RHODES COLLEGE

SCIENCE JOURNAL

VOLUME XI • APRIL 1993

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THE RHODES COLLEGE SCIENCE JOURNAL

VOLUME XI
APRIL 1993

PREFACE

The *Rhodes College Science Journal* is a student-edited, annual publication which recognizes the scientific achievements of Rhodes students. Founded ten years ago as a scholarly forum for student research and scientific ideas, the journal aims to maintain and stimulate the tradition of independent study. We hope that in reading the journal, other students will be encouraged to pursue scientific investigations and research.

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ACKNOWLEDGEMENTS
Dr. David Kesler
Rhodes Biology Department
Alumni and friends whose generosity has made this publication possible

MICROCHARACTERS FOUND TO SEPARATE SUBSPECIES OF *VERBESINA ENCELIOIDES* USING THE SCANNING ELECTRON MICROSCOPE

SUSAN EWART

ABSTRACT

The purpose of the study was to search for characters to separate V. Encelioides using the scanning electron microscope and to reexamine Coleman's treatment of this species. Coleman's key to the subspecies was used to separate specimens used in this study which ranged from Yuma, Arizona to Eleuthera, Bahamas. The surface features of the disk achene separated the population into western and eastern populations. The chaff apex was glandular in the western populations and pubescent, but not glandular in the eastern populations. Disk achene margins were entire in the west and undulating in the east. Geographically these population are separated by the Edward's plateau of Texas. In conclusion, the key microcharacters of the eastern and western populations are as follows:

Predominantly tuberculate disk achene surface, disk achene wing margin entire, and disk achene angle obtuse to intermediate, chaff apex glandular, located west of the Edward's Plateau, Texas.....V. encelioides cana

Predominantly smooth disk achene surface, disk achene margin undulating, and disk achene apex acute to intermediate, chaff apex pubescent, located east of the Edward's Plateau, Texas.....V. encelioides encelioides.

INTRODUCTION

In Coleman's A Taxonomic Revision of Sections *Pterophyton, Sonoricola,* and *Ximensia* of the Genus *Verbesina*, Coleman separated *Ximensia* by auricle height, length, angle to the stem, canescence on adaxial leaves, disk achene apex angle, and involucre length. Coleman found an east/west clinal tendency separated by the Edward's Plateau of Texas. The specimens he measured ranged from Arizona to Texas. Coleman found that the western regions had smaller auricles, less pubescence on adaxial leaves, achene ratings of 1 to 1.5, (Coleman assigned an obtuse angle 1 and an acute angle 3, with 2 as the intermediate) and involucre bract lengths of 6.5 to 10mm. The eastern region had larger auricles, more pubescence on the adaxial leaves, achene ratings of 2.9 to 3.0, and involucre bract lengths were 11.6-16.5 mm. Coleman concluded that *cana* (east of Edward's Plateau) could be characterized by involucre bracts < 12 mm, achene apices obtuse, and oblong auricles. *Encelioides,* found mainly east of Edward's Plateau, was characterized as having > 12 mm involucre bracts, apex of achene acute, and semi-ovate auricles.

In this study the distribution of *V. encelioides* was larger and included several specific herbarium specimens that Coleman remarked would not fit in his existing taxa parameters. These five are: Georgia: McIntosh; Smith 2239, Bahamas: Long Cay Island; JKL Brace 4124, Great Ragged Island; Wilson 7811, Eleuthera Island; Britton and Millspaugh 5508, and Cuba: Santa Clara; Combs 577.

MATERIALS AND METHODS

Gross morphology of the herbarium sheets was measured by the following characters: leaf length and width, auricles present or absent, margin of leaves, pedicel length, and stipules length and width. To further examine characters, the involucre bracts, ray florets, disk florets, disk achenes, ray florets, ray achenes, chaffs, and leaf material of each specimen were immersed in aerosol OT, dehydrated in ethanol, and placed in acetone. The specimens were critical point dried, gold sputter coated, and examined using the 5300 JEOL SEM.

The following characters were examined for the east/west and north/south clinal variations within the eastern and western populations. Characters were examined to identify subgroups with these two divisions. These include: ray floret and ray floret tube length(Appendix A), ray floret surface, ray floret tube pubescence, ray achene shape, disk achene length and width and length to width ratio, and disk achene apex angle (B), disk achene pubescence, disk achene surface, disk shape, disk achene margin, disk achene angle at apex, disk achene pappus morphology, disk floret shape, disk floret and disk floret tube and disk floret to tube ratio (C), disk floret surface cell lengths (D), involucre bract length and width (E), involucre tip, chaff length and width (F), chaff tip, leaf length, width, margin, and presence of auricles (G), abaxial leaf pubescence length and arrangement (H), pedicel length and stipules length and width (I).

RESULTS

The leaf length and width varied throughout the range with the east cline having 3.5 to 12.5 mm, and the west cline 4 to 8 mm. Semi-ovate auricles were assigned a 1 and oblong to lancelot a 2 or 3, respectively. The specimen from the west were predominantly oblong to lancelot, with the exception of population 310, 308, and 314 which registered a 1. The east was predominantly semi-ovate -- the auricles formed a semi-circle lobe at the base of the leaf. The exceptions in the east were 319 and 311. The margins of the leaves were keyed as serrate of varying degrees of incision of the teeth. The east cline had more incised serration than the west. Population 319 was nearly entire except for a few slight serrate edges, and 304 had crennate margins. The margins of *V. encelioides* varied from regularly serrate to double serrate, to irregularly serrate. The length of the pedicel varied across the range from 1.5 - 3.5 cm. with the exception of 304 with 5 cm. pedicels, 325 with 0.6 cm. pedicels, and 311 with 0.3 cm. pedicels. The presence of stipules at the base of the leaf was noted and if present the length and width of the stipules were measured. Specimens with stipules < 0.2 cm. width and 0.2 cm. length and had less than two leaves were considered sparsely stipulated, and > 0.2 cm. in length and width and occurring on more than two leaves, prominently stipulated. The specimens west of the Edward's plateau ranged from 0.4 to 1 cm. in length and 0.3 to 1 cm. in width. The eastern specimens ranged from 0.2 cm. in length and width to absence of stipules all together.

The surface of the disk achene was photographed at 1,000 X to illustrate the

western's pock-marked surface and the eastern's absence of these star-shaped tuberculate formations seen on plate 1:A (populations #316) and plate 1:B (population #321). Exceptions to the split are populations 314, 301, 306, 319. The chaff tip morphology was examined and found to suggest the eastern populations had pubescence at the tip while the western population's chaffs ended in a gland formation as seen in plate 1:C (302) and plate 1:D(305). Disk achene margins were examined and categorized as entire, and the eastern populations proved to be undulating as seen in plates 2:A (316) and plate 2:B (313). Exceptions to the disk achene margin character occur in population 311, 319, and 306 of the eastern group. All western achene margins were smooth.

The achenes were rated 1 for obtuse, 3 for acute, and 2 for intermediate. These specimens fell into the east/west divisions on either side of the Edward's Plateau. The western populations registered 2's and 1's, and the east rated 2's and 3's. The exceptions to these categories are populations 311, and 325. The condition of obtuse and acute for the disk achene apices are seen in plates 2:C (323) and 2:D (309), respectively.

The two populations were then studied for clines within the west and east split for east-west clines and north-south clines. One tendency appeared for clinal tendencies: abaxial lead pubescence arrangement. The arrangement travels west to east following lanate to tomentose at Edward's Plateau, to lanate, to tomentose in the eastern most Bahamas islands. The lanate condition if photographed in plate 3:A (313); the tomentose in plate 3:B (319).

The study also provided significant characters to identify subgroups. The ray floret lengths within the western cline showed one major deviation: 314's ray floret measured 11 mm, standing out of the range of the others (4.5 -8 mm). Specimen 314 did not fit into the disk achene surface character of pock marked for the predominantly smooth surface achene western populations, and had semi-ovate auricles in the predominately oblong auricled west.

Within the western region, 310 the northern most populations tends to have larger measurements. The disk achene length is 6.1 mm compared to the range of 2-5.5 mm. The involucre bract length of 310 is 12 mm compared to the western range of 4.7 - 7.5 mm. The chaff width of 310 is also larger as 2.2 mm compared to the western range of 0.7 - 1 mm. 310 has no gross morphological differences from its region, only a largeness to it suggesting a slight north/south cline.

The population located on Long Cay island in the Bahamas, 319, shows very unique characters from its surrounding populations. The leaf margins of 319 are mostly entire except for a few serrated edges to break the line. The disk achene surface of 319 is pock-marked in the mainly smooth eastern region, and possessed oblong auricles with neighboring populations with semi-ovate auricles. The pubescence of the abaxial leaf surfaces are extremely long, .9 mm out the range of (0.2-0.5 mm). The disk achene pappus is structurally very different from all other achenes studied. The components of the pappus do not resemble the typical bundle of pubescence seen Plate 3:C (302), but a clump of wrinkled cells lengthened for a pappus photographed in Plate 3:D. Population 319's disk achene margin is not undulation like surrounding populations.

The population of 311, located in Webb, Texas is the westernmost populations

of the eastern group and possesses traits to set it apart. 311 is registered a 2-3 oblong to lancelot in a semi-ovate in the eastl. 311 has .3 cm. length pedicels compared to the overall range of 5-.6 cm. The disk achene of 311 has a length to width ratio of 5.6 mm out of the range of (2.1 - 3.6 mm). The disk achene apex angle is also out of place, it registers as 1, obtuse in the acute eastern range. The disk achene margin of 311 is smooth compared to the eastern trait of undulation margin.

Population 304, in Dallas, Texas also stands out with crenate margins and 5 cm. pedicels in the overall range of 1.5 - 3.5 cm.

Population 309, of Georgia also deviates with involucre bract lengths of 188 mm in the range of (4.9 - 10) excluding the other subgroup population #304.

CONCLUSION

In conclusion, the key microcharacters of the eastern and western populations are as follows:

Predominantly oblong to lancelot auricles, serrate margins of leaves, stipules < 0.2 mm in width and length or absent, tuberculate disk achene surface, disk achene wing margin entire, and disk achene angle obtuse to intermediate, chaff apex glandular, located west of the Edward's Plateau, Texas..... *V. encelioides cana*

Predominantly semi-ovate auricles, incised-serrate margins of leaves, stipules > 0.2 mm, smooth disk achene surface, disk achene margin undulating, and disk achene apex acute to intermediate, chaff apex pubescent, located east of the Edward's Plateau, Texas..... *V. encelioides encelioides.*

LITERATURE

Coleman, James Robert. 1964. A Taxonomic Revision of Sections *Pterophyton, Sonoricola,* and *Ximensia* of the Genus *Verbesina.* Indiana University Press.

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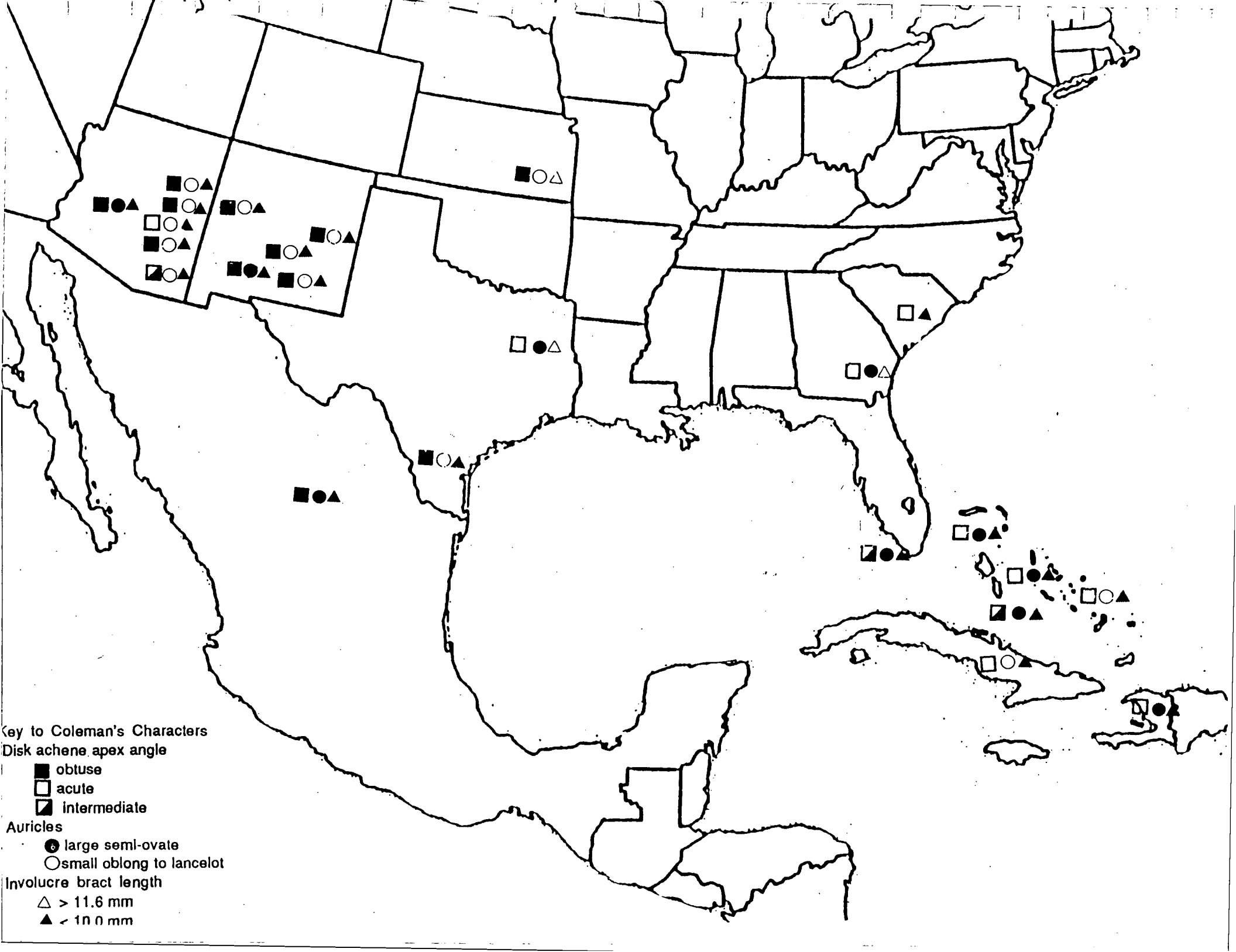
Key to Coleman's Characters
Disk achene apex angle
obtuse
acute
intermediate
Auricles
large semi-ovate
small oblong to lancelot
Involucre bract length
> 11.6 mm
< 10.0 mm

Appendix A: Ray Floret and Tube Length

Population #	tube (mm)	floret (mm)
301	1.4	7
302	1.7	7
303	2.2	8
304	2.4	10
305	1.5	4.7
306	2.2	6.2
307	2	8
308	1.5	5.5
309	2.5	10.5
310	2.5	5.5
311	1	5
312	2	11
313	2.5	8.5
314	---	11
316	1	4.5
317	1.7	6
318	2.1	7.5
319	---	9
320	1	5.2
321	1.5	9
322	2.1	5
323	---	---
324	---	---
325	2	8

Appendix B: Disk Achene Length, Width, and Apice Angle

Population#	length (mm)	width (mm)	length to width	apice angle
301	2	0.8	2.5	2
302	4.5	2	2.3	3
303	4.9	1.7	2.9	3
304	2.3	0.7	3.3	3
305	2.7	1	2.7	1
306	4	1.2	3.3	2
307	5.5	2.2	2.5	1
308	4	1.2	3.3	1
309	4.3	1.2	3.6	3
310	6.1	2	3.1	1
311	4.5	0.8	5.6	1
312	5.2	2.5	2.1	3
313	5.5	1.7	3.2	3
314	4.5	3	1.5	!
316	3	1	3	1
317	3.7	1.5	2.5	1
318	3.5	1.3	2.7	2
319	4.2	1.2	3.5	3
320	4	1.6	2.5	1
321	4	1.8	2.2	3
322	3.2	1.5	2.1	1
323	3	1	3	1
324	3.5	1	3.5	1
325	3	1	3	3

(Apice angle: 1- obtuse, 2- intermediate, 3-acute)

Appendix C: Disk Floret and Tube length and Ratio

Population #	tube (mm)	floret (mm)	floret to tube
301	0.3	3.5	11.7
302	---	---	---
303	2	3	1.5
304	1.5	4	2.7
305	0.7	2.2	3.1
306	0.5	2.2	4.4
307	1.2	3	2.5
308	1.5	2.7	1.8
309	2.8	2.5	0.9
310	1.7	2.4	1.4
311	1.7	4.5	2.6
312	1.7	4	2.4
313	1.2	3	2.5
314	1.2	3.4	2.8
316	1.2	2	1.7
317	1.4	3	2.1
318	1	3.5	3.5
319	1.2	2.2	1.8
320	2	2.7	1.3
321	1.3	2.5	1.9
322	1.5	2.2	1.5
323	1.2	2.5	2.1
324	---	2.7	---
325	1.3	2	1.5

Appendix D: Disk Floret Surface Cell Lengths

Population #	cell length (mm)
301	0.04
302	0.04
303	0.05
304	0.04
305	0.06
306	0.04
307	0.05
308	0.03
309	0.04
310	0.04
311	0.07
312	0.1
313	0.07
314	0.04
316	0.04
317	0.04
318	0.05
319	0.05
320	0.05
321	0.04
322	0.04
323	0.06
324	---
325	0.03

Appendix E: Involucre Bract Length and Width

Population #	length (mm)	width (mm)
301	4.7	5.1
302	5.8	2.1
303	7	1.4
304	18	1
305	5.8	1.5
306	5.5	1
307	6	1.4
308	6	1.2
309	12.5	2
310	12	3
311	4.9	1.3
312	8.2	1.2
313	9	1.3
314	7.5	1.2
316	5.7	1.5
317	---	---
318	9	1.2
319	8	1.5
320	5.2	1
321	10	1
322	6.5	1
323	5.5	0.7
324	7.2	1
325	5.5	1.1

Appendix F: Chaff Length and Width

Population #	length (mm)	Width (mm)
301	7.5	1
302	5.5	1
303	6	1.5
304	7	0.5
305	4.5	0.7
306	3.3	0.6
307	6	0.7
308	5.2	0.7
309	7	1
310	6.8	2.2
311	4.5	0.4
312	7	1
313	7	0.5
314	---	---
316	4.7	1
317	6.8	0.7
318	5	0.7
319	4.3	0.8
320	5.5	1
321	5.5	0.8
322	5.5	0.7
323	4.2	1
324	---	---
325	5.2	1

Appendix G: Leaf Length and Width, Margin, and Presence of Auricles

Population#	leaf length(cm)	leaf width(cm)	leaf margin	auricles present
301	6.5	4	serrate	2
302	6.5	3.5	double serrate	1
303	6	4	serrate	1
304	10	8	crenate	1
305	4.3	2	serrate	1
306	4	3	incised serrate	1
307	4.5	2.3	serrate	2
308	6	3.2	crenate	1
309	12.5	7	double serrate	1
310	5	3	serrate	1
311	3.5	1.2	serrate	3
312	7	3.5	incised serrate	1
313	5	2.5	incised serrate	2
314	8	3.5	irreg. serrate	1
316	5	2.5	serrate	3
317	8	3.5	serrate	2
318	5	2.5	incised serrate	1
319	7	2.5	entire w/serrate	3
320	7	3	serrate	2
321	---	---	---	---
322	3	2	serrate	2
323	4.5	2	serrate	2
324	7	4	irreg. serrate	2
325	4	1.2	serrate	2

Appendix H: Abaxial leaf pubescence length and arrangement

Population #	pub. length(mm)	pub arrangement
301	0.2	L
302	0.5	O
303	0.4	O
304	0.4	L
305	0.2	O
306	0.4	O
307	0.3	L
308	0.3	L
309	0.5	L
310	0.4	O
311	0.4	O
312	0.2	L
313	0.2	L
314	0.3	L
316	0.3	L
317	0.2	L
318	0.3	L
319	0.9	O
320	0.5	O
321	0.3	L
322	0.2	L
323	0.2	L
324	0.2	L
325	0.5	L

Appendix I: Pedicel Length and Stipules Length and Width

Population #	pedicel length(cm)	stipule length(cm)	stipules width(cm)
301	3.5	---	---
302	2	1	0.5
303	2	0.6	0.5
304	5	1	0.5
305	3	0.2	0.1
306	2	0.5	0.3
307	2	---	---
308	2.2	0.1	0.1
309	3.5	1	1
310	2	0.1	0.1
311	0.3	0.4	0.3
312	2	1	1
313	1.5	1	0.5
314	3.5	0.2	0.2
316	2	0.1	0.1
317	2.5	---	---
318	1.5	1	0.6
319	3	1	1
320	2	0.1	0.1
321	---	---	---
322	2.2	0.2	0.2
323	2.5	0.2	0.2
324	3	---	---
325	0.6	0.1	0.1

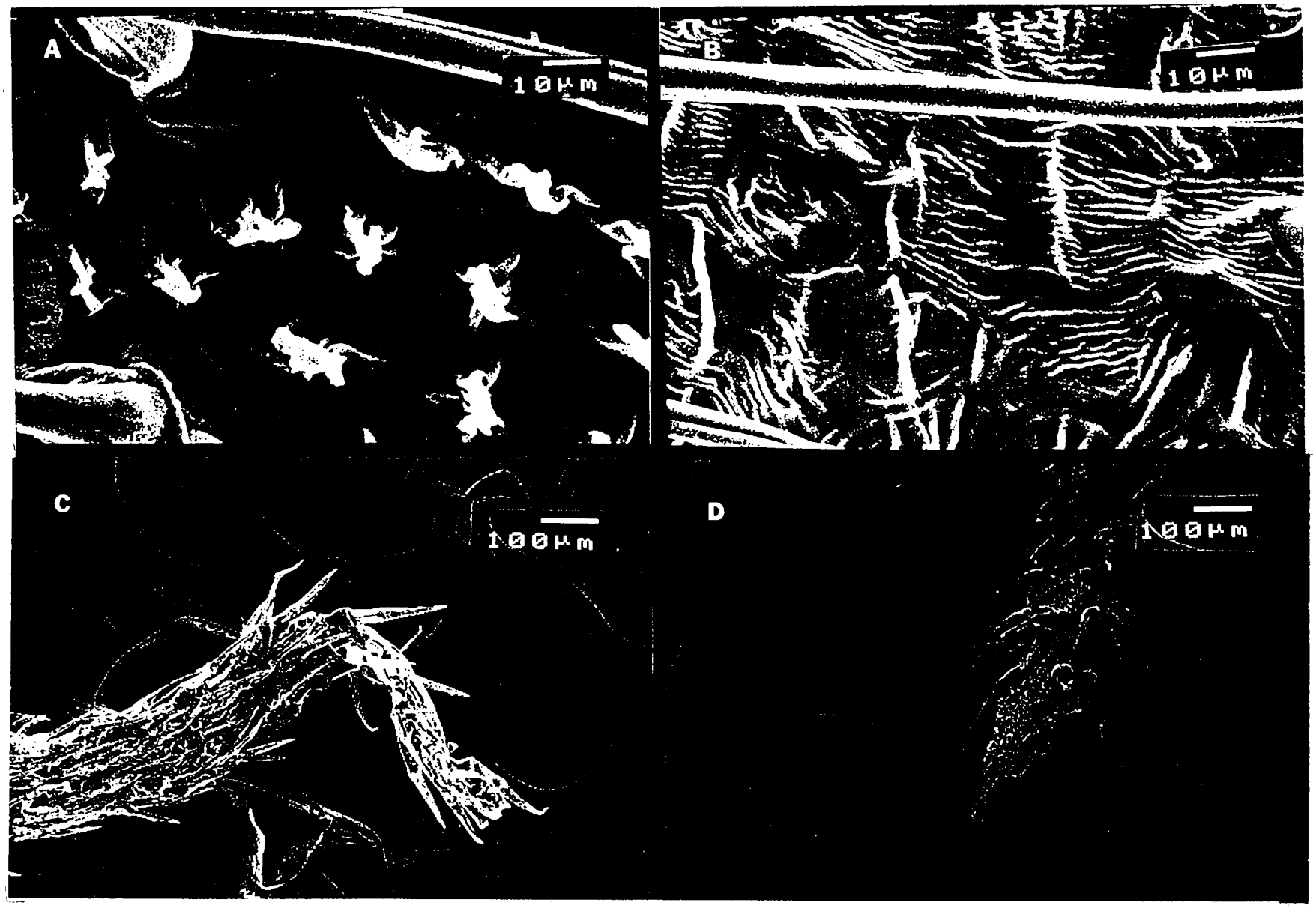

PLATE 1 DISK ACHENE SURFACE AND CHAFF TIP CHARACTERS

A: Pock-marked disk achene surface of population 316 Fochtman 235A V. encelioides cana. B: Smooth disk achene surface of population 321 Boxeman and Radford 11559 V. encelioides encelioides. C: Pubscence on chaff tip of population 302 Britton 5508 V. encelioides encelioides. D: Gland on chaff tip of population 305 Fosberg 53576 V. encelioides cana.

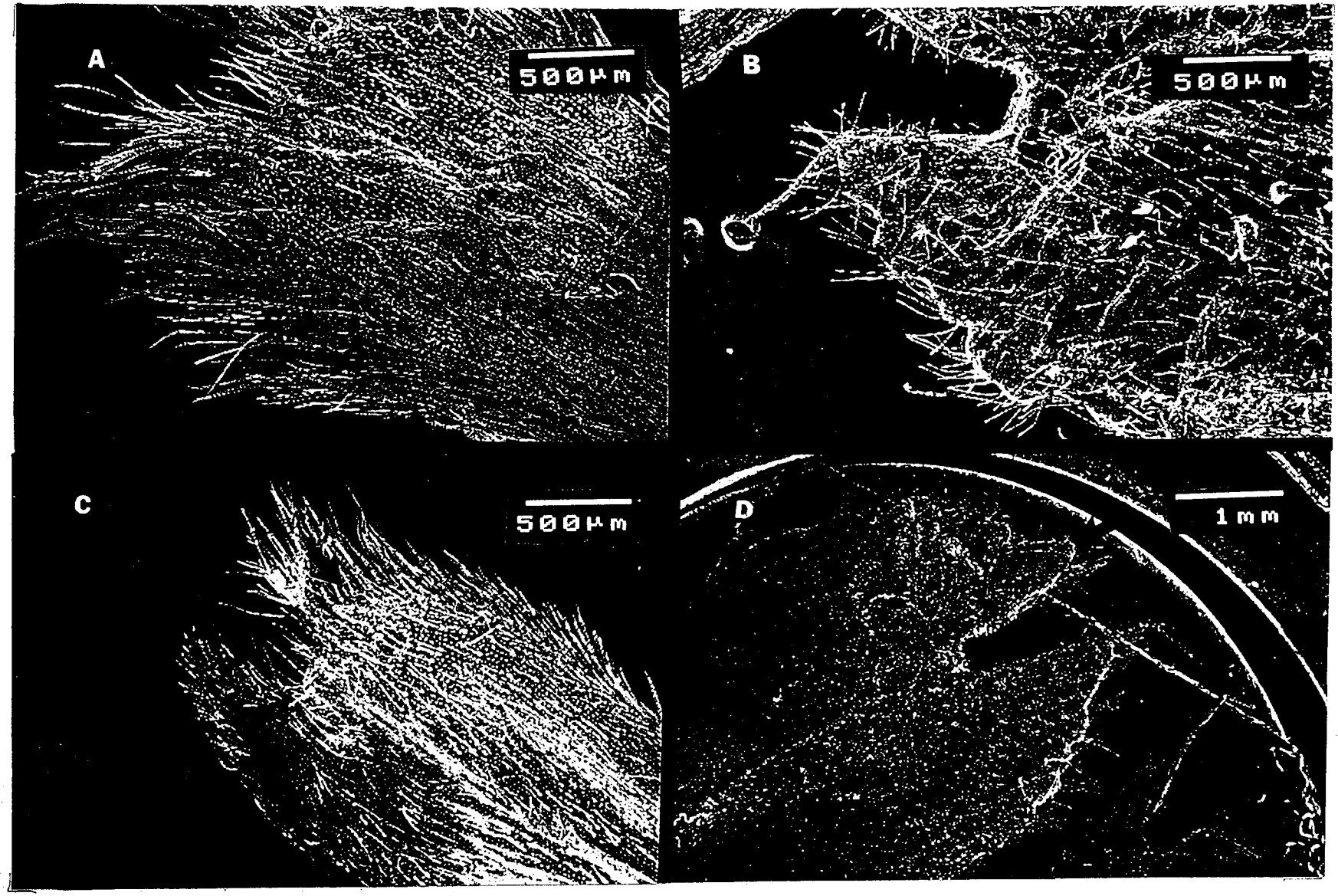

PLATE 2 DISK ACHENE MARGIN AND APEX ANGLE

A: Entire disk achene margin of population 316 Fochtman 235A V. encelioides cana. B: Undulating disk achene margin of population 313 Combs 577 V. encelioides encelioides. C: Obtuse angle disk achene apex of population 323 Fosberg 53874 V. encelioides cana. D: Acute angle of disk achene apex of population 309 Smith 2239 V. encelioides encelioides.

PLATE 3 LEAF PUBESCENCE ARRANGEMENT AND PAPPUS MORPHOLOGY

A: Lanate condition of abaxial leaf pubescence arrangement of population 313 Combs 577 V. encelioides encelioides. B: Tomentose condition of abaxial leaf pubescence arrangement of population 319 Brace 4124 V. encelioides encelioides. C: Typical disk achene pappus morphology of V. encelioides of population 302 Britton 5508 V. encelioides encelioides. D. Atypical disk achene pappus morphology of V. encelioides of population 319 Brace 4124 V. encelioides encelioides.

CATABOLITE REPRESSION OF AMYLASE SECRETION IN *ACHLYA AMBISEXUALIS*

JADD KOURY

ABSTRACT

It has been determined that the fungus-like protist Achlya ambisexualis is capable of using starch as its sole source of carbon and can secrete the starch degrading enzyme amylase into its medium. In tests using a variety of polysaccharides including starch, only the starch served as an inducer of amylase. The presence of free glucose, however, served to reduce the amount of amylase secreted by the organism during growth. This indicates that the secretion of amylase is governed by catabolite repression. Thus, Achlya appears to produce amylase only when starch is present as an inducer, but retains the flexibility to use the more easily metabolized glucose in preference to starch.

INTRODUCTION

Achlya ambisexualis is a fungus-like protist belonging to the phylum Oomycota. The organism is usually found in warm lakes and ponds where it acts as a saprophyte, degrading plant and animal material (Alexopoulos and Mims, 1979). Because *Achlya* and many fungi act as saprophytes, it is essential that they be flexible in the types of substrates they are able to use to obtain carbon (Webster, 1977). Mycologists have discovered that certain types of fungi are capable of using different polysaccharides of varying size as sole sources of carbon; however, there are certain criteria which must be met in order for the fungus to be able to use a carbon source other than glucose. For instance, in all fungi or fungus -like protist, the ability to use a high molecular weight carbon compound (i.e. polysaccharides) depends on the ability of the fungus to have a method for hydrolyzing sugars of longer chain lengths and a carrier system to transport the hydrolyzed sugar into the cells. (Evans Garraway, 1984). The most common method of hydrolyzing the polysaccharide is through the secretion of extracellular enzymes (Evans and Garraway, 1984). One such enzyme of interest is the starch degrading enzyme amylase which is secreted by some fungi. The study of many nonphytopathogenic fungi such as *Aspergillus niger* and *Trichoderma viride* has helped to characterize two major classes of inducible starch-degrading amylases, alpha amylase and glucoamylase (Schellart et al., 1976). Whether or not either of these enzymes is secreted by all fungi is currently not known. Therefore, I decided to focus my research on a particular fungus whose capabilities for using certain carbon sources and for producing certain amylases has not been fully explored, based on the published materials dealing with this organism. My research focused on *Achlya ambisexualis* and was done in order to determine if *Achlya* can produce the substrate-specific enzyme amylase, and to see if the organism can use starch as its sole source of carbon. Other processes investigated were whether or not *Achlya* could use other polysaccharides as its sole source of carbon and how the presence of free glucose and starch in the organism's

environment and growth medium would affect its secretion of amylase.

MATERIALS AND METHODS

One-hundred milliliter volumes of sterile defined liquid growth medium (Mullins and Barksdale, 1965) were made, which varied only in the concentration of carbohydrate present. Single carbohydrates were used at 0.2% weight by volume ratios. To determine the effect of glucose upon amylase secretion, media containing 0.2% soluble starch as an inducer were supplemented with various concentrations of glucose, as indicated in Figure 1. The flasks were inoculated with zoospore suspensions from *Achlya ambisexualis E 87* strain. The suspensions came from first aseptically growing the organism on a series of standard agar plates which contained peptone, yeast extract, and glucose. After a period of approximately 48 hours, the growth obtained on the agar plates was then aseptically cut out and transferred to a series of 250ml Erlenmeyer flasks containing 100ml of a sterile solution of 0.50mM $CaCl_2$. The flasks were allowed to shake for two hours at room temperature (25° C). After two hours, the liquid in the flasks was aseptically poured off and a new solution of 0.50mM $CaCl_2$ was added. The flasks were then allowed to shake overnight for approximately 17 hours, and approximately 100,000 spores were used to inoculate 250ml Erlenmeyer flasks containing 100ml of the defined liquid growth previously mentioned. Spores were counted using a hemacytometer. The cultures were then incubated at 25° C in a shaking water bath and were harvested on weight tared filter papers using a Buchner funnel, after growth periods of 0, 24, 36, 48, 60, 72, and 96 hours of growth. The filter papers were dried in an oven at 80° C for approximately 24 hours, and reweighed to determine mycelial dry weight. The growth media obtained at each harvest were saved and frozen at -80° C so that the necessary assays could be performed.

Reducing sugars were assayed with the DNSA reagent (Miller, 1959). The results for this assay were determined spectrophotometrically by measuring absorbance at 540nm. Soluble starch was assayed with I_2KI using a modified version of the assay used by Bhella and Altosar (1985). The absorbance was measured spectrophotometrically at 550nm. Amylase activity was assayed by monitoring the rate of disappearance of a soluble starch substrate, at 40° C, and pH 6.9. One unit of activity is defined as the amount of enzyme which will cause a 0.10 drop in absorbance at 550nm over a 10 minute period.

RESULTS

After performing all the necessary assays, graphs of the data were made for analysis and interpretation of the experiments. In Figure 1, one can see that the concentration of starch decreased over time. If one then looks at Figure 2, a definite trend can be seen between the concentration of starch and enzyme. It can be seen that as the concentration of amylase increases, the concentration of starch decreases. Figure 3 shows that the presence of free glucose in the growth medium has a definite repressive effect on the amount of amylase secreted. As indicated from

Figure 4, the dry weights obtained from the use of different polysaccharides proves that *Achlya* can use different polysaccharides as its sole source of carbon quite efficiently. In Figure 5, it can clearly be seen that the only polysaccharide associated with the secretion of meaningful amounts of amylase is starch.

CONCLUSIONS

Based upon the data from my experiments upon *Achlya ambisexualis*, the following things were concluded. *Achlya ambisexualis* can use soluble starch as its sole carbon source (Figure 1), and growth on a medium containing starch is accompanied by the secretion of the extracellular enzyme amylase (Figure 2). By analyzing the dry weights obtained (Figure 5), it was further concluded that the organism can use other polysaccharides as sources of carbon for metabolism. However, only growth on starch is accompanied by amylase secretion. This indicates that starch is a highly specific inducer of amylase production. Finally, the presence of free glucose in the growth medium, as indicated by Figure 3, apparently serves to reduce the amount of amylase secreted by the organism during growth. Thus, *Achlya* appears to produce amylase only when starch is present as an inducer, but it retains the flexibility to use the more easily metabolized glucose in preference to starch.

Previous researchers have obtained results which further support my findings. For example, experiments, similar to those presented in this paper, have been performed which the thermophilic fungus *Talaromyces emersonii.* This organism was allowed to grow in the presence of different carbon compounds to be used as its sole source of carbon. Compounds used for these experiments included ribose, xylose, rhamnose, sorbose, glucose, starch etc... (Evans and Garraway, 1984) From these experiments, it was determined that not only could *Talaromyces* use compounds other than glucose as its sole source of carbon, but only starch served as an inducer of amylase production (Evans and Garraway. It can also be noted that just as in my results, the amount of amylase secreted by *Talaromyces emersonii* increased as the concentration of starch in its growth medium increased. These results further support my results which proves that it is possible to induce the production of amylase in some varieties of fungi and fungus-like organisms, specifically *Achlya ambisexualis.*

Since it has been determined that *Achlya ambisexualis* can in fact produce amylase, the next question to ask is how does this ability to secrete amylase allow the organism to fit into its ecological niche? One answer to this question may be that amylase is secreted if or when the organism has begun to grow on the seeds of dead flowering plants. Because these seeds contain starch, amylase is needed by *Achlya* to access this usable source of carbon (Alexopoulos and Mims, 1979). One can see that the ability of *Achlya ambisexualis* to secrete amylase is important for its proper functioning and survival in its ecological environment.

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Figure 1: Starch degradation by the organism

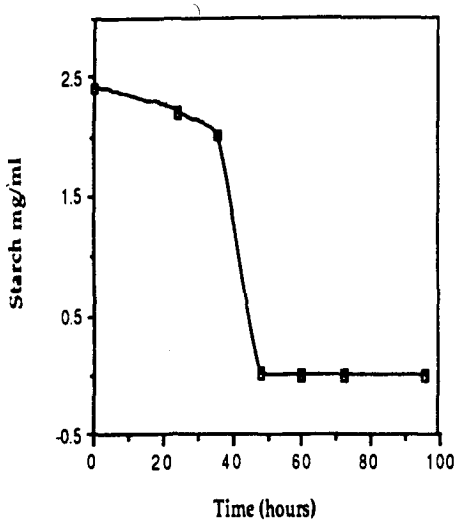


Figure 2: The Inducing Effects of Starch

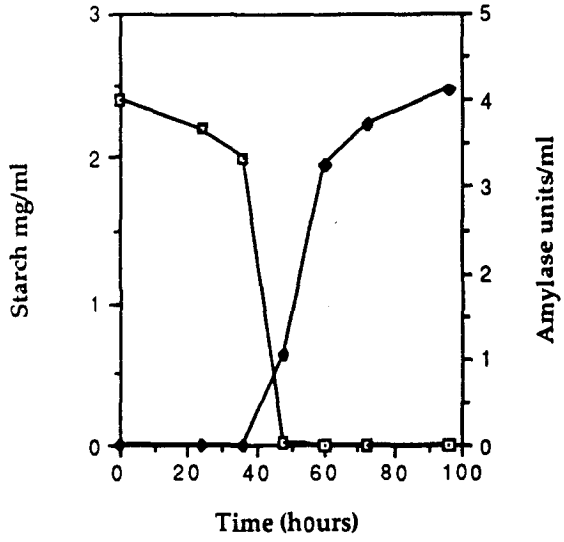


Figure 3: The Repressing Effects of Glucose

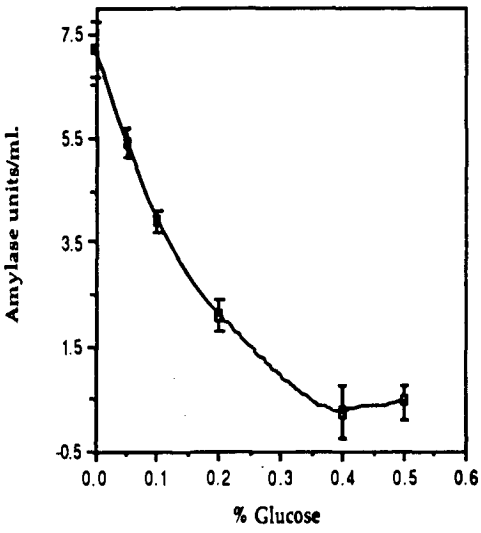


Figure 4: The Use of Different Polysaccharides for Carbon

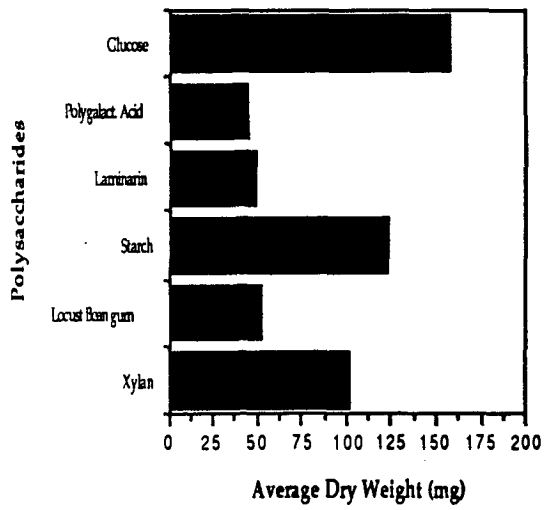
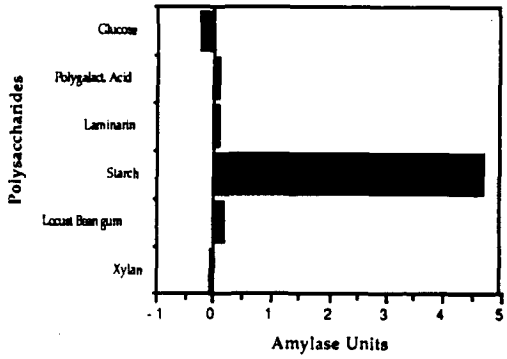


Figure 5: A Comparison of the Inducing Effects of Different Polysaccharides



A STUDY OF SELECTED GENES INVOLVED IN MURINE LEUKEMIA

KRISTEN ROTHAMMER

ABSTRACT

The purpose of this experiment is to analyze two genes involved in murine retrovirus-induced leukemias: Bcl-2 and Hox-11. Bcl-2 and Hox-11 are genes of interest for the following reasons: they are rearranged in certain human leukemias, and they play important roles in cell growth and development. It has been deduced that rearrangement in the regions of these genes could play a significant part in the onset and proliferation of leukemia.

In the first section of this experiment, DNA from different cell lines was probed for rearrangement in the Bcl-2 and Hox-11 loci. No rearrangement was apparent form the results in probing with Bcl-2; therefore, it was deduced that genetic rearrangement of the Bcl-2 gene was not involved in these leukemias. The human Hox-11 probe did not hybridize to the mouse DNA because of lack of significant homology between mouse and human. The experiment was found inconclusive, and will be continued when a mouse Hox-11 probe is available.

INTRODUCTION

When a virus integrates into a region of DNA, it can disrupt normal cell cycle regulation, growth factor response, and the ability of the cell to differentiate [1]. It has been deduced that these factors can lead to the induction and proliferation of leukemia. Oncogenic retroviruses can be divided into two major groups: 1) acute transforming retroviruses, which trigger rapid proliferation of the disease, and 2) non-acute transforming retroviruses, which need a longer time period to induce the disease [2,3]. When a retrovirus inserts itself in the host genome, a number of changes can occur in the host DNA. The retrovirus can induce cells which are normally expressed to turn off, while cells that are not normally expressed can be induced to turn on. Insertions and deletions in the host genome may also result as a consequence of viral integration. Tumor induction relies upon the cellular genes that have been mutated, activated, or inactivated as a result on the integration of the retrovirus [1]. The retrovirus can serve to mutate an existing virus into a form that is still recognizable, or can create a novel gene through multiple mutations.

In studying retroviruses and the genes which are involved in the proliferation of leukemia, one of two primary approaches is taken. One method involves the screening of regions of virus-containing DNA (taken from leukemic cells) for insertions and deletions. The other method focuses on cloning the locus

containing the retrovirus, and looking for novel genes in that region. The first method of screening is implemented in the following study of selected genes involved in murine leukemias.

INTRODUCTION AND PURPOSE (Experiment A):

The purpose of the first section of this experiment is to look for genomic rearrangement in the Bcl-2 and Hox-11 loci of selected mouse cell lines derived from retrovirally-induced leukemias. Bcl-2 and Hox-11 are genes of potential interest for the following reasons: they are rearranged in certain human leukemias (due to a chromosomal translocation), and they play important roles in cell growth and development. Current evidence suggests that Bcl-2 prolongs the life span on a cell, and Hox-11 is known to be a very important developmental gene. Rearrangements in these regions could play a significant role in the onset and proliferation of leukemia. Using the technique of southern blot analysis, I will look for genomic rearrangement of these two genes.

MATERIALS AND METHODS (Experiment A)

DNA from 38 different cell lines was digested overnight at 37 degrees Celsius with the restriction enzyme Sst1. The digested DNA samples were run overnight on a 0.8% agarose gel. The DNA in the gel was transferred to a membrane using the southern transfer technique. The membrane was prehybridized to block nonspecific binding sites, and was then hybridized to a probe specific to the Bcl-2 gene. The membrane was washed and placed in a cassette with X-ray film at -70 degrees Celsius for two weeks. The film was then developed, and areas of radioactive exposure were analyzed.

RESULTS AND CONCLUSIONS (Experiment A)

From the X-ray film, it was deduced that digestion with Sst1 results in two fragments, approximately 3.1 and 13 kb in length. There were some inconsistencies on the X-ray film. In the lane which contained RL-12 DNA, a DNA fragment was found that was not present in the other 37 cell lines tested. RL-12 is a cell line which is derived from a different strain of mouse than the other cell lines, so it is possible that this band represents a polymorphism, a genetic difference between the strains of mice. Since the band is somewhat fuzzy and not very distinct, it is also possible that the band is merely an imperfection or random spot that has appeared in a location that would lead us to think it was a band. To see if this band represents a polymorphism, an artifact, or a true band, further testing will be done using RL-12 genomic DNA.

INTRODUCTION AND PURPOSE (Experiment B)

The purpose of this experiment is to determine if the previously discussed band found in the digestion of RL-12 is a polymorphism, an artifact, or a true band. NFS-112 will be used as a control, since exhibits the expected (normal) restriction fragments when digested. Both RL-12 and NFS-112 will be digested with Sst1, and the same methods will be used as in Experiment A. If the anomalous in RL-12 is an artifact, it will not be seen on the X-ray film (or autorad) in this experiment. Artifacts on autorads are randomly occurring imperfections on the film (caused by water on the film or nonspecific binding of radioactive DNA to the membrane), and there is practically no chance that two such imperfections will occur in the same place or even have the same shape.

The experiment will also be carried out using BamH1 and HindIII restriction digests of RL-12 and NFS-112. These two enzymes cut in sites close in proximity to the Sst1 site. If the band of interest is a polymorphism, the digests with Sst1 will exhibit bands different from NFS-112. The digests of RL-12 with BamH1 and HindIII will probably also exhibit bands different from those of NFS-112 in the case of a polymorphism.

This experiment is not designed to specifically distinguish between results that would suggest a polymorphism and those that would indicate genetic rearrangement. In the event that the result do indicate a polymorphism or genetic rearrangement, testing could be done to compare these results with those that would be obtained for a normal mouse from the same strain as RL-12. This experiment was not done at this time since DNA of this type was not readily available.

MATERIALS AND METHODS (Experiment B)

In this section of the experiment, RL-12 was digested with the enzymes Sst1, HindIII, and BamH1. NFS-112, the control, was also digested with these enzymes. The experimental technique for running the gel, southern transfer, and hybridization was exactly the same as in Experiment A.

RESULTS AND CONCLUSIONS(Experiment B)

The specific band of interest did not appear in the resulting autorad; therefore, we can deduce that the aforementioned band was an artifact. There was no apparent difference in the restriction fragments of NFS-112 and RL-12 for any of the selected enzymes.

From the experiments in parts A and B, we can conclude that genetic rearrangement of the Bcl-2 gene was not involved in these mouse leukemias.

MATERIALS AND METHODS (Experiment C)

In this experiment, 34 cell lines were digested with Kpn1. The rest of the experiment was carried out in exactly the same manner as Experiment A, except that the human probe for the Hox-11 gene was used instead of the Bcl-2 probe.

RESULTS AND CONCLUSIONS (Experiment C)

The autorads show absolutely no indication of hybridization. The lack of any specific hybridization suggests that there is insufficient homology between the human Hox-11 gene and the corresponding mouse gene. A mouse probe for the Hox-11 gene is not available at this time. The project could be continued in the future by finding the region in mouse DNA complementary to the Hox-11 gene and producing a probe complementary to this region. The experiment could then be repeated with this probe. Alternatively, these blots could be rehybridized and washed under conditions of reduced stringency to see if any weak hybridization was occurring.

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SUCKLING BEHAVIOR IN CALIFORNIA SEA LION PUPS AND JUVENILES AT LOS ISLOTES, BAY OF LA PAZ, MEXICO

COURTNEY SCHWARTEN, ROBIN DRAHEIM AND NADINE PAFFETT-LUGASSY

ABSTRACT

Age related changes in suckling strategies of California Sea Lions were studied at Los Islotes, in the Bay of La Paz, from September 21, 1992 through November 24, 1992. Little is known about age related changes in pinniped lactation. Suckling data were recorded for pups and juveniles using one minute scan sampling which looked for three states of behavior; suckling, resting and active. The pup data indicated the mean suckling bout length (MBL) to be 22.97 +/- 1.6 minutes, inter-bout interval (IBI) to be 32.95 +/- 3.6 minutes and percent time spent suckling to be (MTS) 21.32 +/- 1.9 minutes. These data were then compared with prior suckling data collected on the same cohort July through August 1992. T-tests were used to determine the significance of these comparisons. MBL had a significant increase, IBI showed no significant change and MTS showed a decrease in percent suckling time. The increase in MBL was determined to be a good indication of age related changes in the suckling behavior of the California sea lion.

INTRODUCTION

The purpose of this study is to examine the age related changes in the suckling behavior of the California sea lion, Zalophus californianus. Previous studies at the Los Islotes rookery and elsewhere in the Gulf of California have observed suckling of pups 0-3 months of age. Among other things, this study will observe the suckling behavior of the pups the age of 3-6 months and juveniles (1-2 years of age) at the Los Islotes rookery.

The California sea lion (Zalophus californianus) is found in the coastal waters of the Eastern Pacific, ranging from the colder waters of Western Canada to the subtropic climate of Mazatlan, Mexico (Odell 1981). In 1983 the total California sea lion population was estimated at 145,000 animals, 15% of which were located in the Gulf of California (Le Boeuf et al. 1983). A small resident population of sea lions inhabit the island rookery, Los Islotes in the Gulf of California. Censuses taken from June 18, 1992 through July 10, 1992 indicated a population of 227 animals living at Los Islotes (Jones 1992).

On Los Islotes sea lion pups are born in May and June (Aurioles 1988) and maternal care begins immediately with lactation, as suckling begins within hours of birth (Riedman 1990). In the South American Fur Seal the mean length of suckling bouts is related to age. The total proportion of time spent suckling also increases approximately 10% in the first 25 days of life (Harcourt 1990). Lactation is the primary aspect of parental investment in pinnipeds, lasting over 365 days and up to

2 years (Riedman 1990). Parental investment can be defined as anything done by the parents for the offspring which reduces the parents' ability to invest in other offspring (Trivers 1972 - as cited by Alcock 1989). Parental expenditure is the cost which the parent expends on care for their offspring. In Otariids (a family of Pinnipeds that includes the California sea lion), paternal care does not exist (Trillmich 1990). It is therefore necessary to measure maternal effort to calculate parental effort.

The amount of energy provided to a pup by its mother in the form of milk represents the major proportion of maternal effort in pinnipeds (Oftedal et al. 1987b). The mother's milk contains a large percentage of fat and provides the pup the nutritional intake it needs to survive. Trillmich and Lechner (1986) found a correlation between the length of foraging trip of the mother and the milk fat content. Their studies showed that the animals that forage for more days in succession have a much higher milk fat content. Other factors can affect the length of a foraging trip, therefore affecting lactation patterns.

Some environmental changes can affect lactation patterns. For example, the El Niño phenomena is one such occurrence which can cause a significant decrease in numbers of an entire population. The rising of water temperatures corresponding with El Niño in the eastern Pacific ocean reduces the abundance of food resources; the reduction in food availability increases the duration of time the mother must spend foraging, therefore increasing the time the pup must survive on its own. If a pup is abandoned for too much time, it may starve to death. The longer a mother must spend foraging, the greater the risk of the loss of the pup. El Niño can affect whole sea lion populations by reducing food resources, increasing the time and energy it takes to find. However, past studies on El Niño (1982-1983) found that the California sea lion population at Los Islotes was barely affected (Aurioles and le Boeuf 1990).

Another factor that affects lactation patterns in some pinnipeds is differential investment. In some species of polygynous and sexually dimorphic mammals, a lactating mother will show preference to male offspring (Clutton-Brock 1991). If greater investment in the sex with the greater variance in lifetime reproductive success (LRS) can increase that individuals reproductive success (RS), then females may be selected to invest differentially in their offspring according to the sex of the offspring. Sea lions are sexually dimorphic animals with greater variance in male than female RS. Research on differential investment in the resident population at Los Islotes found no difference between the effort put into male and female offspring (Pagana and Sauer 1992).

Larger animals are capable of assimilating greater quantities of milk due to their larger size (Oftedal 1981 - as cited by Clutton-Brock 1991). If bout lengths and proportion of time suckling are related to the quantity of milk intake, as in the Galapagos fur seals, then pup suckling behavior may provide an accurate measurement of maternal expenditure (Trillmich 1986).

Our study on the population at Los Islotes will look at age related changes in suckling behaviors and if these changes in suckling behavior are related to changes

in lactation patterns, and therefore, maternal expenditure.

MATERIALS AND METHODS

Observations were conducted at the Los Islotes rookery (24 35'N, 110 23'W) in the Bay of La Paz, Baja California Sur, Mexico (Aurioles et al. 1984). Data were collected for a total of 35.25 hours (total pup hours were over 157 hours) from cliffs approximately 5 meters above the haul out areas, well out of range of disturbing the seal lions.

Using 35x7 binoculars or the naked eye, data was collected from various observation points, according to where suckling or potential suckling was present. The dates of data collection were 9/21-24, 10/6-7, 11/3, 11/10, and 11/24. Data was taken for a four hour continuous time block starting in the morning and usually ending early in the afternoon, duplicating the methods used by Pagana and Sauer (1992) on the same pup population.

On these 9 days, observations were made using a population scan each minute for a four-hour time block. Minutes were indicated by a wrist watch beeper. Data was collected on the mother-pup pairs and the mother-juvenile pairs observed. The behaviors of the pups and juveniles each minute were recorded as one of three categories: suckling, active, and resting. These categories were defined as follows (Harcourt 1990):

ACTIVE - moving around, not resting

RESTING - lying in a relaxed position

SUCKLING - the off-spring's mouth touches the nipple of its mother and appears to suck

The proximity of mom and pup was recorded as either <1 meter or >1 meter, and additional observations were made when possible on the sex of the pups and juveniles and any identification marks on mothers, juveniles, or pups.

Discrete suckling bouts were distinguished using log survivorship analysis - a graphical method for calculating the minimum interval separating successive bouts (bout criterion interval - BCI) (Martin and Bateson, 1986). From the log survivorship plot, the bout criterion interval was determined to be 6 minutes. Mean bout length and % time suckling were significantly correlated ($r=.61$, $n=52$, $p<.001$) (Figure 1). All mean values will be expressed +/- s.e.

RESULTS

Using the bout criterion interval (BCI) we totalled our data into suckling bouts. Mean bout length (MBL), inter-bout interval (IBI) and mean time spent suckling (MTS) and activity budget data were calculated for pups and suckling juveniles. In pups MBL was found to be 22.97 +/- 1.65 minutes (n=92), IBI was 32.95

+/- 3.6 (n=38), and MTS was 21.32 +/- 1.90 (n=53).

Our data from pups 3-6 months of age were compared with that collected by Pagana and Sauer on pups 0-3 months of age (1992) (Table 1) using two sample t-tests. This comparison showed that no statistically significant changes occurred in the IBI (calculated t, t=0.357, n=80, n.s.), whereas the MBL showed a significant increase (calculated t, t=1.37, n=44, $p<0.05$), and MTS showed a statistically significant decrease (calculated t, t=3.65, n=50, $p<0.05$).

Similar comparisons were carried out between pups 3-6 months of age and juveniles. No significant change was found in MBL (calculated t, t=0.102, n=100, n.s.). There was a significant decrease in MTS for the juvenile animals observed suckling (calculated t, t=2.91, n=35, $p<0.05$)

Activity budget breakdowns were calculated for both the pups and the juveniles observed suckling (Figure 2). Pups were found to spend 21.3% of their observed time suckling, 20.5% resting and 19.5% active. The remaining 38.7% of the time where the pups were out of our site or in the water, and therefore, behavior is unknown. The juveniles observed (n=5) spent 10.9% of their time suckling, 25.0% of the time resting and 12.8% active. 51.3% of the time the behavior was unknown.

CONCLUSIONS

Our research studied changes in suckling behavior of the California sea lion due to age. We compared data on 3-6 month old pups with data on 0-3 month old pups (Pagana and Sauer 1992) and juveniles (1-2 years old), all of the same cohort.

In comparing our observations of 3-6 month old pups to those made by Pagana and Sauer (1992) on pups 0-3 months old, we found a significant increase in mean bout length (MBL). This increase in MBL indicates that age related changes do occur. MBL is an indicator of milk intake and production of milk by the mother. An increase in MBL means the mother is producing more milk for the pup, indicating that maternal expenditure does increase with age (Costa 1991).

Our data collection on juveniles was limited (n=9). Most pups cease suckling after one year, therefore a relatively small number of juveniles can still be observe suckling. In our data, no significant change was found in the mean bout length from 3-6 month old pups to 1-2 year old juveniles. This is accounted for by our small sample size.

In contrast to our findings of increased bout length from 0-3 month to 3-6 month old pups, percent time suckling (MTS) decreased. In comparing the MTS, the older pups (3-6) suckled for a much shorter time overall. We concluded that this difference with data is the result of an inflated pup sample population (n) due to observations of unmarked animals. It is possible that one pup may have been recorded as two separate animals. The large number of unidentifiable pups was due to the molting of previous marked animals during our study time. Without marked animals it was extremely difficult to follow one mother-pup pair for the entire observation time. Juveniles did however show a definite increase in percent time suckling. Juveniles have most likely begun weaning and no longer rely on

milk as their only source of nutrition (Riedman 1990).

The inter-bout interval (IBI) had no significant change in pups 0-3 months to 3-6 months. Considering this data with the increase in mean bout length (from 0-3 months to 3-6 months), this leads us to believe that the 3-6 month old pups may be digesting milk at a faster rate. In Northern fur seals, for example, during the first few months, suckling periods are limited by the pups ability to suckle and process milk. Suckling coordination and efficiency may increases over time, and the pups digestive system requires time to reach maximum capacity and performance (Costa and Gentry 1986). The limited number of juveniles prevented the collection of data on the inter-bout interval of juveniles.

We did not take into consideration either the effects of El Niño or differential investment in our study, since both were found to have little or no effect on our study population, as suggested by David Aurioles (1990) and Field and Fox (1992). Within a species maternal milk and energy output may differ according to factors such as sex of the young, or environmental conditions (Oftedal et al. 1987a - as cited in Oftedal and Iverson 1987). This is not the case in the California sea lion population at the Los Islotes rookery.

The large amount of human activity in the immediate surrounding waters may have been responsible for disturbances that altered suckling bouts and activity budgets of the study animals. Female and juvenile sea lions are known to be disturbed by approaching snorkelers and will leave the area when disturbed. When snorkelers come within 5m of the animals females will leave 51% of the time and juveniles 59% of the time (Cox et al. 1992). Human disturbance must be a consideration. However, the Los Islotes rookery has rather constant traffic, and therefore the disturbance is constant and may not have affected our study. Human disturbance should be a consideration, especially if similar studies are done at another rookery.

A comparison should be done in future studies with the data collected from other California sea lion rookeries in the Gulf of California, since a major shortcoming of the existing information on the duration and frequency of suckling is the almost lack of comparative studies between different populations of the same species (Bowen 1991).

The increase found in mean bout length from 0-3 months to 3-6 months is an important finding. Age related changes do occur in the California sea lion. Studies should be conducted on other rookeries to determine if this measure of change is consistent in the California sea lion population.

note: the original research and paper was completed equally by the three authors. This is a version of the original edited by Courtney Schwarten.

ACKNOWLEDGEMENTS

First and foremost we would like to thank Rob Harcourt for his help, guidance, and all-knowing wisdom. We are, of course, indebted to Jose Sanchez for staying up late with us, answering a million questions about Statgraphics, and teaching us how to imitate sea lions. Thanks also to Lissa Flemming for giving us a better perspective on our project and to Anna Quirk for bringing us cookies and hugs. Thanks to our fellow SFS students who braved the rocks of Los Islotes to help us collect data and keep us sane. Last, but not least, thanks are due to Reggie (SFS mascot) who kept constant vigil with us and licked our faces when we were down (or asleep).

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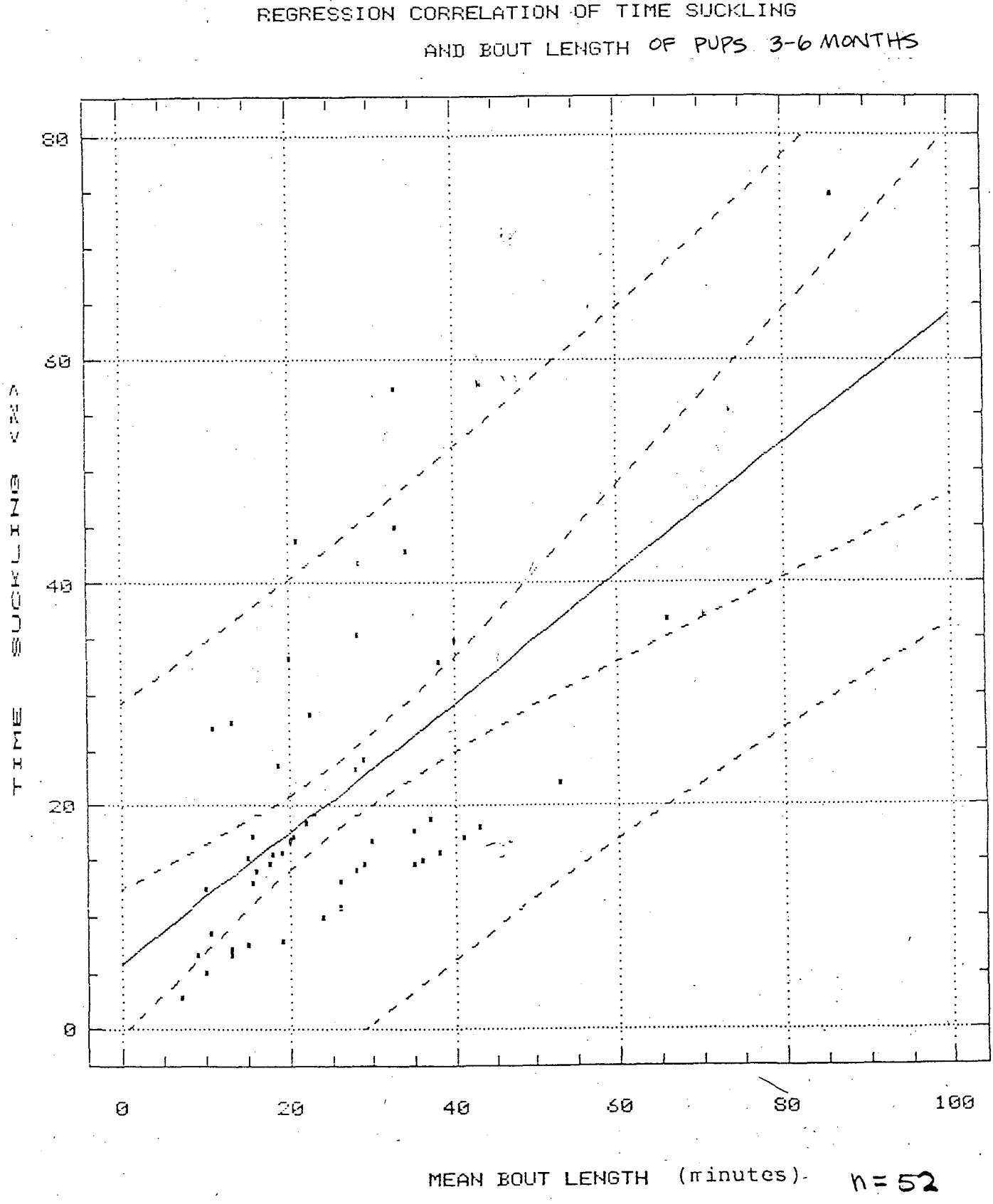


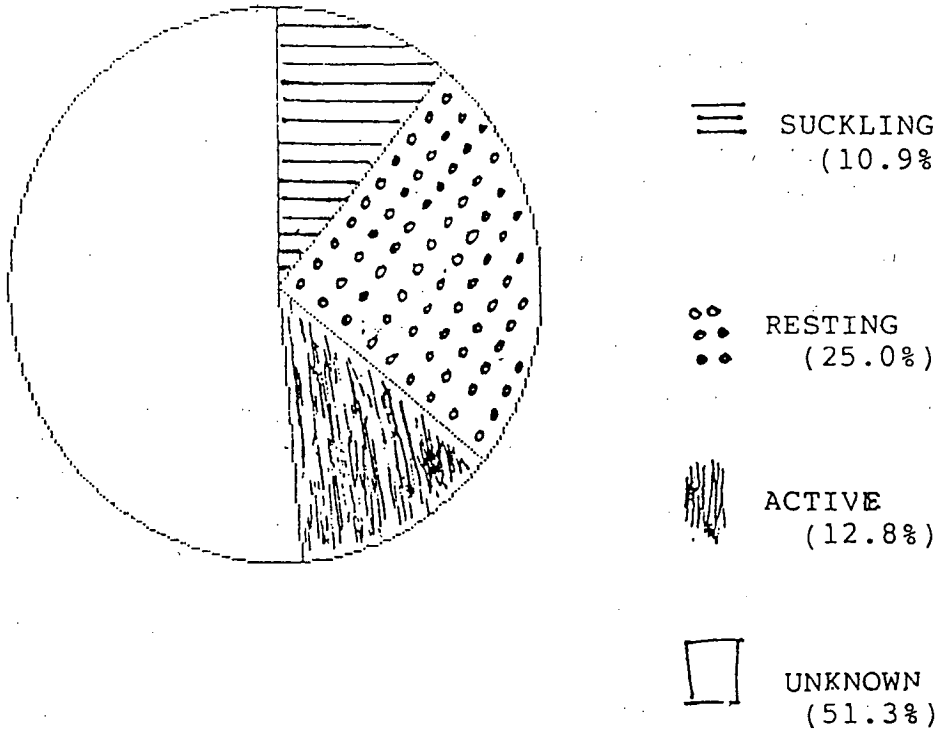
FIGURE 1. Regression correlation of percent time spent suckling and mean bout length (minutes) of California sea lion pups on Los Islotes in the Bay of La Paz, BCS Mexico,

Correlation between Summer 92 and Fall 92 mean suckling determinations of pups		
DESCRIPTION	SUMMER 92 0-3 months	FALL 92 3-6 months
mean bout length	(n=97) 20.4 +/- 0.9	(n=92) 22.97 +/- 1.65
inter-bout interval	(n=44) 34.5 +/- 2.6	(n=38) 32.95+/- 3.6
mean % time spent suckling	(n=43) 44.7 +/- 6.12	(n=53) 21.32 +/- 1.90

TABLE 1. Suckling determination data collected in summer 1992 (Pagana and Sauer, 1992) and fall 1992 on California sea lion nursing behavior of pups on Los Islotes, Baja California Sur, Mexico.

JUVENILE VS. PUP (3-6 MONTHS)

JUVENILE ACTIVITY BUDGET



PUP ACTIVITY BUDGET

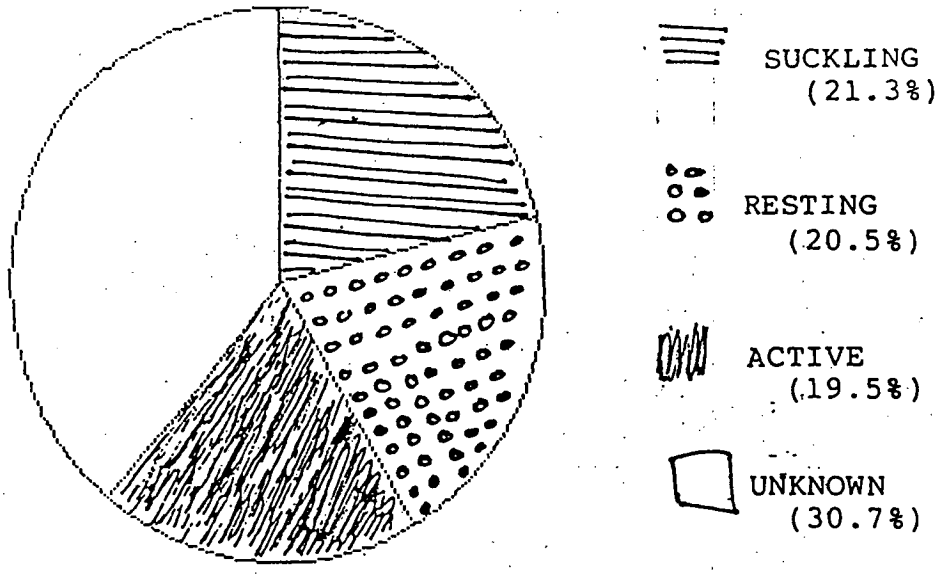


FIGURE 2. Activity budgets of California sea lion juveniles and pups in percent time behavior exhibited during observation.

MEASUREMENT OF ANTI-OXIDANT ABILITY OF DRUGS USED TO TREAT ARTHRITIS

BRIAN J. TIERNEY

ABSTRACT

The goal of this research was to determine the antioxidant properties of several nonsteroidal anti-inflammatory drugs (NSAIDs) and one anti-malarial. These compounds were compared by their ability to compete with carotene for the oxidant, hypochlorite.

The data suggest that some of the newer drugs, such as piroxicam and diclofenac, are more effective anti-oxidants than the older drug, indomethacin. Of the compounds tested, chloroquine displayed the greatest anti-oxidative properties while benoxaprofen showed the most pro-oxidative characteristics.

INTRODUCTION

Arthritis is a disease of unknown cause which results in inflammation of the joints. Rheumatoid arthritis is the most common form. The inflammation may be caused by an excess of prostaglandins which are hormone like substances. The biological effect of prostaglandins are illustrated by stimulation of muscle tissue, regulation of blood pressure, and causing joint inflammation. The production of prostaglandins begins with liberation of arachidonic acid from disrupted lipid membranes catalyzed by phospholipase A_2. Molecular oxygen, with the aid of cyclooxygenase, is added across conjugated double bonds to form cyclic prostaglandin endoperoxides like prostaglandin G_2 (PGG_2). PGG_2 can react to form other prostaglandins which can mediate inflammation (Stinson, 1989). Nonsteriodal anti-inflammatory drugs (NSAIDs), which are widely used in the treatment of arthritis, are known to be inhibitors of cyclooxygenase.

Reactive oxygen species have been implicated in arthritis. In 1988, Hideki Sato, et al., showed that reactive oxygen species can accelerate the degradation of hyaluronic acid in the rheumatoid joint. This is important because the hyaluronic acid seems to play an active role in protecting articular tissues from oxidative damage (Sato, et al., 1988). Many NSAIDs have been evaluated for their effect at reducing the concentration of the reactive oxygen species. In these assays, the reactive oxygen species were generated by enzymes common to inflammation (Twomey and Dale, 1992; Kettle and Winterbourn, 1991). Our concern with the assays is that the NSAIDs could react with the enzymes and lead to a misinterpretation of the results. Therefore, we evaluated the ability of the NSAIDs to remove a model reaction oxygen species, hypochlorite.

An assay based on a competing reaction between NSAIDs and carotene toward hypochlorite was used to rank the antioxidant ability of the NSAIDs. The control was the reaction of hypochlorite with carotene. The rate of reaction of carotene with hypochlorite was followed by the decrease in absorbance with time. A

decrease in the rate of oxidation of carotene indicates that hypochlorite is being removed by the NSAIDs. By comparing the ability of the NSAIDs to compete with the carotene for hypochlorite, we were able to rank the antioxidant ability of the NSAIDs.

Chloroquine, acetaminophen, diclofenac, piroxicam, benoxaprofen, BHT (a anti-oxidative compound found in foods), indomethacin, phenidone and lidocane were evaluated. Of these, diclofenac and piroxicam are among the most commonly used NSAIDs. Chloroquine, an antimalarial drug, and its derivative hydroxychloroquine, have been used to treat arthritis. This drug belongs to a class known as disease modifying antirheumatic drugs. The drugs in this class appear to slow the progress of arthritis (Ward, 1988).

MATERIALS AND METHODS

The carotene was purified by column chromatograph on alumina before using. All NSAIDs used obtained from Sigma Chemical Corporation, St. Louis, MO. The reaction rates were measured by absorbance versus time at a wavelength of 480nm. The measurements were taken on a Hewlett Packard diode array spectrophotometer programmed to take an absorbance reading at 480nm every 20 seconds for 8 minutes (480 seconds). Each evaluation was taken at 30°C. Figure 1 shows some data from the spectrophotometer.

Each trial consisted of 0.1ml of stock drug solution, 0.1ml of stock carotene solution and 2.0ml denatured ethanol. The carotene had a final concentration of 2.9×10^{-7} M in the cuvette. Each sample drug had a concentration of approximately 2.0×10^{-5} M and the NaOCl had a concentration of4.2×10^{-5} M. The sample solution was mixed and then placed in the spectrophotometer for analysis as quickly as possible. The resulting data was then plotted and assigned a best fit third order polynomial line. A tangent was calculated for each set of data at 50, 100 and 200 seconds. The slope of the tangent gave a rate in absorbance units per second. These rates were converted to moles per second.

RESULTS

Of the drugs tested, only two increased the rate of the reaction, as can be seen in Table 1. Figure 2 shows the tested drugs and their effects on the rates of the reaction at 50, 100 and 200 seconds. Indomethacin and benoxaprofen increased the reaction rate with benoxaprofen yielding the fastest rate at all of the times tested. The remaining seven drugs slowed the reaction with the chloroquine showing the slowest rate at all three times. By 200 seconds in the chloroquine reaction, the oxidation of carotene has almost stopped since the hypochlorite is being removed by the NSAID. All of the compounds showed the fastest rate at 50 seconds and slowest rate at 200 seconds except for acetaminophen which gave inverted results with the fastest rate at 200 seconds and the slowest rate at 50 seconds.

CONCLUSION

It is interesting to note that the data suggest there is a correlation between the anti-oxidative properties of the drugs tested and the more widely used NSAIDs. This is to say that the compounds that slowed the reaction more effectively are more popular in the treatment of arthritis. Benoxaprofen and indomethacin yielded unexpected results. Benoxaprofen was the most pro-oxidative compound in the test. Benoxaprofen, was taken off of the market due to problems with its toxicity. We do not known whether there is a relationship between the oxidative qualities of this compound and its toxicity.

The most potent drug in competing with carotene for hypochlorite was chloroquine. This may contribute to its disease modifying antirheumatic properties. We were also surprised by the fact that other NSAIDs like diclofenac and piroxicam are antioxidants. As far as we know, this antioxidant effect has not been reported in the literature. An interesting future study would be to see if this antioxidant effect contributes to their anti-arthritic effect.

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AN ANALYSIS OF CARBON FIXATION PATHWAYS IN THE ROOTS OF SUSPECTED FACULTATIVE C4/CAM EPIPHYTIC ORCHIDS

B.W. WALKER AND J. DAVIS

ABSTRACT

Orchid shoots and roots have been seen to possess different carbon fixation pathways in nature. Some have argued for divergent evolution of carbon fixation pathways in Orchidaceae centered around the idea that form follows function, while others have suggested a linear evolution of carbon fixation pathways based on the premise that more derived CO_2 fixation pathways are simple biochemical modifications of previously existing pathways. An examination of orchid CO_2 fixation pathways was undertaken to see if there was any evidence for the latter by measuring diurnal variation of stomatal resistance in leaves, titration for total acidity in leaf and root tissue, and light microscopy of leaf and root cross sections. We found that it was possible for independent evolution of different carbon fixation pathways to have occurred in the leaves and roots of some tropical epiphytic orchids, though further research is needed to determine the exact nature of C3 or C4 pathways in orchid leaves and roots.

INTRODUCTION

Research shown that orchid shoots can fix carbon dioxide through several pathways: 1) Calvin Benson cycle (C3) 2) Hatch Slack pathway (C4) 3) Crassulacean acid metabolism (CAM) (Salisbury, 1985 and Avadhani, 1982). It has been postulated that orchids which fix CO_2 through the CAM pathway might have evolved from C3 orchids through an evolutionary intermediate C4 form. Although some orchids exhibit C4/CAM photosynthesis in their leaves, no orchid is known to exclusively fix CO_2 via the C4 pathway (Avadhani, 1982).

Aerial roots of many epiphytic orchids contain chlorophyll and have the capacity to fix CO_2, while other aerial orchid roots lack guard cells but are able to fix CO_2 through the CAM pathway (Goh, 1983). An examination of the mode of carbon fixation in aerial orchid roots might reveal more convincing evidence that CAM orchids evolved through C4 intermediates. In this study, we have investigated the carbon fixation pathways in the leaves and roots of tropical epiphytic orchids which were suspected to have facultative CAM or C3/C4 photosynthesis.

MATERIALS AND METHODS

Procedure Determination of carbon fixation pathways in orchid leaves and roots was conducted by: 1) measurement of diurnal variation in stomatal resistance (leaves only), 2) titration for total acidity, and 3) light microscopy of leaf and root cross sections.

Plant Materials Leaf tissue was collected from known C3, C4, and CAM plants (mums, corn, & cacti), while both leaf and root tissues were collected from tropical epiphytic orchids (*Phalaenopsis lueddeuinnani, Brassolva nodosa, Cattleya* Juan Marti, *Cattleya trianae alba, Oncidium spendidum,* and *Cattleya harrisoneae*) that were purchased locally.

Measurement of Diurnal Variation in Stomatal Resistance Stomatal resistance in leaves of both control and experimental plants (which were grown under constant light/dark cycles & temperature) were measured by recording stomatal resistance of five leaves every six hours over a five day period with a transporometer.

Titration for Total Acidity in Leaves and Roots 10 g of leaf tissue was collected from both control & experimental plants 30 minutes before the beginning of each light/dark cycle, while 10 g of tissue was collected from each experimental plant 30 minutes before the beginning of each light/dark cycle. Both leaf and root tissues were ground in a mortar and pestle with a small amount of washed sand, homagenate was then squeezed through cheesecloth into a beaker, and diluted upto 100 ml with distilled H_2O. Malic acid content was then determined by titrating a 50 ml aliquot to the phenophthalein end point with 0.1 **M** NaOH (Johnson, 1985).

Light Microscopy of Leaf and Root Cross Sections Leaf tissue was collected from control plants, while both leaf and root tissues were collected from experimental plants. Both leaf & root tissues then subjected to alcohol dehydration, mounted with pariffin, sliced vertically with a microtone, stained with phloroglucinol, and photographed via light microscope.

RESULTS

Evidence for CAM pathways could easily be seen in the leaves of *Phalaenopsis lueddeuinnani, Brassolva nodosa,* and *Cattleya* Juan Marti (Figures 2 and 4).

Evidence for a probable CAM pathway could be seen in the leaves of *Cattleya harrisoneae,* while a probable C3 or C4 pathway could also be seen in the leaves of *Oncidium spendidium* (Figures 2 and 4).

Contradictory evidence for a C3 or C4 pathway was observed in the leaves of Cattleya triana abla (Figures 2 and 4).

Roots of *Phalaenopsis lueddeuinnani, Brassolva nodosa,* and *Cattleya* Juan Marti were determined to have C3 or C4 pathways, while the roots of *Cattleya harrisoneae* were shown to have a CAM pathway (Figure 5).

Questionable evidence for CAM pathways was seen in the roots of *Cattleya trianae abla* & *Oncidium spendidium* (Figure 5).

No evidence of Kranz anatomy (C4) was observed in the leaves of any tropical epiphytic orchids, while orchid roots displayed visible actinosteles (Figure 6).

CONCLUSIONS

Since different carbon fixation pathways (such as CAM leaves & C3 or C4 roots) were observed in *Phalaenopsis lueddeuinnani, Brassolva nodosa,* and *Cattleya* Juan Marti, it is possible for independent evolution of different carbon fixation pathways to have occurred in the leaves and roots of some tropical epiphytic orchids.

An explaination of questionable CAM pathways in the roots of *Cattleya trianae abla* & *Oncidium spendidum* could be that their aerial roots are mainly heterotropic (Sinclair, 1990) or are capable of "CAM cycling" (Winter, 1985).

Even though no Kranz anatomy was observed in the leaves of any tropical epiphytic orchids, further research is needed to determine the exact nature of C3 or C4 pathways in orchid leaves and roots.

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Figure 1: Transpiration Controls

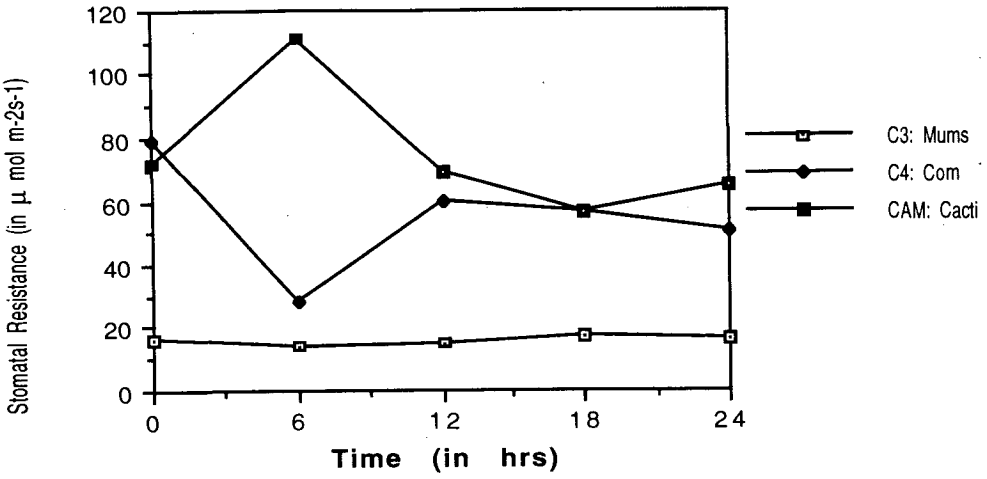


Figure 2: Transpiration Experimentals

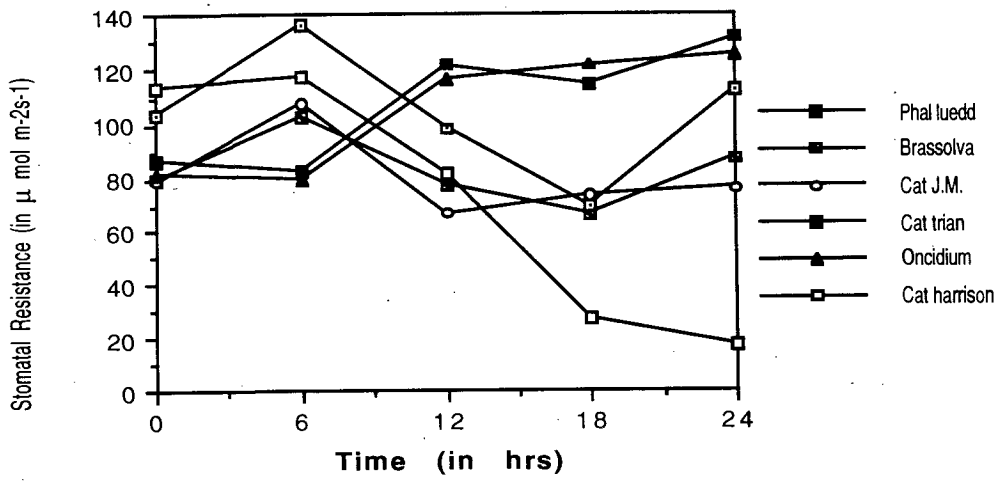


Figure 3: Titratable Acidity in Leaves of Control Plants

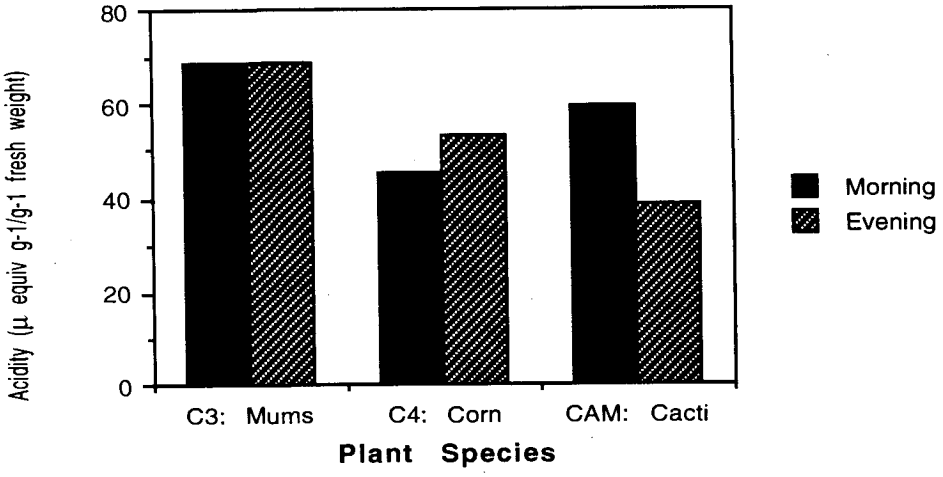


Figure 4: Titratable Acidity in Leaves of Tropical Epiphytic Orchids

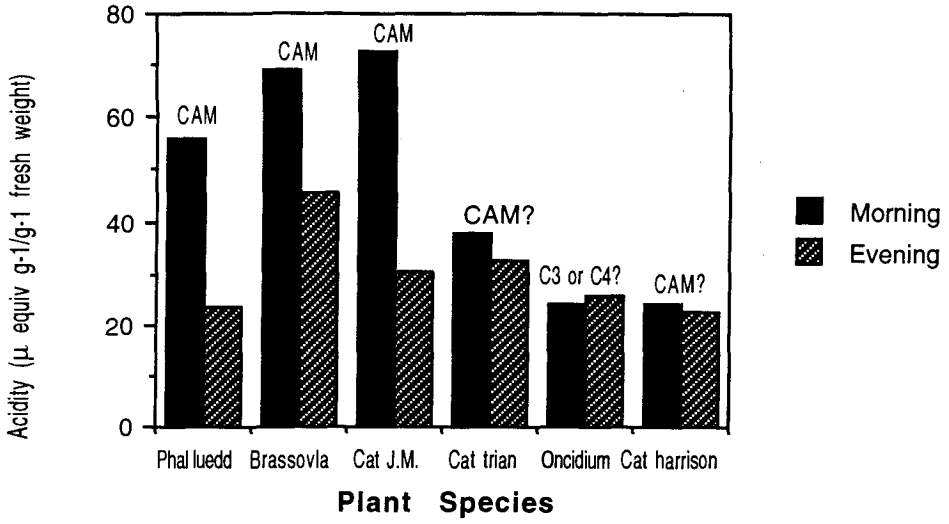
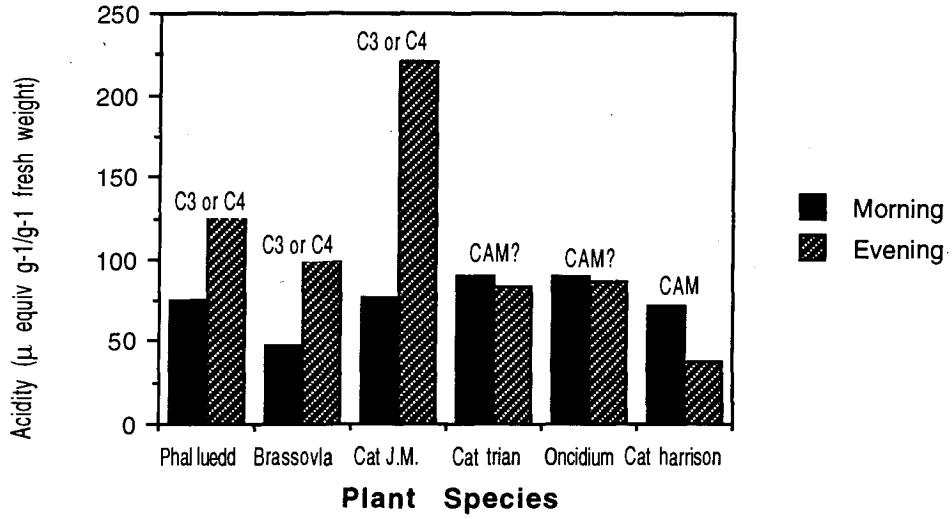
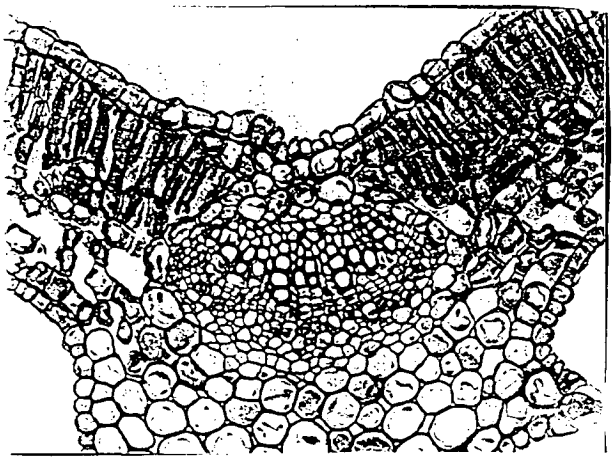
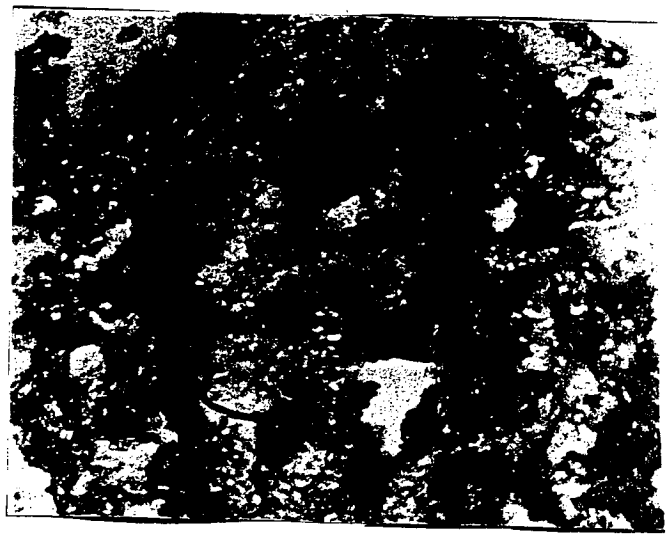


Figure 5: Titratable Acidity in Roots of Tropical Epiphytic Orchids







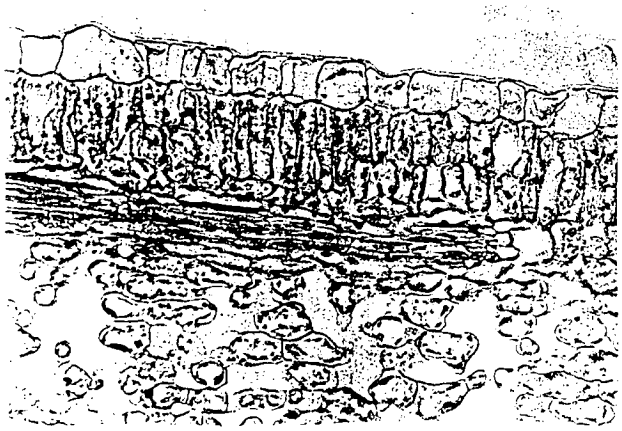


Figure 6: Leaf and Root Cross Sections

From upper left hand corner down to lower right hand corner as follows: C.S. of typical monocot leaf (C3), C.S. of corn leaf w/Kranz anatomy (C4), C.S. of cactus leaf (CAM), C.S. of *Cat* J.M. leaf, and C.S. of *Phal luedd* root.