dying, the reduced carbon in Spartina, stored mainly as structural carbohydrates, is gradually released to the detrital food chain. The energy thus released ultimately supports shrimp and other commercially important species in nearshore and estuarine environments. In addition to biological importance, Spartina marshes are important for sediment stabilization and geochemical and biological interactions with minerals and pollutants carried in river runoff and coastal waters.

Thus, the physiological status of Spartina marshes is of great importance to many biological, geological, and geochemical processes. A rapid and economical method of determining Spartina condition would be useful to governmental agencies and industry in gathering information for impact studies, marsh rehabilitation projects, and for determination of the effects of periodic or chronic "pollution." At present, the large majority of marsh studies deal with measurements of production and the effects on production of nutrient levels, tidal energy, or location (Valiela and Teal, 1971; Odum and Fanning, 1973; Nixon and Oviatt, 1973; McIntire and Dunstan, 1975a).

While the level of organic production of Spartina is of primary importance to the marsh ecosystem, its measurement is laborious and time consuming. Furthermore, natural levels of production vary within marshes as well as between marshes in the same general region (Nixon and Oviatt, 1973; McIntire and Dunstan, 1975a). Thus, the use of production measurements to indicate natural marsh condition is highly unreliable. This study is the culmination of a project to determine base levels of important chemical properties of Spartina alterniflora Loisel from Southeastern marshes in order to establish a "condition index." Application and testing of the values reported in this study are in progress and will be published in a subsequent technical report.

METHODS

Spartina alterniflora marshes were sampled in 5 major areas: Myrtle Beach, South Carolina; Charleston, South Carolina; Savannah, Georgia; Brunswick, Georgia; and St. Augustine, Florida (Fig. 1). Each area was sampled during January-February, May, July and October of 1975. The sampling dates were determined from production levels in an earlier study (McIntire and Dunstan, 1975a). In each area, 5 locations were sampled by clipping three replicate plants from 3 marsh zones determined by the following height categories: less than 30 cm, from 30 cm to 100 cm, and over 100 cm. This resulted in a total of 45 plants (3 plants x 3 heights x 5 locations = 45 plants) from each area which were immediately frozen and maintained in the dark until returned to the laboratory. In

the laboratory, plants from each group were separated into leaves and stems, and dead material was discarded. The plant material was subsequently refrozen, lyophilized, ground in a Wiley Mill to pass a 20 mesh screen and then stored frozen in the dark until analyzed.

Each of the samples of leaves and stems was analyzed for nonstructural carbohydrates (NSC), percent carbon, percent nitrogen, iron content, percent ash, and total pigments including chlorophyll a, chlorophyll b, and carotenoids. The NSC analysis employed a takadiastase enzyme hydrolysis followed by a modified Sheaffer - Somogyi copper iodometric analysis of reducing power (see McIntire and Dunstan, 1975b, for more detail). Sub-samples were weighed into precombusted, preweighed porcelain crucibles and dried at 100°C overnight to determine percent moisture remaining in each sample. After weighing, the dried samples were ashed at 500°C overnight in a Thermolyne muffle furnace and weighed for ash weight. The ash was then digested with 5 ml of 50% HCl in distilled water to solubilize the iron. The liquid was filtered through a Whatman #2 paper into volumetric flasks and a 5 ml aliquot was taken for analysis of iron content using Ferrozine reagent (available from Hach Chemical Co.), (Stookey, 1970; McIntire and Dunstan, 1975b).

Subsamples of dried, ground leaves and stems were analyzed for percent carbon, percent nitrogen, and percent hydrogen using a Perkin-Elmer model 240 Elemental Analyzer. Pigments were analyzed as performed by McIntire and Dunstan (1975a, b). A sample was weighed into a 50 ml stainless steel centrifuge tube. Ten ml of 90% acetone in distilled water and two stainless steel balls were added. The tubes were capped and agitated on a Burrell shaker for 5 minutes and centrifuged for 5 minutes. The acetone was poured off into 50 ml graduated centrifuge tubes and 10 ml of fresh acetone was added. The procedure was repeated. After the second shaking, the two extracts plus sediment were combined and centrifuged for 5 minutes. The extracts were then run on a Bausch and Lomb Model 200 UV scanning spectrophotometer in 1 cm cuvettes against 90% acetone in distilled water. Amounts of pigments were calculated from the equations given below:

$$\text{Chlorophyll } \underline{a} \text{ } (\mu\text{g/ml}) = 11.65e_{663} - 2.14e_{645}$$

$$\text{Chlorophyll } \underline{b} \text{ } (\mu\text{g/ml}) = -4.31e_{663} + 19.97e_{645}$$

$$\text{Carotenoids } (\mu\text{g/ml}) = 7.6(e_{480} - 1.49e_{510})$$

Multiplying the answers by the total volume of extract yielded the amount of pigment/amount of sample analyzed.

Data were listed in tabular form and analysis of variance performed at $\alpha = .05$ to determine significant differences. Where no differences in group means were discovered, all values were averaged to determine the mean value of the given characteristic.

RESULTS

Nitrogen levels are listed in Table 1 and range from .41% (2.56% protein) to 2.68% (16.75% protein). Both leaves and stems were enriched with nitrogen in January. By May, the levels of both leaves and stems had dropped to values that did not significantly change over the rest of the growing season.

Table 2 lists the values of percent carbon for leaves and stems from all samples. There is no significant difference between leaves, stems, or areas on an areal basis. The only seasonal difference appears in January when percent carbon for leaves and stems were lower than the values for May, July and October. An average carbon for all leaf and stem samples is 44.70 (Table 2).

The carbon to nitrogen ratios reflected nicely the seasonal changes in nitrogen and carbon content ranging from 18.52 for January leaves to 70.68 for October stems (Table 3).

Leaf values of ash dropped to the July sample and rose again in October (Table 4). Stem values dropped in July also, but the rise to October was not significant ($\alpha = .05$). Sample values ranged from 12.8 to 5.5%.

Tissue concentrations of iron dropped from January to July in both leaf and stem samples (Table 5). The rise in October was not statistically significant ($\alpha = .05$). Leaves and stems were significantly different ($\alpha = .05$) in each sampling period except October.

Nonstructural carbohydrates (NSC) reflected levels determined in earlier studies (McIntire and Dunstan, in press). Values were stable for January through July for both leaves at 40-50 mg NSC/gm dry wt and stems at 50-60 mg NSC/gram dry wt. October values were significantly lower than preceding values at 10-20 mg NSC/gram dry wt (Table 6).

Pigment concentrations generally decreased from January to October. Exceptions include leaf chlorophyll a values at 1549 μg Chl a/gm dry wt and carotenoid leaf and stem values of 512 and 83 μg pigment/gram dry wt respectively (Tables 7 and 9). Leaf and stem values of chlorophyll b content decreased from 597 to 427 and 142 to 64 μg pigment/gm dry wt respectively (Table 8).

DISCUSSION

Nitrogen levels in Spartina alterniflora from the literature range from .54 (3.38% protein) to 3.68 (23.00% protein) percent (Taschdjian, 1954; Odum and de la Cruz, 1967; Burkholder, 1956; Burkholder et al., 1959). Although levels as high as 4.5% may exist in laboratory grown plants (unpublished data), the values given in Table 1 are representative of normal natural levels. The seasonal averages show Spartina to contain

higher percentages of nitrogen in the early part of the growing season dropping to relatively constant levels in late spring. Similar trends in nitrogen content have been noted in forest herbs (Kieckhefer, 1963), alpine plants (Hadley and Bliss, 1964), and various marine macrophytes (Boyd, 1970). Squiers and Good (1974) found nitrogen concentrations very similar to those in this study although their values represent whole plants rather than individual leaves and stems. They suggested that early uptake of relatively large amounts of nitrogen may enable plants to continue optimum growth through the year even though environmental conditions may not favor the synthesis of proteins or uptake of inorganic nitrogen (Squiers and Good, 1974). It is possible that higher nitrogen levels may reflect more available nutrients early in the year. By taking up large amounts of nutrients early, plants may avoid competition from other species whose period of optimum growth is later in the season (Boyd, 1970).

Values of percent carbon in Table 2 are somewhat higher than those found in the literature although values as low as 35% were present in January (Burkholder, 1956; Burkholder *et al.*, 1959; Odum and de la Cruz, 1967). By freeze drying the samples rather than oven drying, it is possible that some volatile carbon compounds are retained that may be lost in the latter drying method. Lower carbon levels in combination with higher nitrogen levels during January resulted in the lowest carbon to nitrogen ratios of the year. The significant rise in carbon to nitrogen ratios of stems can be attributed to reduced nitrogen levels as carbon levels remained constant. Values from this study are equal to or higher than most in the literature reflecting the greater percent carbon reported here (Burkholder, 1956; Odum and de la Cruz, 1967). In general, carbon to nitrogen ratios for leaves are within the range of terrestrial plants (30-40) which contain a high percentage of structural carbohydrates. Stems are higher reflecting the accumulation of cellulosic compounds during the year. All of the values illustrate the generally nitrogen poor status of Spartina in its living state.

Although it has been generally acknowledged that Spartina accumulates large amounts of iron, no studies have been performed to determine exactly how much iron the plant requires. One study has suggested a limiting nutrient relationship between soil iron concentration and plant height (Nixon and Oviatt, 1973). Production data from a previous study (McIntire and Dunstan, 1975a) combined with iron data from this study show that like nitrogen, well over 50% of total standing crop of Spartina iron may be present in the plant early in the year (Table 10). Iron data were graphed as ordinate points against tissue nitrogen content from the same sampling date (Figure 2). There is a good correlation ($r = .56$) between tissue iron content and tissue nitrogen content again indicating that iron as well as nitrogen may be taken up early in the year.

Contrary to earlier studies, ash content in Spartina was rarely over 12% (Williams and Murdoch, 1969). Values as high as 20% ash have been reported in Spartina with even higher values in dead material (Keefe and Boynton, 1974; Williams and Murdoch, 1959). It must be remembered that in our data no dead plant material was included. Many studies report little or no pattern to ash content of Spartina (Squiers and Good, 1974;

Williams and Murdoch, 1969). Results of this study (Table 4) show that percent ash decreases during the year probably to the time of peak standing crop, increasing thereafter with the death of the plants. This pattern can be seen in data from Squiers and Good (1974) where values ranged from 14% ash to a low of 9.5% ash in September and rising to 11.5% ash in November. Similar patterns are evident in other metal concentrations as well (Williams and Murdoch, 1969). Williams and Murdoch (1969) suggested that the decreases of iron, zinc, and other metal concentrations with increasing production levels were related through a "dilution" effect of increased structural carbohydrate content on relatively stable metal concentrations. Such a "dilution" concept agrees with present results indicating early uptake of most of the total iron and nitrogen standing crop in Spartina.

Nonstructural carbohydrates (NSC) include storage products and the small amounts of monosaccharides involved in active photosynthetic processes (Smith, 1986). NSC levels in Spartina are usually less than 100 mg NSC/gram dry wt while average values for leaf and stem material are 40-50 mg NSC/gram dry wt (McIntire and Dunstan, in press). Results of this study agree with previous work in that values for leaves and stems average approximately 45 and 55 mg NSC/gram dry wt respectively through the July sample. The reduced levels in late fall may reflect greater percentages of dead and dying material within the samples. Temperature and environmental effects on photosynthesis and/or translocation of photosynthetic production may also be responsible for reduced NSC levels in leaf and stem tissues.

Little information is available on pigment concentrations in aquatic plants and almost none on Spartina alterniflora. Boyd (1970) determined concentrations of chlorophyll a, chlorophyll b, and carotenoids in submerged, floating, and emergent aquatic vascular plants. Values for leaves in Table 7 compare well with those for emergent plants. Pigment concentrations of whole plants of Typha latifolia and Scirpus americanus decrease with time (Boyd, 1970). Decreasing levels of chlorophyll a and b in stem tissues can be accounted for by the rapid buildup of cellulosic material during the late spring and summer months. Leaves seem to maintain relatively constant levels of all three pigments during the year.

The results of this study are currently being used to establish a "condition index" for Spartina alterniflora. Further sampling of natural and polluted marsh sites as well as laboratory experiments with Spartina responses to given perturbations will test and clarify important relationships between the chemical parameters in this study. With this study, the seasonal ranges of values have been established for normal marshes. Future testing will enable marsh ecologists to determine harmful situations from chemical symptoms below or possibly above expected values.

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FIGURE 1

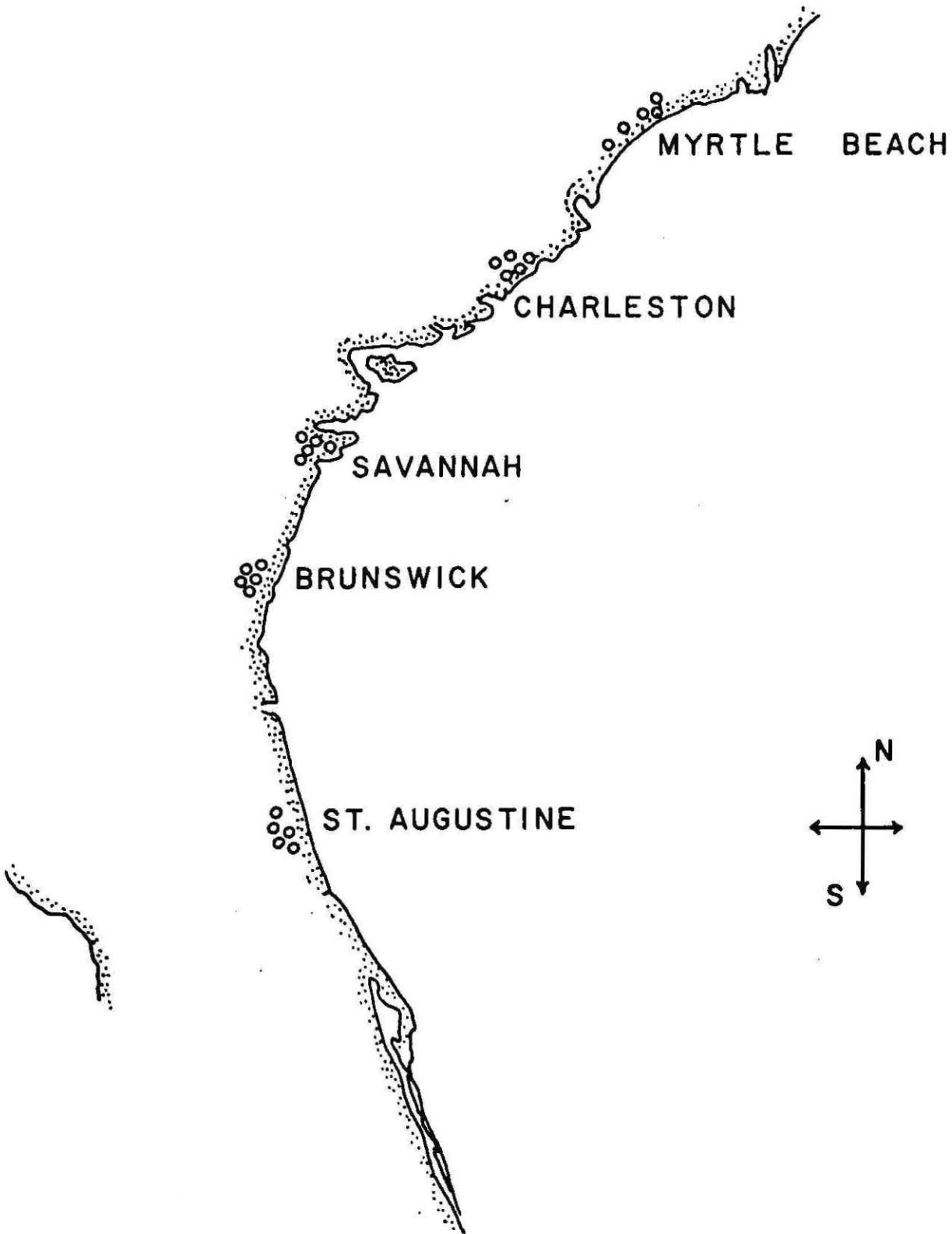


FIGURE 2

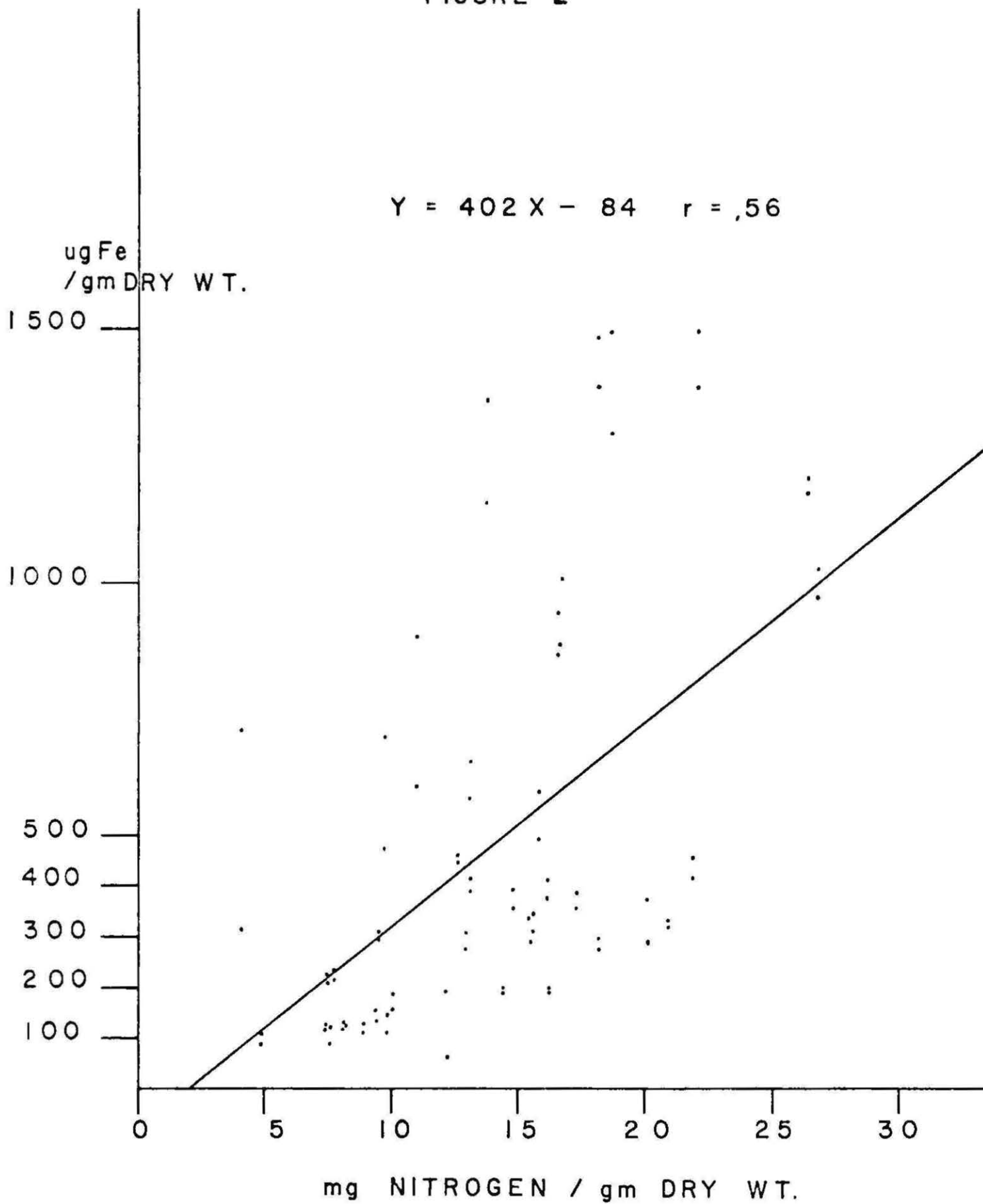


TABLE 1

PER CENT NITROGEN

LOCATION		JAN	MAY	JULY	OCT	LEAF	STEM
Myrtle Beach	Leaf	2.21 †	1.31	1.29	1.82	1.66 b*	
	Stem	1.56	.78	.89	.75	.44 †	1.00 a b c d .38
Charleston	Leaf	2.64	1.66	2.09	1.58	1.99 a	
	Stem	1.62	.95	.99	1.22	.49	1.20 a b c d .31
Savannah	Leaf	1.82	1.38	1.31	1.73	1.56 a b c	
	Stem	1.48	.93	.76	.41	.25	.90 b c d .45
Brunswick	Leaf	1.87	1.26	1.62	1.44	1.55 a b c	
	Stem	1.10	1.00	.81	.49	.26	.85 d .27
St. Augustine	Leaf	2.68	1.67	1.55	2.19	2.02 a	
	Stem	2.01	.97	.74	.81	.52	1.13 a b c d .59
Leaf \bar{X}		2.24 m	1.46 n	1.57 n	1.75 n	1.76	
σ		.41	.20	.32	.28	.42	
Stem \bar{X}		1.55 n	.93 o	.84 o	.74 o		1.01
σ		.33	.09	.10	.32		.39

*Values followed by similar letters are not significantly different at $\alpha = .05$.

† Standard deviation

‡ All values are % of dry weight.

TABLE 2

PER CENT CARBON

LOCATION		JAN	MAY	JULY	OCT	LEAF	STEM
Myrtle Beach	Leaf	41.19 †	44.46	46.13	49.01	45.20 a* 3.27 †	44.72 a 4.27
	Stem	38.47	46.55	45.81	48.04		
Charleston	Leaf	49.00	47.48	48.68	47.90	48.27 a .70	42.33 a 4.27
	Stem	45.29	35.98	44.15	43.88		
Savannah	Leaf	41.77	49.13	47.87	46.51	46.32 a 3.22	44.79 a 3.55
	Stem	40.24	46.89	48.22	43.81		
Brunswick	Leaf	35.89	48.45	45.76	46.34	44.11 a 5.60	42.96 a 6.03
	Stem	34.63	47.26	42.41	47.52		
St. Augustine	Leaf	35.39	48.43	47.29	47.23	44.59 a 6.15	43.72 a 4.84
	Stem	36.65	47.59	45.07	45.46		
Leaf \bar{X}		40.65 mn	47.59 _o	47.15 _o	47.24 _o	45.70	
σ		5.51	1.85	1.21	1.06	4.08	
Stem \bar{X}		39.06 m	44.85 _{n o}	45.13 _o	45.74 _o		43.64
σ		4.06	4.99	.74	1.98		4.36
Mean of all samples		44.70					
σ		4.24					

*Values followed by similar letters are not significantly different at $\alpha = .05$.

†Standard deviation

‡All values are % of dry weight.

TABLE 3
CARBON/NITROGEN RATIO

LOCATION		JAN	MAY	JULY	OCT	LEAF	STEM
Myrtle Beach	Leaf	18.70	33.85	35.77	26.94	28.82 d 7.74	48.96 de 16.58 †
	Stem	24.63	60.02	52.26	58.93		
Charleston	Leaf	18.52	28.61	23.30	30.38	25.20 d 5.38	36.59 de 6.76
	Stem	28.03	37.95	44.44	35.92		
Savannah	Leaf	22.95	35.48	36.68	26.88	30.50 d 6.66	61.89 e 33.20
	Stem	27.28	50.62	63.26	106.39		
Brunswick	Leaf	19.23	38.50	28.21	32.19	29.53 d 8.07	56.83 e 27.60
	Stem	31.56	47.28	52.44	96.03		
St. Augustine	Leaf	13.21	28.94	30.59	21.53	23.57 d 7.59	46.16 e 19.22
	Stem	18.26	49.25	61.01	56.13		
	Leaf \bar{X}	18.52 a*	33.08 a	30.91 a	27.58 a		
	σ	3.48	4.27	5.53	4.08		
	Stem \bar{X}	25.95 a	49.02 b	54.68 b	70.68 c		
	σ	4.96	7.89	7.57	29.48		

* Values followed by similar letters are not significantly different at $\alpha = .05$.

† Standard deviation

TABLE 4

PER CENT ASH

LOCATION		JAN		MAY		JUL		OCT	
Myrtle Beach	Leaf	11.3	11.9†	11.0	10.8	9.4	9.4	9.1	9.0
	Stem	10.7	10.7	11.5	11.7	8.4	7.8	6.0	5.8
Charleston	Leaf	12.8	12.8	11.2	11.5	8.0	7.8	10.7	10.7
	Stem	10.5	10.4	11.1	11.1	7.9	7.7	9.8	10.0
Savannah	Leaf	12.1	12.1	10.4	10.1	8.6	8.2	10.4	10.2
	Stem	12.8	12.7	8.7	8.6	5.7	5.5	8.1	8.3
Brunswick	Leaf	11.3	11.0	10.8	11.2	7.5	7.8	10.5	10.6
	Stem	10.3	10.3	9.9	9.9	7.8	7.5	7.2	7.2
St. Augustine	Leaf	9.5	9.2	10.1	11.9	8.4	8.4	10.7	10.7
	Stem	9.6	9.6	9.4	9.4	6.8	7.5	9.1	8.2
Leaf \bar{X}		11.40 a*		10.9 a b		8.3 c		10.3 a b	
σ		1.24		.6		.6		.6	
Stem \bar{X}		10.76 a b		10.1 b		7.3 c		8.0 c	
σ		1.12		1.2		1.0		1.4	

*Values followed by similar letters are not significantly different at $\alpha = .05$.

†All values are % dry weight.

TABLE 5
IRON*

LOCATION		JAN	MAY	JUL	OCT
Myrtle Beach	Leaf	1497 1384	647 571	277 302	295 272
	Stem	341 304	232 227	108 125	209 220
Charleston	Leaf	1208 1179	856 940	317 324	586 492
	Stem	410 373	298 304	143 104	58 193
Savannah	Leaf	1481 1383	1360 1157	418 389	381 353
	Stem	352 391	132 150	118 87	705 316
Brunswick	Leaf	1495 1285	444 451	298 182	198 188
	Stem	597 897	154 182	130	105 87
St. Augustine	Leaf	968 1021	1005 877	332 289	418 455
	Stem	374 289	696 475	123 112	117 122
Leaf \bar{X}		1290.1 a	830.8 b	303† c e	363.8 c e
σ		192.6	303.1	74	128.7
Stem \bar{X}		432.8 c	285.0 c d e	117 d	213.2 d e
σ		183.8	176.7	16	189.2

*Values are $\mu\text{g Fe/gm dry wt.}$

†Values followed by similar letters are not significantly different at $\alpha = .05.$

TABLE 6

NON STRUCTURAL CARBOHYDRATES*

LOCATION		JAN		MAY		JUL		OCT†	
Myrtle Beach	Leaf	7.8	3.4	3.8	3.2	4.8	4.7	1.1	1.2
	Stem	6.1	6.9	3.9	6.0	5.3		1.8	1.7
Charleston	Leaf	4.2	3.6	4.0	4.0	4.0	4.2	1.5	1.4
	Stem	2.9	3.9	4.3	6.1	5.8		1.8	1.5
Savannah	Leaf	4.2	3.2	4.3	4.9	3.8	4.9	1.9	1.8
	Stem	3.9	4.7	5.5	6.2	10.1		2.0	2.8
Brunswick	Leaf	4.5	4.6	5.2	4.7	4.7	5.0	1.8	1.8
	Stem	5.6	5.7	5.3	5.8	5.6		1.6	1.6
St. Augustine	Leaf	5.2	4.9	3.7	3.5	4.4	4.8	1.7	1.3
	Stem	6.0	4.6	6.1	6.8	3.9	4.7	2.0	1.9
Leaf \bar{X}		4.6		4.1		4.5		1.6	
σ		1.3		.6		.4		.3	
Stem \bar{X}		5.0		5.6		5.9		1.9	
σ		1.2		.9		2.2		.4	

*Values are mg non-structural carbohydrates/gm dry wt.

†October is significantly different at $\alpha = .05$ from the earlier sampling dates.

TABLE 7

CHLOROPHYLL a *

LOCATION		JAN	MAY	JULY	OCT	LEAF	STEM
Myrtle Beach	Leaf	1672	2122	1407	1899	1775 306 [†]	289 75
	Stem	295	370	303	188		
Charleston	Leaf	1189	1573	1398	1225	1346 177	262 111
	Stem	263	402	253	130		
Savannah	Leaf	1755	1540	1514	1430	1560 138	283 98
	Stem	384	334	254	161		
Brunswick	Leaf	1477	1211	1492	1483	1415 137	242 52
	Stem	285	239	275	170		
St. Augustine	Leaf	1488	1763	1629	1926	1701 187	291 51
	Stem	284	291	357	232		
Leaf \bar{X}		1516	1642	1488	1592	1549	
σ		218	334	94	308	251	
Stem \bar{X}		302	327	288	176		274
σ		47	64	43	38		75

*Values are μg chlorophyll a/gm dry wt.

[†]Standard deviation

TABLE 8

CHLOROPHYLL b *

LOCATION		JAN	MAY	JULY	OCT	LEAF	STEM
Myrtle Beach	Leaf	640	586	439	489	539 91†	121
	Stem	152	157	100	76		40
Charleston	Leaf	544	417	510	297	442 110	105
	Stem	136	146	95	42		47
Savannah	Leaf	651	464	444	394	488 112	115
	Stem	154	144	103	59		43
Brunswick	Leaf	559	345	427	382	428 93	95
	Stem	133	92	101	54		33
St. Augustine	Leaf	593	498	471	572	534 58	115
	Stem	136	113	121	90		19
Leaf \bar{X}		597	462	458	427	486	
σ		48	90	33	106	97	
Stem \bar{X}		142	130	104	64		110
σ		10	27	10	19		35

*Values are μg chlorophyll b/gm dry wt.

†Standard deviation

TABLE 9

CAROTENOIDS *

LOCATION		JAN	MAY	JULY	OCT	LEAF	STEM
Myrtle Beach	Leaf	645	631	440	679	599 108 †	
	Stem	79	90	100	80		87 10
Charleston	Leaf	216	484	457	444	400 124	
	Stem	65	106	86	60		79 21
Savannah	Leaf	522	476	563	568	532 43	
	Stem	121	86	83	64		89 24
Brunswick	Leaf	521	384	496	521	481 65	
	Stem	61	69	88	70		72 11
St. Augustine	Leaf	370	589	555	670	546 127	
	Stem	53	79	129	86		87 32
Leaf \bar{X}		454	513	502	576	512	
σ		65	98	56	100	112	
Stem \bar{X}		165	86	97	72		83
σ		39	14	19	11		20

*Values are μg Carotenoids/gm dry wt.

†Standard deviation

TABLE 10

MONTH	Production gm/m ²	Per Cent Leaf % Total Dry Wt	Leaf Iron mg/gm	Iron/m ² mg/m ²
January	900	11	1.290	127.7
May	350	30	.830	87.2
July	1250	41	.303	155.3
October	850	31	.364	95.9

Standing crop was determined from average plant height as follows: From Figure 3 (McIntire and Dunstan, 1975a), average plant height for January, May, July, and October at the Savannah River site was determined. Each height was matched to a value of standing crop from the graph in Figure 6 (McIntire and Dunstan, 1975a).

Percent leaf values from the Savannah River site are from Table 1, McIntire and Dunstan, 1975a.

Iron values are for leaf tissue.

Iron standing crop of leaves was determined by taking the percent leaf from Table 1 (McIntire and Dunstan, 1975a) and finding the weight of leaf tissue/m² in each sampling period. This was multiplied by the iron content per gram leaf resulting in iron production values.

