Beetle drawings provide insight for chapel design at Subiaco Abbey

Page 46
Message from the Provost

At the University of Arkansas we believe the process and results of research and subsequent scholarship are critical to modern undergraduate education. The talent that our graduates will take into the workplace is evident in their research, exhibited in this issue of Inquiry. The work of these talented students reflects vividly how the University of Arkansas is contributing to undergraduate education at its best and to the broad economic development of Arkansas.

I marvel at the diversity and excellence featured for your reading interest. The journal committee draws three major category parameters for submission: Arts and Humanities; Social and Health Sciences; and Sciences and Engineering.

If your interest is in extracting silymarin compounds from thistle seed using hot water techniques, or investment ideas for Eastern bloc countries like Bulgaria, or landscaping your property with plants native to Arkansas, or novels written about the women’s movement following World War I, or creating a greenhouse that can travel to Mars — read on.

These articles are one small indication of the way in which research, education and service flow from one to the other at the University of Arkansas. This process enriches the students, the faculty and the community of which they are a part.

Sincerely,

Bob Smith
Provost and Vice Chancellor for Academic Affairs
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FOREWORD

This journal, the third in an annual series, is a project of the Teaching Academy of the University of Arkansas and is testimony to the Academy's belief that a function of good teaching is to encourage good research and creative thinking on the part of students.

This issue of Inquiry records the individual research exploration of 17 UA student/faculty mentor pairs during the 2001/2002 academic year. The projects included here are drawn from disciplines from each of the university's six undergraduate colleges and schools — the Dale Bumpers College of Agricultural, Food and Life Sciences; the School of Architecture; the J. William Fulbright College of Arts and Sciences; the Sam M. Walton College of Business; the College of Education and Health Professions; and the College of Engineering — and are representative of the quality of research done by the honor students in the various disciplines represented on campus. The breadth of subject matter included here is testimony to the commitment made throughout the university to honors study and research at the undergraduate and graduate level. These 17 articles were chosen by Inquiry's publication board from nearly 50 abstracts received as a result of a call for papers. They vary in subject, writing style, and manner in which they reference their research sources, but they are uniformly excellent in content. In each case, the paper published herein is a précis of the student's larger research product.

The intent of the journal is to record the depth and breadth of the scholarly activities of the university's best undergraduate students. I believe that it does this. We hope that readers will see that there is no limit to what can be accomplished by the university's best students and teachers working together. I hope you will be as excited as I am with the quality of the work presented here.

Murray Smart, Jr.,

Editor

University Professor of Architecture, Emeritus

Inquiry Publication Board, 2001-2002 Academic Year

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SECTION I: ARTS AND HUMANITIES

LANDSCAPE ARCHITECTURE, ARCHITECTURE AND ENGLISH
NATIVE PLANTS: THE PRESERVATION AND RESTORATION OF NATIVE PLANTS IN DESIGNED LANDSCAPES IN NORTHWEST ARKANSAS

Janet L. Coleman
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University of Arkansas

University of Arkansas Faculty Mentors:

Karen Rollet-Crocker, MLA
Department of Landscape Architecture

David Lewis, MLA
Ph. D. candidate in Environmental Dynamics

Jon Lindstrom, Ph. D.
Department of Horticulture

Abstract:

A decline in Northwest Arkansas’s native plant population has occurred over the past 50 years, as documented by the U.S. Forest Service in the Ozark-Ouachita Highlands Assessment. This decline has been caused by increased human development in natural areas and the replacement of native plants with exotic, non-native plants. As a result, a generation has grown up not knowing what an Ozark wake Robin trillium (Trillium pusillum var. ozarkanum) or Blood root (Sanguinaria canadensis) look like, because these plants are difficult to find in nature, are not commonly grown in designed landscapes, and are largely unavailable in garden centers.

The loss of Arkansas’s native plants has led to a second problem: the loss of the region’s landscape identity. A proposed solution focuses on restoring the region’s landscape identity by landscaping with native plants. The benefits of native plants include the following: the creation of native habitats, an increase in biodiversity, the conservation of natural resources, a reduction in long-term landscape costs and a decrease in landscape maintenance requirements and costs. Interviews with regional suppliers and users of native plants identified sources of information and methods of growing these plants. As a result, guidelines on how to incorporate native plants into designed landscapes were developed and are presented with a listing of native plant experts and nurseries, a partial listing of plants for Northwest Arkansas (organized by site conditions), a suggested reading and reference list, and a listing of public gardens that emphasize native plants.

Problem:

In this project two related problems were identified in Northwest Arkansas: the loss of natural areas due to increased population growth and development and the loss of the region’s landscape identity. Industrial, commercial and residential construction is on the rise in Northwest Arkansas. Lands that were previously undeveloped are rapidly becoming developed, resulting in a loss of natural habitats. This is followed by the replacement of preexisting native plants with commonly available non-native plants, thereby resulting in loss of the region’s native landscape identity.

Methods:

Information was gathered by a review of literature, personal interviews with native plant experts, data collection from the native plant questionnaire, and from native plant workshops. This research clarified the problems and proposed solutions. It also suggested methods of data collection and analysis.

Goal of Project:

The goal of this project was to study the use of native plants in designed landscapes in Northwest Arkansas.

Obstacles:

A lack of knowledge about local native plants is the largest obstacle in the use of native plants. Many homeowners, and even landscape designers, lack the horticultural knowledge to make optimum plant choices and are easily influenced by the generic, non-native offerings of plants available through local nurseries or catalogs. Another obstacle to using native plants is the creation of a standard concept of aesthetics for American landscapes. This phenomenon of homogenization is occurring throughout the United States. "Somehow we have arrived at the point in which homogenization and monotony are called 'aesthetically pleasing' and not cutting the lawn is called 'un-American.' " The last obstacle that stands in the way of native
plant usage is the lack of local resources and the associated high cost of native plants. However, this is changing as more native plant nurseries enter the market.  

**Proposed Solution:**

To use native plants in designed landscapes instead of commonly available non-native plants.  

**Benefits:**

Psychologists Stephen and Rachel Kaplan have clearly documented throughout their 20 years of research that humans have lower stress levels and improved quality of life if they live and/or work near natural settings. Books they have written on this subject include the following:

*Humanscape: Environments Are For People and With People In Mind: Design and Management For Everyday Nature.* The benefits of natural environments are related to other living things as well. Native plants and their communities are the foundation of natural habitats. In addition, native plants provide a framework of associations that other living creatures can then build upon.

The more species living within an ecosystem, the higher its productivity and the greater its ability to withstand drought and other kinds of environmental stress. Since we depend upon an abundance of functioning ecosystems to cleanse our water, enrich our soil, and manufacture the very air we breathe, biodiversity is clearly not an inheritance to be discarded carelessly [however] biodiversity is our most valuable but least appreciated natural resource.  

There have been numerous studies done that clearly demonstrate that loss of habitat directly correlates to declining numbers of insects, birds and other forms of wildlife. Scientists have documented alarming declines in the number of songbirds, “warblers, vireos, flycatchers, and thrushes have declined by 50 percent or more in the past few decades.” According to associate director of conservation for the National Audubon Society, Vincent Muehler, “the number one reason for the declining bird population is habitat degradation” In addition, many of the non-native plants that are commonly planted do not provide adequate habitats for local wildlife. Author Sara Stein so aptly suggests, “Remove a pheasant’s cover or the butterfly’s flower and you have erased its space.”  

Native plants in designed landscapes also decrease the amount of time and energy required to maintain that landscape, thereby saving natural resources. Once established, most native plants do not require herbicides or pesticides, nor extra water or fertilizer, and they need only occasional mowing or pruning.  

**The Preservation of Regional Landscape:**

Gardening with native plants serves as a link to the regional landscape and fosters a true sense of place, “something that is missing from most of our homogenized, look-alike landscapes.” The sense of place of Northwest Arkansas is threatened because of the rapid growth that has occurred in recent years. University of Georgia, Landscape Architecture Professor, Darrel Morrison proposes to solve this problem “by utilizing patterns and processes that are intrinsic to naturally evolved landscapes, we can create designed and managed landscapes that are clearly of the place and that approach the ideal of sustainability.” The identity of a place, like Northwest Arkansas, is defined by its geology, terrain, climate, soil conditions, native vegetation and wildlife. This knowledge is necessary to design a landscape using native plants.

**Questionnaire:**

Fourteen (local and national) native plants people were interviewed ranging from residential homeowners to nurserymen to professors in horticulture and landscape architecture. As a result, their experiences related to native plants varied significantly. However, a common factor was an overriding desire to promote the use of native plants, because they promote a sense of place, often cost the same as non-native plants, and native plants are typically easier and cheaper to maintain.

**Guidelines for Starting a Native Plant Garden:**

It is recommended that interested persons read books and journals on the subjects of garden design, garden installation and maintenance, native plants and plant communities. Local lists of regional suppliers and sources should be gathered. To begin the plan obtain the lot dimensions and draw a base map of the site. Complete an inventory of the existing site including all features and document the findings. Make a wish list of the desired elements and features. The design should consider the type of setting that the site is located in (rural, suburban, urban), the natural characteristics of the site, the information derived from the site inventory, the desired formality of the design, items from the wish list, the available finances and time that can be invested into the project. The design of the garden can be naturalistic or formal as native plants can be used in either setting.

**Conclusions and Recommendations:**

This project supports the use of native plants in designed landscapes to promote the over all health of the planet and its human inhabitants. This information was based on the literature search and was expanded by interviewing suppliers and users of native plants. As a result, guidelines on how to incorporate native plants into designed landscapes were developed. A listing of local and national experts and nurseries, a partial listing of native plants for Northwest Arkansas (organized by site conditions), a suggested reading list, and a listing of public gardens that emphasize native plants, are available from the author.

Future recommendations to further the discussion on native plant use in Northwest Arkansas include informal discussions at
local venues, a continuation of native plant workshops, the development of a Web site and future research projects related to the use of native plants in designed landscapes that create specific habitat types.

Endnotes:
2 See list of native plant nurseries. (Omitted due to space limitations. Contact the author.)
8 Wasowski, Sally. 1994. Gardening with Native Plants of the South. (Dallas: Taylor) x.

Addenda:
1. Native ferns:

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common Name</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adianthum pedatum</td>
<td>Maidenhair Fern</td>
<td>Moist rich soil, med to hvy shade, ht 1 1/2'</td>
</tr>
<tr>
<td>Asplenium platyneuron</td>
<td>Ebony Spleenwort</td>
<td>Rocky, limestone, shade, small plant</td>
</tr>
<tr>
<td>Athryrium filix-femina</td>
<td>Lady Fern</td>
<td>Moist rich, shade. Water edges. Ht 2-5'</td>
</tr>
<tr>
<td>Asplenium trichomanes</td>
<td>Maidenhair Spleenwort</td>
<td>Fine texture, small fern</td>
</tr>
<tr>
<td>Cystopteris bulbifera</td>
<td>Bulblet Bladderfern</td>
<td>Rocky water edges, wet or moist, neutral ph</td>
</tr>
<tr>
<td>Cystopteris protrusa</td>
<td>Lowland Bladderfern</td>
<td>Shade, rocky sites.</td>
</tr>
<tr>
<td>Dryopteris marginalis</td>
<td>Marginal Shield Fern</td>
<td>Well drained woods, evergreen, leather texture, ht 1-2'</td>
</tr>
<tr>
<td>Onoclea sensibilis</td>
<td>Sensitive Fern</td>
<td>Moist swamps/ woods sun to shade, colonies, ht 1-2'</td>
</tr>
<tr>
<td>Phegopteris hexonoptera</td>
<td>Broad Beechfern</td>
<td>Rich woods, acid, deciduous, ht 16 - 24&quot;</td>
</tr>
<tr>
<td>Polypodium polpodioides</td>
<td>Polypody</td>
<td>Loamy soil, moist, part to full shade, deciduous</td>
</tr>
<tr>
<td>Polystichum acrostichoides</td>
<td>Christmas Fern</td>
<td>Evergreen, moist, rich soil, lite to heavy shade, ht 2 1/2'</td>
</tr>
<tr>
<td>Pteridium aquilimum</td>
<td>Brackenfern</td>
<td>Sun to part shade, acid, inferitile soil, ht 3 - 5'</td>
</tr>
<tr>
<td>Woodsia obtusa</td>
<td>Bluntlobe Cliff Fern</td>
<td>Moist, acid, shady, rocky sites, evergreen, small</td>
</tr>
</tbody>
</table>
### 2. Native Grasses

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common Name</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arundinaria gigantea</strong></td>
<td>Giant Cane</td>
<td>Large grass, wet or dry, wildlife food</td>
</tr>
<tr>
<td><strong>Bouteloua curtipendula</strong></td>
<td>Side Oats Gamma</td>
<td>Lite shade, birds and butterflies</td>
</tr>
<tr>
<td><strong>Carex flaccosperma</strong></td>
<td>Blue Wood Sedge</td>
<td>Part shade, drought tolerant, blue leaves</td>
</tr>
<tr>
<td><strong>Carex grayi</strong></td>
<td>Gray’s Sedge</td>
<td>Sun to lite shade, moist rich soil</td>
</tr>
<tr>
<td><strong>Carex lurida</strong></td>
<td>unknown</td>
<td>Moist soils, along ponds &amp; streams</td>
</tr>
<tr>
<td><strong>Carex stricta</strong></td>
<td>Tussock Sedge</td>
<td>Fine leaves, wet soils, water</td>
</tr>
<tr>
<td><strong>Chasmanthium latifolia</strong></td>
<td>River Oats</td>
<td>Moist sun or dry shade, dangling seed heads</td>
</tr>
<tr>
<td><strong>Elymus virginicum</strong></td>
<td>Virginia Wild Rye</td>
<td>Bunch grass, sun to lite shade</td>
</tr>
<tr>
<td><strong>Eragrostis spectabilis</strong></td>
<td>Purple Love Grass</td>
<td>Showy clumps, low growing, sun to lite shade</td>
</tr>
<tr>
<td><strong>Panicum virgatum</strong></td>
<td>Switch grass</td>
<td>Sun to lite shade, many cultivars</td>
</tr>
<tr>
<td><strong>Sorghastrum nutans</strong></td>
<td>Indian Grass</td>
<td>Sun, tall, butterflies &amp; birds, flower spikes</td>
</tr>
<tr>
<td><strong>Spartina pectinata</strong></td>
<td>Prairie Cord Grass</td>
<td>Aggressive in moist conditions, contain in pots</td>
</tr>
<tr>
<td><strong>Sporobolus cryptandrus</strong></td>
<td>Sand Dropseed</td>
<td>Sun, not attractive, sandy, use as soil stabilizer</td>
</tr>
<tr>
<td><strong>Sporobolus heterolepis</strong></td>
<td>Prairie Dropseed</td>
<td>Sun to lite shade, small and cascading form</td>
</tr>
<tr>
<td><strong>Triden strictus</strong></td>
<td>Longspike Tridens</td>
<td>Sun, golden flower heads, birds</td>
</tr>
</tbody>
</table>

### 3. Native Herbaceous Plants for Sunny Sites

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common Name</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amsonia tubernaemontana</strong></td>
<td>Blue Star</td>
<td>Star shaped spring flowers, light shade ok</td>
</tr>
<tr>
<td><strong>Apocynum cannabinum</strong></td>
<td>Dogbane or Hemp</td>
<td>Dry areas, bark used to make rope, white summer flowers</td>
</tr>
<tr>
<td><strong>Aquilegia canadensis</strong></td>
<td>Columbine</td>
<td>Moist, part shade to sun, red/yellow spring flowers</td>
</tr>
<tr>
<td><strong>Asclepias incarnata</strong></td>
<td>Pink Milkweed</td>
<td>Pink summer flower clusters, dry sites, butterfly attractor</td>
</tr>
<tr>
<td><strong>Asclepias purpurascens</strong></td>
<td>Purple Milkweed</td>
<td>Grows 4’, rocky dry sites, purple summer flower clusters</td>
</tr>
<tr>
<td><strong>Asclepias tuberosa</strong></td>
<td>Butterfly Weed</td>
<td>Bright orange summer flowers, +butterflies</td>
</tr>
<tr>
<td><strong>Aster anomalus</strong></td>
<td>Aster</td>
<td>Dry sites, idle land, wildlife food, fall lavender flowers</td>
</tr>
<tr>
<td><strong>Aster patens</strong></td>
<td>Spreading Aster</td>
<td>Purple fall flowers, rocky dry sites</td>
</tr>
<tr>
<td><strong>Aster pilosus</strong></td>
<td>White Heath Aster</td>
<td>Dry open areas, white late fall flowers</td>
</tr>
</tbody>
</table>
Astranthium interfrifolium  Western Daisy  Rocky, sandy soils, pink spring flowers
Bidens aristosa  Tickseed Sunflower  Adaptable, grow in large stands, yellow summer flowers
Baptisia australis  Blue False Indigo  Purple summer flowers, grow from seed, limestone
Baptisia alba  White Wild Indigo  White summer flowers, 4-5', dry open sites
Baptisia sphaerocarpa  Yellow Wild Indigo  Bright spring yellow spikes, pinelands, sandy soils
Callirhoe involucrata  Purple Poppy Mallow  Pink summer flowers, dry open areas, good drainage
Camassia scilloides  Wild Hyacinth  Adaptable, long stalks of white spring flowers
Castilleja coccinea  Indian Paintbrush  Rocky, alkaline, limestone, red spring flower
Ceanothus americanus  New Jersey Tea  White sping flower clusters, wildlife food, dry soil
Clitoria mariana  Butterfly Pea  Pale pink summer flowers, dry sandy soils, wildlife food
Coreopsis lanceolata  Lance Leaf Coreopsis  Wildlife food, dry open areas, yellow late spr. Flowers
Delphinium carolinianum  Carolina Larkspur  Purple summer flower spikes, open, well drained soil
Delphinium treleasei  Trelease's Larkspur  Glades, open areas, limestone, purple summer spikes
Echinacea pallida  Pale Purple Coneflower  Pinelands, disturbed soil, tall pinkish summer flowers
Echinacea purpurea  Purple Coneflower  Idle ground, deep pink summer flower, insect attractor
Erigeron philadelphicus  Philadelphia Fleabane  Wildlife food, open areas, pink daisy like spring flowers
Eupatorium coelestinum  Mist Flower  Moist area, fuzzy lavender late summer flowers
Eupatorium fistulosum  Joe Pye Weed  Damp, very tall plant, large groups of pink sum. flowers
Gaurna longiflora  Biennial Gaura  Open sunny areas, small summer flowers open in the evening
Gentiana puberulenta  Downy Gentian  Prairies, dark purple summer-fall flowers
Helianthus annuus  Common Sunflower  Dry idle areas, large summer flowers, wildlife food
Heliotropium tenellum  Heliotrope  Drought resistant, white summer flowers, colonies, rocky
Hibiscus laevis  Rose Mallow  Wet areas, large pink summer hibiscus flowers
Hypericum spp.  St. John’s Wort  Dry, open areas, wildlife food, yellow summer flowers
Hypericum hypericoides  St. Andrew’s Cross  Yellow summer cross flowers, adaptable, low growing
Iris brevicaulis  Short Stemmed Iris  Wet areas, ponds, marshs, sun, purple spring flowers
Iris cristata  Crested Iris  Rocky, sandy, streams, hillsides, lavender spring flowers
<table>
<thead>
<tr>
<th>Species</th>
<th>Common Name</th>
<th>Habitat and Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iris virginica</td>
<td>Southern Blue Flag</td>
<td>Wet, swampy areas along ponds, pink spring flowers</td>
</tr>
<tr>
<td>Justicia americana</td>
<td>Water Willow</td>
<td>Moist, lake edges, thick growth, lavender summer flowers</td>
</tr>
<tr>
<td>Liatris aspera</td>
<td>Rough Blazing Star</td>
<td>Rocky, sandy soils, short stalk lavender summer flowers</td>
</tr>
<tr>
<td>Liatris pycnostachya</td>
<td>Prairie Gayfeather</td>
<td>Open areas, pink-purple summer flower stalks</td>
</tr>
<tr>
<td>Linaria canadensis</td>
<td>Blue Toadflax</td>
<td>Fallow or disturbed soil, shades of blue summer flowers</td>
</tr>
<tr>
<td>Lithospermum canescens</td>
<td>Orange Puccoon</td>
<td>Rocky, sandy, dry soils, orange spr-summer flowers</td>
</tr>
<tr>
<td>Monarda fistulosa</td>
<td>Beebalm</td>
<td>Dry soil, pink summer flowers, wildlife food</td>
</tr>
<tr>
<td>Oenothera fruticosa</td>
<td>Sundrops</td>
<td>Yellow summer day flowers, dry soil, sun or shade</td>
</tr>
<tr>
<td>Oenothera speciosa</td>
<td>Showy Primrose</td>
<td>Pink spr-summer flowers, drought resistant, easily grown</td>
</tr>
<tr>
<td>Opuntia humifusa</td>
<td>Prickly Pear</td>
<td>Cactus, showy summer flowers, wildlife food, dry sites</td>
</tr>
<tr>
<td>Oxalis violacea</td>
<td>Violet Wood Sorrel</td>
<td>Nice lavender flower, shamrock like foliage, adaptable</td>
</tr>
<tr>
<td>Penstemon digitalis</td>
<td>Foxglove Beard Tongue</td>
<td>Tall plants, crisp white summer flowers</td>
</tr>
<tr>
<td>Phacelia hirsuta</td>
<td>Hairy Phacelia</td>
<td>Damp areas upright lavender spring flowers</td>
</tr>
<tr>
<td>Phlox bifida</td>
<td>Sand Phlox</td>
<td>Rocky or sandy areas, lavender spring flowers</td>
</tr>
<tr>
<td>Phlox pilosa</td>
<td>Downy Phlox</td>
<td>Dry thin soils, openings in woods, pink spring flowers</td>
</tr>
<tr>
<td>Physostegia virginiana</td>
<td>Obedient Plant</td>
<td>Moist open areas, showy pink summer flowers</td>
</tr>
<tr>
<td>Pycnanthemum albescens</td>
<td>Mountain Mint</td>
<td>Dry soil, interesting green summer bracts &amp; flowers</td>
</tr>
<tr>
<td>Pycnanthemum tenuifolium</td>
<td>Slender Mtn. Mint</td>
<td>Slender leaves, white-lavender summer flowers</td>
</tr>
<tr>
<td>Ratibida pinnata</td>
<td>Gray Head Coneflower</td>
<td>Open idle areas, drooping yellow summer flowers</td>
</tr>
<tr>
<td>Rudbeckia grandiflora</td>
<td>Large Coneflower</td>
<td>Dry areas, yellow summer flowers, grow in large stands</td>
</tr>
<tr>
<td>Rudbeckia hirta</td>
<td>Black Eyed Susan</td>
<td>Wildlife food, eroded land, yellow summer flowers</td>
</tr>
<tr>
<td>Salvia azurea</td>
<td>Blue Sage</td>
<td>Tall plants, lite blue summer-fall flowers</td>
</tr>
<tr>
<td>Salvia lyrata</td>
<td>Cancer Weed</td>
<td>Adaptable plant, lite blue-lavender spr-summer flowers</td>
</tr>
<tr>
<td>Satureja arkansana</td>
<td>Calamint</td>
<td>Rocky soil, small lavender summer flowers, mint odor</td>
</tr>
<tr>
<td>Schrankia nuttallii</td>
<td>Sensitive Brier</td>
<td>Wildlife food, trailing habit, pink puffy summer flowers</td>
</tr>
<tr>
<td>Sedum nuttallianum</td>
<td>Yellow Sedum</td>
<td>Sun, dry, rocky areas, grows in low clumps</td>
</tr>
<tr>
<td>Silene virginica</td>
<td>Fire Pink</td>
<td>Open areas or lite shade, red flowers, hummingbird attr.</td>
</tr>
</tbody>
</table>
### Native Plants

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common Name</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silene regia</td>
<td>Royal Catchfly</td>
<td>Full sun, red flowers, slow grower, hummingbird attractor</td>
</tr>
<tr>
<td>Silphium perfoliatum</td>
<td>Cup Plant</td>
<td>Large leaves form a cup, moist, yellow summer flowers</td>
</tr>
<tr>
<td>Silphium terebinthinaceum</td>
<td>Prairie Dock</td>
<td>Lg. Spade leaves, limestone, yellow summer flowers</td>
</tr>
<tr>
<td>Sisyrinchium campestre</td>
<td>Blue Eyed Grass</td>
<td>Not a true grass, grass like leaves, clumps, blue flowers</td>
</tr>
<tr>
<td>Solidago petiolaris</td>
<td>Goldenrod</td>
<td>Rocky, yellow late summer flower spikes</td>
</tr>
<tr>
<td>Talinum calycinum</td>
<td>Fame Flower</td>
<td>Rocky, dry sites, cedar glades, bright pink flowers</td>
</tr>
<tr>
<td>Tephrosia virginiana</td>
<td>Goat's Rue</td>
<td>Pale pink pea like flowers, vetchlike plants, wildlife food</td>
</tr>
<tr>
<td>Teucrium canadense</td>
<td>Wood Sage</td>
<td>Dry or moist soil, elongagted lavender summer flowers</td>
</tr>
<tr>
<td>Glandularia canadensis</td>
<td>Rose Vervain</td>
<td>Sandy, rocky, open areas, lavender spring-summer flowers</td>
</tr>
<tr>
<td>Veronicastrum virginicum</td>
<td>Culver's Root</td>
<td>Adaptable open or shade, white summer flower spikes</td>
</tr>
<tr>
<td>Yucca arkansana</td>
<td>Arkansas Yucca</td>
<td>Dry, uplands, large spikes of white summer flowers</td>
</tr>
</tbody>
</table>

### Native Shrubs for Dry Sites

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common Name</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amelanchier arborea</td>
<td>Serviceberry</td>
<td>White flowers, +fruit, sun/shade</td>
</tr>
<tr>
<td>Amorpha canescens</td>
<td>Lead Plant</td>
<td>Dry or moist sun, compound leaves, gray green color</td>
</tr>
<tr>
<td>Ceanothus americanus</td>
<td>New Jersery Tea</td>
<td>Part shade, dry</td>
</tr>
<tr>
<td>Hamamelis vernalis</td>
<td>Ozark Witch Hazel</td>
<td>Winter frag. flower, wildlife attractor, yellow fall</td>
</tr>
<tr>
<td>Hamamelis virginiana</td>
<td>Witch Hazel</td>
<td>Fragrant winter flowers, fall color, forms colonies</td>
</tr>
<tr>
<td>Philadelphus pubescens</td>
<td>Gray Mock Orange</td>
<td>Fragrant white flowers, tough shrub.</td>
</tr>
<tr>
<td>Physocarpus opulifolius</td>
<td>Common Ninebark</td>
<td>Tough adaptable shrub, white flowers, nice bark</td>
</tr>
<tr>
<td>Ribes missouriense</td>
<td>Missouri Gooseberry</td>
<td>Adaptable shrub, wildlife food</td>
</tr>
<tr>
<td>Ribes odoratum</td>
<td>Golden Current</td>
<td>Frag yellow flowers, wildlife food, sun to part shade</td>
</tr>
<tr>
<td>Rhus aromatica</td>
<td>Fragrant Sumac</td>
<td>Hot,dry sun, red fall, good for massing, wildlife food</td>
</tr>
<tr>
<td>Rhus copallina</td>
<td>Winged Sumac</td>
<td>Dry, sun or shade, orange fall, wildlife food</td>
</tr>
<tr>
<td>Rhus glabra</td>
<td>Smooth Sumac</td>
<td>Forms thickets, common, red fall, wildlife food, slopes</td>
</tr>
<tr>
<td>Rosa arkansana</td>
<td>Prairie Rose</td>
<td>Pink rose, drought tolerant, wildlife food</td>
</tr>
<tr>
<td>Rosa caroliniana</td>
<td>Pasture Rose</td>
<td>Pink rose, drought tolerant, wildlife food, compact</td>
</tr>
</tbody>
</table>
Rubus species | Blackberry | Thorns, edible fruit, tolerates sun, dry soil
Rubus occidentalis | Black Raspberry | Edible fruits, thorns, tolerates dry, sun
Salix humilis | Prairie Willow | Dry open areas, pussy willow buds, short shrub
Sambucus canadensis | Elder-Berry | Wildlife attractor, edible fruit, dry or moist soil
Symphoricarpos orbiculatus | Coral Berry | Wildlife attractor, red fall fruit, dry soil

4. Native Shrubs for Moist Sites

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common Name</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aesculus glabra</td>
<td>Ohio Buckeye</td>
<td>Pale yellow flowers, nuts, occ fall color</td>
</tr>
<tr>
<td>Amelanchier arborea</td>
<td>Serviceberry</td>
<td>White flowers, fruit, sun/shade, adaptable to soil</td>
</tr>
<tr>
<td>Amorpha canescens</td>
<td>Lead Plant</td>
<td>Dry or moist sun, compound leaves, gray green color</td>
</tr>
<tr>
<td>Amorpha fruiticosa</td>
<td>Indigobush Amorpha</td>
<td>Pinnate foliage, tough plant, purple flowers, moist</td>
</tr>
<tr>
<td>Cornus racemosa</td>
<td>Gray dogwood</td>
<td>Moist or rocky soil, white flower</td>
</tr>
<tr>
<td>Dirca palustris</td>
<td>Leatherwood</td>
<td>Shade, yellow flowers and fall, woodland</td>
</tr>
<tr>
<td>Euonymus americana</td>
<td>Strawberry bush</td>
<td>Moist woodlands, bright red fruit</td>
</tr>
<tr>
<td>Euonymus atropurpureus</td>
<td>Burning Bush, Wahoo</td>
<td>Moist, fall color, part to full shade</td>
</tr>
<tr>
<td>Hamamelis vernalis</td>
<td>Ozark Witch Hazel</td>
<td>Winter frag. flower, wildlife attractor, yellow fall</td>
</tr>
<tr>
<td>Hamamelis virginiana</td>
<td>Witch Hazel</td>
<td>Fragrant winter flowers, fall color, forms colonies</td>
</tr>
<tr>
<td>Hydrangea arborescens</td>
<td>Wild Hydrangea</td>
<td>Moist, part shade or sun, fall color</td>
</tr>
<tr>
<td>Hypericum prolificum</td>
<td>Shrubby St Johnswort</td>
<td>Yellow flowers, sun to part shade, well drained</td>
</tr>
<tr>
<td>Lindera benzoin</td>
<td>Spicebush</td>
<td>Acid soil, sun to part shade, yellow flowers</td>
</tr>
<tr>
<td>Physocarpus opulifolus</td>
<td>Common Ninebark</td>
<td>Tough adaptable shrub, white flowers, nice bark</td>
</tr>
<tr>
<td>Rhododendron viscosum</td>
<td>Texas Azalea</td>
<td>Frag white/pink flowers, moist rocky soil, part shade</td>
</tr>
<tr>
<td>Salix eriocephala</td>
<td>Rigid Willow</td>
<td>Good for wet areas, sun, fine texture</td>
</tr>
<tr>
<td>Sambucus canadensis</td>
<td>Elder-Berry</td>
<td>Wildlife attractor, edible fruit, dry or moist soil</td>
</tr>
<tr>
<td>Sapindus drumondii</td>
<td>Soapberry</td>
<td>Limestone, fruit, pinnate leaves, gold fall</td>
</tr>
<tr>
<td>Staphylea trifolia</td>
<td>Bladder-Nut</td>
<td>Interesting fruit, striped bark, part shade to sun</td>
</tr>
<tr>
<td>Viburnum prunifolium</td>
<td>Blackhaw</td>
<td>White flowers, fall color, wildlife attractor</td>
</tr>
<tr>
<td>Viburnum rufidulum</td>
<td>Southern Blackhaw</td>
<td>White flowers, fall color, glossy leaves, wildlife</td>
</tr>
</tbody>
</table>
### 5. Native Shrubs for Shady Sites

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common Name</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aesculus glabra</em></td>
<td>Ohio Buckeye</td>
<td>Pale yellow flowers, nuts, occ. fall color, part shade</td>
</tr>
<tr>
<td><em>Amelanchier arborea</em></td>
<td>Serviceberry</td>
<td>White flowers, +fruit, sun/shade</td>
</tr>
<tr>
<td><em>Ceanothus americanus</em></td>
<td>New Jersey Tea</td>
<td>Part shade, dry</td>
</tr>
<tr>
<td><em>Cephalanthus occidentalis</em></td>
<td>Buttonbush</td>
<td>Part shade or sun, moist, attracts butterflies, birds</td>
</tr>
<tr>
<td><em>Cornus racemosa</em></td>
<td>Gray dogwood</td>
<td>Moist or rocky soil, white flower</td>
</tr>
<tr>
<td><em>Dirca palustris</em></td>
<td>Leatherwood</td>
<td>Shade, yellow flowers and fall, woodland</td>
</tr>
<tr>
<td><em>Euonymus americana</em></td>
<td>Strawberry bush</td>
<td>Moist woodlands, bright red fruit</td>
</tr>
<tr>
<td><em>Euonymus atropurpureus</em></td>
<td>Burning Bush, Wahoo</td>
<td>Moist, fall color, part to full shade</td>
</tr>
<tr>
<td><em>Hydrangea arborescens</em></td>
<td>Wild Hydrangea</td>
<td>Moist, part to full shade, fall color</td>
</tr>
<tr>
<td><em>Lindera benzoin</em></td>
<td>Spicebush</td>
<td>Acid soil, sun to part shade, yellow flowers</td>
</tr>
<tr>
<td><em>Physocarpus opulifolus</em></td>
<td>Common Ninebark</td>
<td>Tough adaptable shrub, white flowers, nice bark</td>
</tr>
<tr>
<td><em>Sapindus drumondii</em></td>
<td>Soapberry</td>
<td>Limestone, fruit, pinnate leaves, gold fall, part shade</td>
</tr>
<tr>
<td><em>Staphylea trifolia</em></td>
<td>Bladder-Nut</td>
<td>Interesting fruit, striped bark, part shade</td>
</tr>
<tr>
<td><em>Symphoricarpos orbiculatus</em></td>
<td>Coral berry</td>
<td>Wildlife attractor, red fall fruit, dry soil, part shade</td>
</tr>
<tr>
<td><em>Rhododendron viscosum</em></td>
<td>Texas Azalea</td>
<td>Frag white/pink flowers, moist rocky soil, part shade</td>
</tr>
<tr>
<td><em>Ribes odoratum</em></td>
<td>Golden Current</td>
<td>Frag yellow flowers, wildlife food, part shade</td>
</tr>
<tr>
<td><em>Rhus copallina</em></td>
<td>Winged Sumac</td>
<td>Dry, sun or shade, orange fall, wildlife food</td>
</tr>
<tr>
<td><em>Vaccinium arboreum</em></td>
<td>Huckleberry</td>
<td>Fruit, low ferd. acid soil, red fall, sun or part shade</td>
</tr>
<tr>
<td><em>Viburnum prunifolium</em></td>
<td>Blackhaw</td>
<td>White flowers, fall color, wildlife food, part shade</td>
</tr>
<tr>
<td><em>Viburnum rufidulum</em></td>
<td>Southern Blackhaw</td>
<td>White flowers, fall color, glossy leaves, wildlife</td>
</tr>
</tbody>
</table>

### 6. Native Shrubs of Sunny Sites

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common Name</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aesculus glabra</em></td>
<td>Ohio Buckeye</td>
<td>Pale yellow flowers, nuts, occ. fall color</td>
</tr>
<tr>
<td><em>Amelanchier arborea</em></td>
<td>Serviceberry</td>
<td>White flowers, +fruit, sun/shade</td>
</tr>
<tr>
<td><em>Amorpha canescens</em></td>
<td>Lead Plant</td>
<td>Dry or moist, attracts butterflies, gray green color</td>
</tr>
<tr>
<td><em>Amorpha fruticosa</em></td>
<td>Indigobush Amorph</td>
<td>Pinnate foliage, tough plant, purple flowers, moist</td>
</tr>
</tbody>
</table>
Cephalanthus occidentalis  Buttonbush  Part shade or sun, moist, attracts wildlife
Cornus racemosa  Gray dogwood  Moist or rocky soil, white flowers
Hamamelis vernalis  Ozark Witch Hazel  Winter frag. flower, wildlife attractor, yellow fall
Hamamelis virginiana  Witch Hazel  Fragrant winter flowers, fall color, colonies
Hydrangea arborescens  Wild Hydrangea  Moist, part shade or sun, fall color
Hypericum prolificum  Shrubby St Johnswort  Yellow flowers, small shrub, well-drained soil
Lindera benzoin  Spicebush  Acid soil, sun to part shade, yellow flowers
Philadelphus pubescens  Gray Mock Orange  Fragrant white flowers, tough shrub.
Physocarpus opulifolius  Common Ninebark  Tough adaptable shrub, white flowers, nice bark
Ribes missouriense  Missouri Gooseberry  Adaptable shrub, wildlife food
Ribes odoratum  Golden Current  Fragrant yellow flowers, wildlife food
Rhus aromatica  Fragrant Sumac  Hot,dry, red fall, massing, wildlife food
Rhus copallina  Winged Sumac  Dry, sun or shade, orange fall, wildlife food
Rhus glabra  Smooth Sumac  Forms thickets, common, red fall, wildlife food
Rosa arkansana  Prairie Rose  Pink rose, drought tolerant, wildlife food
Rosa caroliniana  Pasture Rose  Pink rose, drought tolerant, wildlife food
Rubus occidentalis  Black Raspberry  Edible fruits, thorns, tolerates dry, sun
Rubus species  Blackberry  Thorns, edible fruit, tolerates sun, dry soil
Salix eriocephala  Rigid Willow  Good for wet areas, sun, fine texture
Salix humilis  Prairie Willow  Dry open areas, pussy willow buds, short shrub
Sambucus canadensis  Elder-Berry  Wildlife attractor, edible fruit, dry or moist soil
Sapindus drummondii  Soapberry  Limestone, fruit, pinnate leaves, gold fall
Staphylea trifolia  Bladder-Nut  Interesting fruit, striped bark, part shade to sun
Symphoricarpos orbiculatus  Coral berry  Wildlife attractor, red fall fruit, dry soil
Vaccinium arboreum  Huckleberry, Farkleberry  Fruit, low fert. acid soil, red fall
Vaccinium pallidum  Low Bush Blueberry  Delicious fruit, wildlife food, acid soil, colonies
Viburnum prunifolium  Blackhaw  White flowers, fall color, wildlife attractor, sun
Viburnum rufidulum  Southern Blackhaw  White flowers, fall color, glossy leaves, wildlife
Native Plant Book List


American Plants for American Gardens. Edith Roberts & Elsa Rehm ann. 1996

Gardening with Native Plants of the South. Sally Wasowski. 1994.


Penelope Hobhouse’s Natural Planting. Penelope Hobhouse. 1997.


Redesigning the American Lawn: A Search for Environmental Harmony. F. Herbert Bormann. 1993.


Editor's Note:

Ms. Coleman's addenda also included the following lists of plant materials:

Native trees for dry sites

Native trees for moist Sites

Native vines

Native woodland herbaceous plants

She also included extensive lists of individuals and agencies from whom information on native plants can be secured, as well as local, state and national sources for native plant materials listed, a native plant book list, an annotated questionnaire used in collecting her data, and plans of three local gardens that use native plants extensively. Space limitations of this journal do not allow the inclusion of this material. It is available, however, from the author who can be reached at 123 Snapfinger Way, Athens, GA 30605.

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greenet/arbs/salt/salt.html>.

Faculty Comments:

Professor Laurie Fields, Chair of the Department of Landscape Architecture, made these comments about Ms. Coleman's work:

Though I did not work directly with Ms. Coleman on her research project, I have read her paper and believe that it is thorough, significant and worthy of broad dissemination.

With the prestigious SILO Research Grant for undergraduates and numerous awards and scholarships, the faculty and the university have recognized Ms. Coleman potential as a designer and achievements as a scholar. Ms. Coleman is a non-traditional student whose intelligence is reflected in design work marked by rigor and originality, and in the research project on native plants that she is currently completing. Having participated in her process of investigating and applying to graduate schools, it is clear that she is thorough, organized and thoughtful.

Ms. Coleman potential as a scholar is evident in this paper and in the fact that she was accepted at every graduate school to which she applied. She has decided to attend the University of Georgia where she will continue work on native plants with Professor Darrell Morrison. This paper represents the beginning of a graduate research agenda and application to landscape architectural design.

Professor David Lewis worked closely with Ms. Coleman throughout the duration of the project. He had this to say about his experience:

While teaching in the University of Arkansas Department of Landscape Architecture it was my pleasure and good fortune to work with many good students and one extraordinary one: Ms. Janet Coleman. As a student and budding scholar, Ms. Coleman positioned herself at the top of her class, carefully completing assignments in a timely and appropriate fashion, but looking beyond, or through, the assignments to examine what she was learning and how to fit that knowledge with what she knew and what she wanted to know about the profession of landscape architecture. Janet was thoughtful, motivated and curious; she pushed herself forward, taking her classmates and the profession along with her.

Ms. Coleman's interests forced her to work with other professionals, particularly horticulturists. Her interest in the use of native plant species in residential applications suggest that her impact on the world will extend beyond traditional practices of landscape architecture and make her valuable as a designer and teacher of new ways to conceive individual landscapes.

As Ms. Coleman's mentor, I have followed her research into the use of native plant materials. The work that she is submitting for publication in Inquiry is important not only as a representation of how designed landscapes are influenced by and are significant to multiple disciplines (environmental design, environmental science, psychology, horticulture, and others), but it also demonstrates how to make connections not only among the various disciplines but with the public as well. Her attempt is to identify what is significant about the use of native plants in designed landscapes, not for the specialists but for the people who could be utilizing them everyday. I strongly encourage the consideration of Ms. Coleman's paper for publication as her work attempts to educate the public about how to make a difference in the quality of the environments in which we all live.
Ms. Coleman's faculty mentor, Karen Rollet-Crocker, describes the value of Ms. Coleman's research as follows:

Janet Coleman's research project is part of an effort in the landscape architecture profession to create a new area of knowledge that can be applied to landscape design: the use of native plants in designed landscapes. This requires an understanding of native plant associations, ecology, and landscape preservation in unique regional environments. Robert Grese of the University of Michigan and Darrel G. Morrison of the University of Georgia have done academic research in this area. The University of Arkansas Landscape Architecture Horticulture departments have begun developing courses and research in this subject and applying this information to specific projects such as the Garvan Botanical Garden in Hot Springs and the Compton Gardens in Bentonville.

Ms. Coleman's study developed information on the subject through interviewing experts and attending workshops. A literature search was done based on suggested categories. This initial work produced an understanding of general issues about the use of native plants in designed landscapes. It also resulted in a list of constraints created by current landscaping practices. The current American garden aesthetic with its flowerbeds and lawns wastes resources and degrades habitats for both plants and animals. A number of economic issues, including the limited availability of native plants, add to the problem.

A questionnaire helped to develop a methodology for using native plants. It allowed Janet to discover what information and techniques were most helpful to people who were actively involved in this kind of landscaping. She found that self-education and experimentation were keys to their success.

The summation lists guidelines from all aspects of her study, framed in a format typically used by landscape architects for design purposes. She then applied these guidelines to several small design projects.

The importance of this project lies in the application of general knowledge about the value of using native plants to design landscapes in a specific region: Northwest Arkansas. The study process could be replicated in any regional environment, using regional source material. This would tailor the plant and habitat information to a specific location while following the general goals and guidelines listed in the study.
MARSPOrT DEPLOYABLE GREENHOUSE

Iova Dineva
Department of Architecture
Faculty Mentor: Dr. Jerry Wall
Professor of Architecture

Abstract:

To sustain a Mars exploration team, NASA's reference mission IV includes a greenhouse facility requirement to supplement the crew's food supply. This research project explores strategies for building, delivering, deploying, operating, and maintaining a greenhouse as a supplemental structure for the Mars environment. The MarsPort Deployable Greenhouse (MDG) addresses the issues of atmosphere, sunlight, energy, deployment mechanism, water and nutrients, crop collection, modular development for expansion, research module for Mars experimentation, and crew recreation.

Heaven on Mars:

The planet Mars has been tantalizing mankind since the dawn of time. The Red Planet has been given attributes that range from a mankind-welcoming place to a deadly force and trap for explorers. In 1998 NASA refined the Mars Design Reference Mission (DRM), which will enable humanity to finally set foot on the mysterious planet. In preparation of such a prolonged mission many innovations are put in use.

The given problem:

The Reference Mission v 3.0 defines the requirements and strategies for executing human exploration on Mars. Although DRM specifies the use of non-perishable food supply reserves as a means for executing the near 900-day mission segments, it also indicates the possible development of regenerative life-support systems, including food production in situ. In search of creative solutions, NASA commissioned the 2002 MarsPort Engineering Design Student Competition, establishing a set of requirements for a greenhouse structure. As a result of an independent study at the University of Arkansas School of Architecture, this paper presents a theoretical response to the competition based on application of known materials and adoption of existing design strategies. The design adheres to the NASA requirements to explore strategies for building, delivering, deploying, operating, and maintaining a greenhouse as a supplemental structure for the Mars environment, but it also augments the given program by introducing the requirement of human habitation.

The MarsPort Deployable Greenhouse (MDG) engages issues of atmosphere, sunlight, energy, deployment mechanism, water and nutrients, crop collection, modular development for expansion, research module for Mars experimentation, and crew recreation. These issues were considered equally for their technical and architectural value and priorities.

Multiple studies have been conducted in universities and various agencies across the globe, from state-supported institutions (Utah State University, University of Florida, Purdue University) to private agencies and groups (the Moon Society Artemis Project, the Mars Society, etc.) to NASA and her contractors. Most of these projects are highly specialized in a certain area and lack continuity and overall integration of the issues. Building on architectural education, this proposal utilized research publications by these specialists to synthesize a more comprehensive view of the problem. The design strategy linked solutions of structural integrity with deployable delivery, Mars native chemistries with a sustainable Earth ecosystem, and last, but not least, human comfort with efficient mixed-use of facilities.

Traditionally in the development of a life-supporting mechanism like the MarsPort, practicality of the systems, robust mechanics, and maximum utilization are given priority over the architectural, aesthetic or psychological effect of the environment. The MarsPort will be stationed on the planet surface for a prolonged period of time (the projected life-span for the MDG is twenty years; the crew stay is 600 days), so it must be equipped with possible technologies to utilize native resources for fuel and building materials, and certainly the opportunity for linear surface expansion of the complex. Thus, the MarsPort does not have the size limits and confines of space stations or transportation crafts, and it presents the opportunity for expanding the engineering side of design to take into account the human variables.
Based on a belief that there is little human satisfaction derived from living in a ‘tin can’, it can be concluded that prolonged missions like the Mars project call for special care for the provision of privacy, exercise, and a relaxing environment. While ideally this can be achieved in a specialized module apart from the main surface habitation and laboratory, the efficiency of all systems should be augmented by designing particularly Earth-recalling spaces such as the MDG to serve the double role of functional element and dwelling-place where people can regenerate their physical and psychological condition.

**Work methods:**

In an effort to maintain a wide margin of ideas built upon existing practical research on the subject, the theoretical findings of this document generated a catalogue of possible solutions for each issue of the overall problem (atmosphere maintenance, light, heat and energy, vegetation, deployable structure and methods of construction) and described the basic advantages and disadvantages of each system. During this phase of the project there was no attempt to present conclusive evidence for one system over another. Limitations on time, funding and equipment made it impossible to develop functional prototypes for each solution and test them for satisfactory performance in structural integrity, deployment mechanism, and vegetation sustainability.

The project consisted of several phases: data collection, preliminary design, development of construction strategies and sample prototype design. Phase One provided the basic knowledge of life-support systems, requirements for growth of vegetation in extreme conditions, parameters for crew necessities and comfort and the initial parameters for structural design. Phase Two developed the preliminary design of several schemes ranging in programmatic layout from a fully automated utility device to a leisurely garden that doubles as crew quarters. At this point the selection and design of general lighting and plant-growing systems was included. Phase Three focused mainly on the development of construction methods for each scheme, inclusive of installation, execution, and support techniques. Phase Four was the design of a sample structure utilizing the schemes that were determined to be most appropriate. Strategies were considered for the physical linkage of the MDG to the main MarsPort complex, for clustering of multiple greenhouses as a system of multi-function modules, and for integration of the life-support systems between the main complex and the MDG.

**Issues and Proposal Summary:**

The MarsPort Deployable Greenhouse presents a set of requirements, which, although closely related to greenhouses on Earth, also make the structure radically different from traditional greenhouses. The term greenhouse is loosely used as meaning an enclosed space for growth of vegetation. Besides the main requirement as a vegetation growth chamber requiring certain atmospheric, energy, nutrition and storage needs, the MDG also has to include provision for protection from the harsh environment of Mars and for the possible utilization of native resources for sustainability, thus minimizing the dependency on Earth-imported material. Although it will depend on highly scientific support mechanisms as does everything else of human origin on Mars; the intent of the design is to detach the MDG as much as possible from the feeling of a laboratory and to make it similar to a peaceful garden.

**Atmosphere maintenance:**

The specified atmosphere can be maintained through the use of a commercially available system for close monitoring of air chemistry. Such systems can control every aspect of the atmosphere: chemical makeup (amount of different gases and water vapor), relative humidity, and temperature. Air quality can be tailored to the vegetable crops in the greenhouse at any given time.

An initial amount of Earth life-supporting substances will be shipped with the MDG. A sample system is designed that efficiently stores, uses, and supplants imported material that cannot be easily extracted from the Mars environment. The most important elements are oxygen (produced and consumed by the plants), water vapor can be introduced into crew quarters for breathing or into a biochemical reactor for breakdown of other substances by oxidation), carbon dioxide (used by the vegetation for photosynthesis, carbon dioxide can be introduced directly from the Martian atmosphere which is 95% CO₂, and nitrogen (used by the vegetation as nitrite or ammonium only, nitrogen can be extracted from the Martian atmosphere as gas (2.7%) and fixed by bacteria or chemical processes). By-products of the greenhouse such as ethylene gas and water vapor can be removed in the air-monitoring systems and routed to other services for utilization.

**Light, heat and energy:**

A major concern of the design is meeting the energy requirements for the greenhouse. NASA prescribes the minimum requirements to be 125 – 50 W/m² for at least 12 hours per day, with a temperature range of 10-30 degrees Celsius. The fact that Mars’ iridescence is 589.2 W/m² means that a 21% transparency of the enclosure will be adequate to produce the mid-day intensity required. Few materials can maintain this level of transparency and still keep a high R value (resistance to heat transfer). Furthermore, transparent materials that are flexible on Earth, therefore ideal for a deployable tensile structure, loose such qualities in the cold Martian climate (as low as -140 degrees Celsius). A more viable solution is an opaque enclosure that satisfies the requirements for structural integrity, deployment, and heat-transfer isolation in which the lighting can be achieved through a system of light-collecting devices (focusing lenses, mirrors, sun-tracking apparatuses), distribution devices (fiber optics, mirrors), and conservation devices (photothermic/photovoltaic...
plates, batteries) that maximize the light collection and utilization levels for both natural and artificial lighting (Figure 1).

Temperature in the MDG must be maintained between 10-30 degrees Celsius. Part of the heat requirement will be satisfied from the natural operation of the equipment – lamps, pump engines, biochemical reactor – all of which emit heat in their normal mode of operation. Provided there are adequate energy sources, the temperature can be further supplemented by the previously discussed air system.

Energy for the MDG may be harnessed in situ with photovoltaic/photothermal systems, other natural systems like wind or geothermal energy, or the air system may be plugged into the main power grid of the complex, utilizing either fuel cells with (ideally) native materials or the two nuclear power plants prescribed in the DRM.

Vegetation:

NASA studies have shown that the ALS (Advances Life Support) greenhouse can fully meet the dietary needs of one adult with 12 m² of planting trays. The MDG is designed to produce 25% of the needed food for the crew; therefore, for design purposes, 3m² planting space per person is allocated. As the NASA brief and DRM specify crew size of 6, 18m² is the minimum required planting space.

The most efficient growth system is hydroponics – either static medium (inert "soil" for root support and aeration) or NFT (Nutrient Film Technique, a shallow channel) with a slow moving solution of nutrients that washes the plants' roots (Figure 2). Hydroponics requires a system of reservoirs for the nutrient solution and a monitoring mechanism for nutrient content. One of the major advantages is that the system can optimize the nutrient content for any of the desired crops. Systems for collection, primary preparation, and storage of the foods produced may include both human and robot labor. If the greenhouse is dispatched to arrive before the crew, it would be beneficial if it were designed to be self-deploying and fully automated so that an initial amount of food would be ready for consumption at crew landing. A selection of foods with a high harvest index is desired for optimizing dietary supplements.

The Utah State University Crop Physiology Lab has developed a series of dwarf cultivars – tomatoes, rice, and wheat. Such space-efficient plants mean a more compact planting area in the enclosure, thus allowing for a more liberal allocation of space for human activities not directly related to the operation of the greenhouse.

Deployable structure and methods of construction:

The discussed technological requirements produce the need for a mechanical core that houses all the equipment and storage units needed for the operation of the greenhouse. In architectural terms this translates into a separation of systems into the mechanics core, the enclosure, and the planting space. The plant-trays and the enclosure system can become the deployable portion of the structure, backed-up by a rigid mechanical core that contains all the necessary materials and machinery for deploying and operating the MDG during transit. For space efficiency during transit and operation, geometrical forms that optimize the ratio between internal space and enclosure surface are desirable, therefore cylindrical or near-spherical forms are desirable.

There are multiple ways to handle the deploying systems. They can generally be separated into three categories – inflatable, telescoping, and smart-materials derivations. Some of the options available include air-supported structure and enclosure, inflatable structure and tensile enclosure, tensile enclosure and telescoping structure, telescoping sliced rigid shells, rigid telescoping enclosure, smart material sheets corresponding to open-closed situation under different amounts of agitation, and programmable or propelled resins on a rigid skeleton structure (Figure 3).

The designed sample prototype focused mainly on the cylindrical 'backbone' version of the system with inflatable enclosure and ribs filled with foam, which has the necessary rigidity after curing to provide the compression strength needed by the structure (Figure 4). All life-support systems are enclosed within the mechanics bay in the center of the developed torus, while the fiber optic light distribution system, the enclosure, and the plant trays are integrated into an inflatable skin and structure. Depending on the size of the crew, the area and height of the torus may be modulated for efficiency and comfort.

Crew life:

While the MDG is primarily a utility building, it also provides a unique opportunity to introduce a node for crew interaction that is not easily achievable in any other part of the MarsPort. It provides an option for mimicking a piece of Earth for private use as a garden for relaxation or solitude or for another mode of working that is different from the crews' highly scientific everyday life.

In the worst-case scenario of the mission window 2007-2009, transit time for the crew is 180 days, with a surface stay in the order of 600 days, which means about three years in the exclusive company of five crewmates without the certainty of returning to Earth. While acquired discipline, training, and everyday work will be a constant protection from the tension among crew members and between the crew and mission control, the addition of a purely low-speed and low-stress environment with edible and decorative vegetation should be able to provide for necessary privacy and humane ambiance.

Work Value:

In conclusion, this research focused on obtaining and cataloging information and design ideas and collating them into
a useful document to serve as a starting point for prototype development of the MDG. While my background in botany, engineering, chemistry and psychology is limited, this project allowed me to gather the basic information needed to design a prototype structure for a Martian greenhouse. This project can also serve as a base for future student projects and build upon the University of Arkansas relationship with NASA. I maintain a great interest in the subject of space exploration and design for human comfort in sterile environments, and my intention is to continue research with prototypes of the proposed deployable structures for use in a wider sphere of human habitation.

This project has proved to me the need of architectural design for any and all human environments, and iterates the importance of close integration between technology and design. Extreme environments call for extreme strategies. While on Earth many aspects of the architectural design are taken for granted (sunlight, openings, access, and sustainability), design for environments like that on Mars take on the character of ultimate problem-solving and can easily slip into the realm of engineering. An extraordinary need for enclosure, and yet openness to the outside world, is manifested by the pioneers that go out in space to learn as much as they can; yet they must physically isolate themselves from their subject just to be able to perform a basic human action – to breathe.

References:

Endnotes:
1 Mars Design Reference Mission v.3.0/1998
2 Mars Fact Sheet
3 For example vinyl sheets, 89% transparent and highly elastic at normal temperature, become brittle at -58 degrees Celsius
4 As far as the density and speed of growth is concerned, as well as the amount of growth labor and collection. The most labor-intensive part is the seeding stage, which can be automated. On Earth with NFT lettuce can give between 9-12 harvest per year.
5 My former professor Ted Krueger established a successful dialogue between the School of Architecture and NASA. Professor Jerry Wall is continuing his efforts.
Figure 1 - Lighting system - collected sunlight is tunneled through fiber optic cables to the plants, excess is piped to photovoltaic plates; the produced electricity is stored in batteries and used to supplement the lighting when necessary. The electric light is distributed through the same piping system as the natural light.

Figure 2 - Hydroponics diagram

Re-circulating nutrients tank
Figure 3 - Structural systems – fully inflatable, telescoping structure with tensile enclosure, rigid sliced shell, rigid telescoping, bi-metal smart plates.

Figure 4 - a. An MDG prototype – (functionalism preferred)
  b. Integration of deployable systems
Faculty Comments:

Julianna Preston, who has had Ms. Dineva as a student in both design and technology classes, made the following comments about her work:

I first met Ms. Dineva in the midst of interviewing for my current position at University of Arkansas School of Architecture. She was the lone student/expert willing and able to sort through several layers of computer technological challenges between my presentations and the building network system. Despite being in the middle of preparing her own final studio presentation, she attended to my needs in a cool and collected manner. The way in which she solved these problems is emblematic of her design method, rigor and willingness to experiment.

I later taught Ms. Dineva in several of my design studio courses and in an experimental elective course focused on computer technology and ornament. In each of these courses she forged her own unique path in response to the course objectives. Her proposals have consistently stood out among her fellow students for their intense consideration of technology (structural, material, geometry) and for their numerous iterations toward refinement. It is my observation that this is not the norm in this school or in other students of architecture around the country.

The work on deployable greenhouses demonstrates Ms. Dineva's ability to identify a program, research its extension as a material and spatial construction and feed the project with knowledge of technical data, architectural design and known precedents. The fact that she developed this proposal almost completely on her own initiative and critical skills is credit to her enthusiasm, drive and professional attitude.

Ms. Dineva's faculty mentor, Jerry Wall, had this to say about her:

Iova Dineva is an excellent student, creative designer, exhaustive researcher, and responsible leader. As her faculty mentor I knew that she always gives her best effort. She has been extremely helpful with our NASA research on exercise equipment for zero gravity. She was instrumental in preparations for the exhibit of "Work surfaces in space," in Zurich, Switzerland. Ms. Dineva is definitely one of the top students in the School of Architecture. I support her wholeheartedly.
A WORLD OF THEIR OWN: WOMEN AND FOLKLORE 
IN INTER-WAR BRITAIN

Natalie Holub
Department of English

Faculty Mentor: Debra Rae Cohen
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Abstract:

The period between the First and Second World Wars was an unsettling time for women in Great Britain. After the First World War, the media, governmental acts, and everyday society urged women to return to the home. This was an especially difficult concept for women to accept after they had played a very public role during the war actively contributing to the war effort. My thesis explores three novels of interwar England that feature female characters seeking purpose in places outside of the traditional role of housewife. Ashe of Rings by Mary Butts, Harriet Hume by Rebecca West, and Lolly Willowes or the Loving Huntsman by Sylvia Townsend Warner feature females who employ untraditional methods in their quest for both stability and power in post World War I England. The women of these novels do not comply with the then popular notion that women belong in the role of wife and mother tucked back neatly into the domestic sphere. Rather, they relate to the mystical realm of nature to empower them. In Ashe of Rings, a woman returns to her homeland to reestablish herself as the rightful heir to the power of the Badbury Rings, a megalithic site in southern England. Harriet Hume is the story of a woman who discovers she has a special psychic power to read a masculine mind. A single woman, tired of being shuffled from home to home within her family, finally flees to the countryside and becomes a witch in Lolly Willowes or the Loving Huntsman. In carefully examining these texts as other literary criticism on the topic, I have found that these women do indeed find solace in the mysticism of nature and the power of myth. They discover a powerful connection in a country whose certainties have been demolished by the First World War. They do not find this connection in the much-encouraged role of housewife or mother, and they must search outside the boundaries of "traditional" society to find this stability. These characters possess the power to inhabit a world of their own where their strength is intertwined with the mysteries of nature and the might of the myth. Therefore, this study has implications for the potential of women to find empowerment outside the traditional patriarchy.

Introduction:

This other self knew that life need not be bitter, nor worthless, nor bounded by a narrow casement, but could be limitless, infinite... Daphne du Maurier, from Frenchman's Creek

During February 1919, as troops returned home to England after World War I, an advertisement for Oatine Face Cream featured attractive women applying the product to their faces. Underneath the picture was the legend, 'Back Again to Home and Duty.' The advertisement went on to say, "Now that the war is won, many women and girls are leaving work, their war job finished. They are naturally desirous of regaining their good complexions and soft white hands freely sacrificed to the National need. Oatine is invaluable for this purpose" (qtd. in Beddoe 13). The "war jobs" that women had performed were crucial to the stability of England during the Great War. Their contributions ranged from that of Mrs. Rosanna Foster, who took over her husband's chimney-sweep business to women munitions workers who hand-filled artillery shells in factories throughout the country (Condell and Liddiard 10, 111). Whether they were filling the jobs that soldiers left behind or creating aerial bombs, these women assumed very public roles in the workforce of England. But as men returned from the trenches, there was tremendous pressure for women to return to domesticity. Deirdre Beddoe says that "the single most arresting feature of the inter-war years was the strength of the notion that women’s place is in the home" (3). The media, the governmental acts, and often the public urged women to return home where they could regain their "good complexions and soft white hands." There was a push for women to "bear and raise children in a generation tragically depleted by the war" (Beddoe 4). Thus, while the idealized image of the housewife was praised by British society at the time, images of other culturally defined "genres" of women were shrouded with negative connotations. During the war, many women felt a sort of liberation as they came out into the workplace. Their prewar roles as passive, private maternal figures gave way to the more empowering positions of active, public members of the British workforce. "It was now permissible for women to be physically
courageous, enduring, responsible, conscientious, cheerful, and outgoing” (Condell and Liddiard 157). However, much of Britain longed for the familiarity of the world before the destruction of the war, and “properly” putting the gender roles back into their previous state was a way of restoring this. Men were to reenter the workforce, and women were to return to the domestic sphere where they would find their satisfaction from being wives and mothers. But the wartime need for women in the workforce had already shown women that they could play an important role in society outside of the home. Even before the war, women had worked for the vote and begun to examine their roles in society and question the validity of traditional notions of gender. Although women did receive limited voting rights in 1918, expectations for a society that allowed a more public women were dashed as the general social notion became that they should return to the home.

The opposition between societal expectations for women and the longing for a sense of purpose outside of the traditional role of housewife and mother created tension for English women. Literature was, of course, a method of voicing such anxieties and concerns. According to Alison Light, “The search for her own room, for a place which could be both domestic and public, private and professional, suggests how much the act of writing has itself had special meaning for women given their situation both in the house and in history” (5). Indeed, literature reflected the women’s need to achieve a balance between traditional definitions of femininity and their own desire for connection to the world. World War I sparked a wave of such female writing on the topic of gender roles and cultural standards. According to Dorothy Goldman, “If war acts as a stimulus to make women openly reconsider their roles and to write about that reconsideration, it also makes them reconsider what they have been told about their inherent natures, and to write about that too” (42). Thus, the question of women’s role in the war effort sparked questions about essential feminine nature. Goldman goes on to say that “one of the threats to women’s ability and freedom to undertake war duties came from their families, and here, too, women began to question the familial identity that encompassed them” (43). Many works by female authors question this familial and societal identity by presenting heroines who accept their own version of femininity, one that relates less to the patriarchal public or private spheres than to the mystical realm of nature.

Ashe of Rings by Mary Butts, Harriet Hume by Rebecca West, and Lolly Willowes or the Loving Huntsman by Sylvia Townsend Warner feature female figures who employ untraditional methods in their quest for both stability and power in post-World War I England. The women of these fantasy novels find solace in the mysticism of nature and the power of myth. One woman is priestess of an ancestral monolithic site, one finds that she has a strange ability regarding insight into her former lover, and one escapes from her family into the wilderness to become a witch. These heroines possess the power to inhabit a world of their own where their strength is intertwined with nature. The study of these novels has implications for the potential of women to find empowerment outside the realm of traditional patriarchy.

Mary Butts, author of Ashe of Rings, was born December 13, 1890, in the Salterns of England to Mary Briggs and Frederick Butts. Salterns, the name of the house at Parkstone, was a tremendous influence on Mary. She described it as a temenos, or a sacred enclosure, and called it “Perfectness” in the autobiography of her childhood, The Crystal Cabinet: My Childhood at Salterns (14). She described her isolated childhood as being “saturated with the arts. With the visual arts as with nature, a foundation of classic and to some extent of modern literature” (31). While Mary had an unstable relationship with her mother, her father was a respected source of knowledge. While she described her father as “old soldier and something of a scholar, the inheritor and the treasurer of Blake, the admirer of Whistler and of Wilde,” she spoke of her mother as having a “vigorous, romantic, emotional nature, truly religious, but uncoordinated by the least touch of subtlety or intellectual love” (31). Butts’s brother, Anthony, was born in April 1901, and her father died in 1905. Even after her father’s death, Mary’s interest in the classical world continued to flourish. Of her intimate relationship with Greek tradition, she said “to remember Greek life is not to adventure into a delicious ideal but to go home to something so familiar it can bore me” (Blondel 22). Although she felt a deep connection to these ancient traditions, she still practiced a disregard for contemporary conventions and rules all her life through her rejection of popular religion, her numerous relationships (one of which resulted in the birth of her daughter, Camilla, in November 1920), and her experimentation with drugs. During her life, Mary Butts wrote five novels, three short story collections, around thirty poems, an account of her childhood, and many essays and reviews (Wagstaff xii).

A journal entry by Mary Butts written on September 18, 1917, says, “Notes for Ashe. Take for the central theme the hatred of age for youth, of the old for the new, of yesterday’s ‘rose’ for today’s, expressed in a cruelty, part intuitive, part consciousness” (Wagstaff xvii). Ashe of Rings does present a series of battles, between war and peace, destruction and construction, and good and evil. But the novel also serves as a slightly altered reflection of Mary Butts’s childhood: “Friends and family are barely disguised in all her work, and speed of her impressions is translated at once to her paper” (xviii). Indeed, Ashe of Rings contains a woman raised in a mystical countryside who is taught by a wise father and criticized by her mother. But the novel also parallels Butts’s relationship with the mysterious. Once as she explored the ancient countryside with her brother she “experienced an epiphany of supernatural patterning, ‘the makings of a correspondence, a translation which should be for ever valid, between the seen and the unseen’” (Wagstaff xix). Set during World War I, Ashe of Rings explores what can be understood by some and rejected by others.
In 1912, a young woman named Cicely Fairfield renamed herself Rebecca West. The reason for this formal change was practical: “She had begun writing in earnest for the radical suffragist journal the Freewoman and dropped her original name to spare her sister Lettie professional embarrassment over this association” (Scott, Gender 560). Rebecca West was born to Charles Fairfield and Isabella Mackenzie in 1873. Her father deserted her family in 1901, but West inherited from her mother “a love of music that would serve her literary metaphors, and the character type of a brave, though stern, female provider” (561). She was a feminist and socialist journalist and book reviewer, wrote studies and lectures on men such as Henry James and Shakespeare, and produced a vast array of short stories and novels. Her personal life was tainted by a “heterosexual crisis, bequeathed in part by the sexual liberation of the new woman. She endured unwed maternity, lifelong identification as the mistress of the sexual libertarian H.G. Wells, and persecution as a bad mother by her only child, Anthony West” (Scott, Refiguring xi). Harriet Hume figures into West’s gender studies as a “lighter ‘fantasy’ novel that sets the male ambition to rise in political endeavors against feminine art” (Scott, Gender 564). The novel explores a woman who has a supernatural relationship with her garden and her music. The heroine of Harriet Hume is fully feminine while remaining a single woman in the patriarchal society of London.

Sylvia Townsend Warner, born to George Townsend Warner and Nora Hadleston in 1893, also explored the theme of feminine options in an inter-war society of domestic pressure. Sylvia was an “abnormally intelligent child, even at an early age,’ eager, observant and self-possessed” (Harman 7). But her mother was disappointed in her because “Sylvia was not a son, she was not going to be a beautiful daughter, she was rather off-puttingly clever and rapidly becoming the apple of her father’s eye” (13). Much like the heroine of Lolly Willowes, Warner adored her father and had no interest in marriage whatsoever. Warner was “unmarriageably intellectual” and chose to live her life defying societal standards (23). After her father’s death in 1916, Sylvia’s unmarried status continued to bother Nora. But Sylvia Townsend Warner’s great romance came later in life with her dedicated relationship to woman poet Valentine Ackland with whom she published the work Whether a Dove or a Seagull, dedicated to Robert Frost.

During her life, Sylvia Townsend Warner wrote seven novels, 10 volumes of short stories, nine books of poetry, a biography of T.H. White, a translation of Proust’s Contre Sante-Beuve, and many essays and reviews (Marcus, Intro 531).

Lolly Willowes, written in 1926, is a novel whose main character has a special relationship with her father, a distaste for marriage, and a special connection to the natural world around her. These attributes reflect Warner’s own life, and the reader has little doubt after careful comparisons that she pulled from her own experience to write the fantasy novel. Warner’s special connection to the peace of nature is exemplified by one of her letters:

The day before yesterday, I appeased a life-long ambition: I held a young fox in my arms... I held him in my arms & sniffed his wild geranium smell, and suddenly he thrust his long nose under my chin, and burrowed against my shoulder, and subsided into bliss. His paws are very soft, soft as raspberries. Everything about him is elegant— an Adonis of an animal (qtd. in Marcus, Intro 531-32).

It is clear that Warner had a simple appreciation for the natural such as animals and plants. Warner never had children, but this passage suggests the maternal aspect of her nature. Similarly, Lolly Willowes depicts a woman that has a private relationship of wonder and respect for nature. It explores the theme of women “struggling for privacy, not power” (Harman 64).

Sixty new women’s magazines were launched between 1920 and 1945. In October 1932, Woman’s Own was introduced to the public and prefaced with the following:

How do you Do?

We introduce ourselves and our new weekly for the modern young wife who loves her home.

Woman’s Own will be a paper with a purpose— a paper thoroughly alive to the altered conditions of the present day. The home paper that makes any girl worth her salt want to be the best housewife ever— and then some (Beddoe 14-15).

But many women were discovering that they did not need an instruction manual on how to be the perfect housewife. Instead, they needed a place to find their own power. This place might be in the English countryside or a small garden in a big city as it was in the fantasy novels of Mary Butts, Rebecca West, or Sylvia Townsend Warner. These stories recommended that women return to their own form of the wilderness, to the essential feminine, to find the ancient traditions within themselves, those of goddess, artist, or witch. But the place could be anywhere, as Mary Butts says, that was “enchanted— technically— concretely— if there is such a thing— by reputation, by experience, by tradition” (Blondel 114). Women needed the opportunity to protect, create, or examine themselves in their own way. The fantasy novels of this time provided an example of this power and showed that this place was available, if only within the pages of a book.

Chapter One:

The notion is that life involves maintaining oneself between contradictions that can’t be solved by analysis.— William Empson, note to Bacchus (The Gathering Storm, 1940)

On Badbury Rings... We went up the hill to them. I
walked first saying it is 1 who have given them life. Then inside the first fosse is steep, and I leaned on Cecil’s shoulder and said I need you to enter the Rings... There was the quiet. I lay stretched out on the ground, and understood that the Ring’s signature is written in its quiet. Its quiet is made audible by the sound in the grove. — Mary Butts, on her 12 March 1922 visit to the Rings with friends.

John Gould’s essay, “On Making Sense of Greek Religion,” attempts to explain a complex concept that has particular significance in understanding Mary Butts’s novel, Ashe of Rings, and the novel’s relationship to myth. Gould points out that to more accurately understand the religious experience of the ancient Greeks, readers must dismiss what they understand to be the traditional concept of religion. For instance, Gould states that “Greek religion is not ‘revealed; as Christianity is; there is no sacred text claiming the status of the ‘word of God’” (7). Greek religion did not feature a belief in “divine omnipotence,” nor did it demand an “organized body persisting through time comprising those with dogmatic authority.” Thus, there are few sacred requirements. Gould explains that, “the central Greek term, theous nomizein, means not ‘believe in the gods,’ but ‘acknowledge them,’ that is, to pray to them, sacrifice to them, build them temples, make them the object of cult and ritual” (7). The gods of ancient Greece did not require the belief of humanity. Instead, they needed only to be the center of ritual functions.

If they did nothing else, these ritual functions gave the people of ancient Greece a connection to a higher being. Gould goes on to say in his essay that the Greeks perhaps pursued their religion to handle chaotic events in the world around them (5). It is not outrageous to believe that they encountered the same basic experiences as other groups of people. They sought out a method to handle the uncertainty they encountered, and their mystic rituals of worship and offering provided this. Author of the “War-fairy-tale,” Ashe of Rings, Mary Butts studied the ancient Greeks and the way they maintained balance in their society (Butts 232). Indeed, Butts felt a “profound familiarity” with classical mythology; she states in a book review, “we feel that their delights and desires and difficulties were in the same terms as ours: they were examining, shaping, using and being used by life in a way we understand” (Blondel xi). In her introduction to Ashe of Rings, Nathalie Blondel discloses that Butts “acted out Greek fables and historical events with her father in the garden of Salterns, her home in Parkstone on the Dorset coast” (ix). The heroine of Ashe of Rings also acts out Greek myths as a child, yet this is not the only instance where Greek myth appears in the novel. Ashe of Rings is centered around the concept of human beings inheriting a connection to powerful, ancient forces. Although these forces are not entirely explained, they are able to be harnessed through ritual and humanity’s reverence for their mysterious powers. Butts parallels the rituals and myths of the ancient Greeks to the British family of Ashe and their respect for the ancient powers of nature. The novel is the story of the Ashe woman who becomes heir to this power and by implication, helps keep the world regulated. Ashe of Rings features the strong feminine presence of Vanna Ashe, who learns that her strength as a guardian of the Rings involves reinforcing traditions that are worth preserving.

The novel is constructed around the Badbury Rings, a megalithic structure consisting of concentric rings found in south Dorset, England. Rings is referred to in the novel not only as the actual stone structure but also as the property on which are the Badbury Rings. Throughout the novel, there is a constant feeling that there is something powerful yet mysterious attached to these Rings. The forces of this structure bring empowerment to whomever can grasp them and the surrounding aura. Vanna Ashe attempts to explain them to an unbeliever: “Think of Rings as a ship, full rigged, full of treasure, every sail set, plunging over the back of the world. The seas hiss and slide, and she cuts them; and crosses the horizon where the moon comes out of the sea” (Butts 95). If the Rings are a grand ship, then the Ashes are the crew and the hissing seas are the chaotic world. However, maintaining the Rings is also crucial for maintaining the order of the world itself. Therefore, there is an important connection between the stability of the Rings and the existence of the world. In this aspect, the fate of the seas depends on how the ship is sailed by the crew. The Ashe family has an essential role as protector of the Rings because they also assume the role of guardians of the cosmos.

It is clear that the Ashe family has always been consciously attached to their obligation at the Rings. “Its triple circle was the sole device on their shield, represented from the hatchment of their dead to the coral and bells each baby chewed and shook” (Butts 6). Anthony Ashe, father of Vanna, is constantly referring back to the history of the Ashe family at the Rings. The many Ashe ancestors form a strong network of accessible power that reminds the living family of this enabling link. The family had a serious duty to the world through their connection to the Rings. They must be protected by the proper person, but they offer something to that person as well. They can be seen as a living capsule of protection for the Ashe family. They are not only on the family shield, they are the shield itself, offering options for coping in an inherently chaotic universe. At the beginning of the novel as the Rings and the Ashe home are being described, there is a description of their near proximity to the ocean: “The waves rang within earshot of the lodge. In storms they covered it with spray. There Rings ended and the world began” (Butts 5). The world cannot fully penetrate the Rings. This passage suggests that the Rings are not necessarily of this earth. The Rings are a universe of their own where accountable forces guard against the storms of our world.

It is important to remember that Mary Butts wrote Ashe of Rings as a “War fairy-tale.” Good and evil are more easily defined in this context. Death, destruction, and violence are evil. World War I included all of these things, and Judy Marston, the
villianesse of the story, embodies the war. On the other hand, vitality, generation, and stability are good. Vanna Ashe, the heroine, represents these benevolent forces. Butts comments on the fairy-tale status of her novel in an afterword: “as a story, it is entirely an invention; yet one finds that one had good reason for everything that one puts in” (232).

Clearly, there is a reason for every literary technique of the novel. The story can be seen as an allegory, a tale that translates the tensions of the world into symbols. Therefore, the novel is more than a fairy-tale; it is a depiction of the strain in inter-war Britain where the aftermath of the war threatens to unravel the threads of tradition. The Rings are a traditional, stable force of good. The guardian of the Rings protects what is right while destructive forces threaten this system. Guardians are not powerful for their own sake; rather, there is a suggestion that their power can help the world. The Ashe family must not use this power for their own purpose. Instead, they must understand the Rings’ beneficial role and use them accordingly.

In her introduction to the novel, Blondel offers the idea that, “Ashe of Rings is an allegorical contest between those who understand this prehistoric landscape (and who thus see themselves as the Eumolpidae, inheritors of the Eleusian Mysteries) and those antagonistic to it” (x). The aristocratic Eumolpidae served as “custodians of religious tradition and customary law” (Easterling 7). Perhaps the family of Ashe does not completely understand the mysteries of the rings; rather, they are able to fully recognize their power much as the central term of Greek religion discussed earlier: theos nomizein, demands that humans “acknowledge” the gods. Blondel even suggests that Butts thought herself to be part of this descent: “She was increasingly convinced that Ashe of Rings had been ‘all prophecy’ and... she traced the lineage of ‘A priestly house. Alkmionids — Eumolpidae — Blake — 1 Camilla — ?’” (99). N.J. Richardson gives an explanation of the Mysteries that proves helpful in understanding the Ashes’ relationship with the Rings:

In the classical period initiation into the Mysteries (as they were called) at Eleusis was open to anyone who could speak Greek, provided that he was not polluted by bloodshed, and participation was later extended to the Graeco-Roman world in general. Consequently secrecy, although always very solemnly stressed (with the penalty of death if it was broken), was to some extent a nominal matter, and the real point of it seems to have been not so much to limit the range of those who could hope to benefit from initiation, but rather to stress the awe-inspiring character of the deities who were being honoured and of the benefits which they could confer. (58).

The function of the Rings becomes more apparent when they are likened to the Eleusian Mysteries. The Mysteries, a sacred ritual honoring deities, offered a means for the ancient Greeks to empower themselves and control chaos through close relation to “awe-inspiring” beings. Similarly, the Ashes are intimately connected to the “awe-inspiring” power of the Rings. Again, it is important to acknowledge that the power that these beings provide is not to be misused. The forces should not be used only to empower the guardians; instead, this power should be used to protect what is right in the world.

Throughout the novel, the Rings are shown to be an active force. For example, they are referred to as living creatures. Humans talk to them (7), they sleep (53), and they show their teeth (54). The constant personification of the Rings signals that they are alive and very much in control. In one instance, the Rings actually hide Vanna from danger. As her attacker moves in closer, her naked body blends in with the stone and the blood of a dead dog turns into a “white poison” and moves the stone (Butts 189). This is a result of Vanna’s prayers to “Florian and Ursula, my father and my mother in Ashe” and her realization that her bare white body could match the paleness of the stone. She has combined herself with the Rings and her faith in this union has saved her. Still, it is the combination of her confidence in the Rings, her ancestors, and her practicality that pull her through the situation. As with the Greek deities, it is not as important to believe in the Rings as it is to acknowledge them. The Ashes have faithfully done this, and they have remained the steadfast guardians of their beneficial power.

Vanna is the heroine of Ashe of Rings, and the novel traces her life from conception to adulthood in three sections. Ashe of Rings begins in 1892 as the aged Anthony Ashe seeks a young woman to marry in the nearby village of Gulltown. His intentions for the girl quickly become obvious. She will bear his child, the heir to the power of the Rings. As he ponders a particular village woman as a potential bride, he says, “She’d fill the gilt cradle, and the oak cradle” (Butts 9). Anthony Ashe eventually marries the “ripe virgin,” Muriel Butler, whom he renames Melitta. The meaning of this new name is significant, according to Roselyn Reso Foy: “Melitta comes from the Greek, suggesting bees and the sweetness of honey, and the implication is that Melitta is chosen because of her fertile childbearing abilities and not for any other reason” (38). From the start of their marriage, Melitta is uncomfortable in the house of Rings. She cannot understand her role in the Ashe family. Furthermore, when she finally becomes pregnant and has a baby girl, she fails to connect with the child. She feels as though “the baby [does] not like her” (28).

This daughter is Vanna Elizabeth Ashe, heir to the power of the Rings. Vanna’s early childhood is filled with the myths and legends, both Greek and local, that her father passes on to her. From playing “Phoenix persuading Achilles” in the garden to explaining the function of the Lares, household spirits of Roman religion, Anthony Ashe is careful to reveal the power of myth to his young daughter (30-1). Meanwhile, Melitta, confused and unsure of her position within the realm of the Rings, begins an affair with Morice Amberton, a neighbor. The affair is terribly insulting to Anthony Ashe, not only in and of itself, but because it is consummated on the Rings. After Anthony discovers them there, he attempts to clean the tainted area by using “clean leaves
to cover the place where they had lain." The affair does not
surprise him, but the fact they disgraced the area is deeply
disturbing. However, he realizes that "this, too, had its place in
the cycles" (39). Anthony understands that this violation of the
Rings has a purpose in the overall string of events that will lead
to Vanna's eventual reign as heiress to their power. Anthony
Ashe dies shortly after the incident. Melitta discovers her second
pregnancy and marries Morice Amberton. Melitta's second
child is a boy, Valentine Evelyn Ashe. The children spend their
time between Amberton and Rings. Section I ends as Vanna has
been sent away at school, the rest of the family dwells at the
Amberton house, and the Rings rest in silence. The Sections II
and III, set in 1917, explore the life of an older Vanna and the
effects of the war. Foy states that, "the stage is set for the adult...
Vanna's struggles to search for answers and to begin her attempt
to heal and pity those who need her magical solace" (39). The
Rings are the source of the "magical solace" that Vanna can
provide for those who have been effected by the war-torn and
evil-filled environment. In this section it becomes clear that
Vanna is the guardian of the Rings and the priestess who is able
to draw from their power. The energy she receives from them has
the potential to heal those who have been affected by the war.
In this way, she has a certain responsibility to use her inherited role
for the survival of the world.

One of the main forces antagonistic to the power of the
Rings is Judy Marston. Although Judy first appears as Vanna's
friend, it soon becomes apparent that she is Vanna's antithesis.
Although she is a strong feminine presence, she spends her
powers on trying to undo what is stable and secure. She
unsuccessfully tries to control the power of the Rings for her own
ruinous purposes. Section II begins as Vanna must share a flat
with Judy due to a limited income. The section introduces Serge,
a Russian Emigré who has avoided military service in the war.
Judy seduces Serge and fights to control and undo him. Vanna
tries to save Serge when Judy leaves him for Peter Amberton, the
shell-shocked nephew of Lord Maurice Amberton. Although
Judy desperately tries to introduce Serge to the power of the
Rings, he cannot comprehend their significance. The novel
suggests that this is due to the impact that the war's destruction
has on Serge. During a conversation with Clavel, the
groundskeeper at Rings, Serge admits that he has not found access to the Rings:

"Miss Vanna had the Rings to help her. They're no
good to me."

"Perhaps you haven't got what Mr. Anthony Ashe
called the words of power, Mr. Serge."

"Magic is no good unless you believe in it, Clavel."

"Quiet, sir. The only good in it is to take out of you
what is already there.

Inside out is the rule." (Butts 220).

Serge is a young man who has a history of fleeing from
military service. He continues this practice during the First
World War. As an artist, he might be viewed as a creator, one
who produces things or ideas. He cannot be a part of the war
because war is inherently destructive. The role of a soldier would
contradict his beliefs as an artist. However, no matter how much
he tries to accept the healing nature of Vanna Ashe and the Rings,
he has already been claimed by Judy and her destructive powers.
Throughout the novel, Serge is in limbo between the "magical
solace" that Vanna has to offer through the Rings and the ruinous
magic of Judy.

Judy, as Vanna explains in the novel, represents the war and
its shattering properties. "Consider the war. Have you known
anyone who loves the war as Judy loves it? Stoop then and wash.
She dips her tall, white body in the blood and rolls it in her mouth,
and squeezes it out of her hair" (Butts 149).

If Ashe of Rings is a "War-Fairy-Tale," then Judy is the
wicked witch of the story. She is the enemy of the forces of good,
and will stop at nothing to undo Vanna and the power of the
Rings. Furthermore, if the Rings are a ship, then Judy is struggling
to gain control of the vessel and tap into its powers. Vanna must
protect the power of the Rings as the Eumolpidae guarded the
Eleusinian Mysteries. But although the female heir to the Rings
successfully maintains the positive forces, she cannot claim the
soul of Serge from Judy. Judy is the strong power of evil in Foy's
mind: "She is the antithesis of Vanna's white magic (change for
good); she is a throwback to ancient aggression and sorcery that
Vanna must struggle to combat" (Foy 41). If Judy can be seen as
an embodiment of the war, Vanna is a return to what was stable
before the war. Her character offers a method of regaining what
World War I stole from the hearts of the British.

One of the effects that the war had on the British was a
feeling of displacement. Vanna handles her displacement in the
city by a return to her country home, and this reflects a long
tradition of seeing rural life as the heart of Britain, what Martin
J. Weiner terms "idealization of the countryside" (47). According
to historian R. C. K. Esnoro:

The human wealth of a populous countryside in
which all classes lived, and could live, at peace, for
centuries—that is our arch-achievement as a nation,
the source and condition of our other greatnesses, the
base on whose fragments, 'majestic though in ruin,'
we can still sound, if not our loudest, at least our most
legitimate fame (qtd. in Weiner 61).

The Rings, set near the ocean in the British countryside,
represent for Vanna a near-Eden. It is there where she can find
her strength as a goddess who protects a mystical tradition.
Although Judy, the evil goddess of ruin and decay, can tap into
their power, Vanna is able to reestablish the Rings as a symbol
of peace and order. Butts provides a scenario in which, while
World War I threatens to destroy all that was traditionally
British, the strength of the British myth of stable rural life finally triumphs.

Triumph comes from Vanna’s position as the “embodiment of an ancient priestess” who is “also closely connected to the animism of the land” (Foy 42). Vanna firmly acknowledges that the Rings indeed have a soul that supports the human life that praises it. Even as a child she begs her tutor to tell her stories of the Ashe family and “say it again. Make a magic of it. I think I know what it is. It’s in the tower on the lawn” (Butts 47). She understands the rituals of the Rings as she offers to help Serge: “Serge—I will do for you what we do sometimes. I will cut a turf strip off the Rings, and we will stick our knives in it and you shall be seized of this country.” Serge is aggrieved at these “local enthusiasms” (Butts 145). But Vanna remains steadfast in her inherited commitment to the Rings. As discussed earlier, they save her from the danger of Judy and Peter Amberton as she actually becomes one with the stone. At the end of the novel, Vanna prays to the land for a calm stability: “Rings. Rings. Upon the bells of your horses. Pray for the peace” (231). She goes on to her room where there is “a red fire; the air in suspense, like the veil over a cradle.” Just as when she was cocooned as the sacred baby heir to the Rings, she falls fast asleep in this magical realm of protection. The fire in her room dies, and the “sea wind pour[s] over her” (Butts 232). The ship of the Rings rocks her to sleep as the wind from the ocean peacefully blows. She is established as the present priestess of the Rings. Vanna Elizabeth Ashe has defeated the evil forces of Judy Marston, although she failed to save the soul of Serge. However, even her powers are not strong enough to convince those who do not acknowledge the Rings. Vanna’s failure to rescue Serge from Judy’s grasp symbolizes the continuing dangers of modern life. Even though Vanna has regained control of the Rings, there are some who cannot be brought back to the stability that they represent.

Ashe of Rings is an allegory, as Nathalie Blondel explains: “Forces are portrayed through the ‘masks’ (in the Greek sense of the word) of the other characters” (146). Indeed, each character is fighting for something. Anthony and Vanna Ashe fight to defend the honor of the Rings. Melita Ashe struggles to comprehend this phenomenon. Judy Marston battles to destroy the guardians of the Rings while Serge struggles to save his own soul from the devastation of Judy. Either a character has inherited an intimate understanding of the Rings or he or she has not. On the allegorical level, the story is about a country of people who are trying to defend the only thing they have left that is stable, their traditions and myths. The effects of war and modernity threaten to end even this, and some will be lost as they try to maintain peace. However, in Mary Butts’s fairy tale, the peace and order found in nature prevails through the feminine spirit of Vanna. Elizabeth Ashe. The solution for the war-torn country lies in the strong feminine presence whose understanding of the mystical in life can reestablish what the degeneration of the war has stolen.

Chapter Two;

I have a garden of my own,
But so with roses overgrown
And lilies, that you would it guess
To be a little wilderness;
And all the springtime of the year...

—Harriet Hume, from Harriet Hume

While Ashe of Rings involves a heroine who finds the stability and power of nature in the English countryside, Harriet Hume features a female musician who has located her own “wilderness” within the city of London. The heroine of Rebecca West’s novel inhabits a spiritual sphere where she can fully realize her supernatural capacities. One of her powers is that of mind-reading, and there is only one particular mind she can read, that of her former lover, Arnold Condorex. The novel traces Harriet Hume’s encounters with this man. While Harriet establishes stability and discovers her spirituality through her music and garden, Arnold quests for monetary and political success. Much to his dismay, Harriet can see all his dishonest schemes involved in his rise to power. The novel ends in a confusing fantasy sequence as Arnold shoots and kills himself. His spirit travels across the city to Harriet’s home where he encounters ghost policemen and the spirit of the deceased Harriet Hume.

Harriet Hume is very much a fantastic novel complete with the mystical phenomenon of mind-reading, the fantasy episode at the end of the novel, and many curious tales of the supernatural told by Harriet. However, woven into this fantasy are comments on the role of women in inter-war Britain. Harriet Hume explores the purpose of women in a society that is adjusting to a shift in gender roles. Published in 1928, the novel is described by Victoria Glendinning as a “fable for adults, a vehicle chiefly for entertainment but also for the conveying of social and psychological observations” (1). As the tale of a single woman’s supernatural relationship with her masculine opposite, it comments on gender and addresses inter-war social unease in Britain. In this way, the novel transcends its position as a “vehicle chiefly for entertainment.”

Indeed, Harriet Hume is a novel centrally concerned with gender roles. Harriet Hume is a single woman who has found her niche through her piano playing and life in her private garden. Harriet is the embodiment of a curious category of woman who fits neither the newly re-encouraged role of housewife, nor the stereotyped notion of a 1920s “flapper.” She is single, but her role in life is self-affirmed and she is fulfilled. She is not a maternal figure, but the reader soon realizes that West means her to represent the essence of femininity. In a society that idealized motherhood in the wake of the war as the most meaningful part of a woman’s existence, Harriet’s place in society is not a traditional one.
Like Vanna in *Ashe of Rings*, Harriet evinces a bond with nature. Her home is accessible only through the back door entrance, and visitors must walk through the garden to enter her home. In this way, a visit to Harriet is a call on nature. Her special connection to her garden can be examined in the following passage:

...led the way down the steps into her garden, which was bloomy with deep shadows. At the first flower bed she stooped to dismiss a weed from service, brushed the mould from her fingers with some distaste and grumbled because the duties of a gardener could not be performed with a feather duster (West 37).

Note the way Harriet bends to “dismiss a weed from service.” The wording presents the weeds as the subjects of Harriet the Queen. These plants obey and respect her. However, they do not fear her. Nature is controlled, not controlled, by Harriet as at the end of the novel when she happily sings and she thinks of the arrival of spring (279). Later in the passage, Harriet complains that she is not able to dust as she does inside her house. The garden is an extension of the inside of the house, and Harriet grumbles because she cannot clean it as she does the inside. The precious natural world deserves as much respect as does her inside quarters, for it is her home, her living environment. Arnold also observes that Harriet seems to actually dwell in her garden just as much as she does her indoor home. He says she lives “like a gypsy, half in her garden.” He goes on to ponder that Harriet leaves her gloves strewn about, but perhaps they are not gloves at all. “There was a race of gazelles with a snake-like habit of casting their skins at certain seasons of the year” (53). Harriet is portrayed as fluttering about her garden, sometimes shedding her skin here and there. What is clear is that she has the same complex relationship with nature that an animal would be it a bird, cat, snake, or gazelle.

Indeed, throughout the novel, Arnold Condorex views Harriet as connected to particular animals. When Arnold and Harriet are lovers, he sees her as a little creature who might take flight into the air at any moment with her shoulders that “might have been wings folded in on themselves and packed away for reasons of prudence” and her little feet compared to those of “a bird–woman built by a magician expert in fine jewelers’ work and ornithology” (11). The image of Harriet as a flighty entity who almost floats instead of walks comments on her sense of connection. She is not bound by the rationality of the earth, or limited by a domestic setting. Instead, she is a willowy character who might fly away when she pleases and float around if she so chooses. “If she indeed desired to look an ordinary woman, walking on earth and of much the same specific gravity she had better not have cut her skirts so full, for theirswaying buoyancy seemed to be supporting her” (11). Harriet is not an “ordinary woman” but one with aerial possibilities.

It is essential to realize, however, that these are Arnold’s observations of Harriet. What does West show about their relationship in Arnold’s observation of her? These characterizations from his perspective are, in a way, disparaging. As Arnold reveals to the reader glimpses into Harriet’s character, he never truly recognizes their ramifications for himself. Arnold reduces Harriet to animalistic terms without ever realizing the positive qualities they imply. While the reader is able to connect Harriet to the amazing qualities of nature through these comparisons, Arnold simply dismisses Harriet by them. Instead of seeing Harriet as empowered through her similarities to animals, Arnold reduces her to something he can control.

Late in the novel, Arnold compares Harriet to a feline: “She rubbed against him in the way of a pleased cat.” As she adjusts her position on the sofa during a conversation with Arnold, she “nimibly” curls herself up on the cushions and keeps an intent and “steadfast” gaze on him (201). Here it seems that Harriet has been granted the skills of a cat, and her movements have become sly and stealthy. She is not described as having the same ethereal quality as she did when compared to a bird. Rather, Harriet is shown as an animal that is very much grounded and has a skillful relationship with the earth. Cats always keep their balance and cleverly maneuver over obstacles. However, it is important to note that now Harriet is not seen by Arnold as an innocent, fluttering bird but as the creature that preys on birds as he becomes more paranoid that her insights into him will result in his evil schemes being revealed, “...you are an animal, and cannot be saved. For you dip your lips to your glass as a cat dips its muzzle to a saucer, and not raise your glass to your lips, as houseleaved ladies do” (West 201). Here is an interesting set of contradictory images. One is that of a primal animal with no civilized urges such as raising a glass to drink. Arnold denies that Harriet can be “saved,” and thus implies that Harriet is somehow a doomed predator. The other image is that of the “houseleaved lady” who raises her glass to her lips. This woman is what Arnold implies that Harriet should strive to be, if at all possible. If Harriet occupied this role, she would be “trapped” into a household where she would not have the power of insight that frightens Arnold.

In this scenario, Arnold would feel safe from being discovered. The “houseleaved lady” perhaps is married, and, if not, longs to be. As a passive, polite woman, she gladly accepts her role within traditional society. Arnold’s relationship with Harriet has at this point begun to sour. He is no longer impressed with her strange relationship to her home. He cannot see her as an innocent, beautiful creature anymore; instead, she is a predator, and specifically, dangerous to him. From this fate he does not believe that she can be redeemed. As far as Harriet is a creature with a close, complex relationship with nature, Harriet cannot be saved. Furthermore, Arnold’s opinion of felines is explored later in the story when he approaches a neighborhood cat: “...what a vulgar animal you are in your craving for publicity, and, in considerateness, how inferior to man!” (236). In this context, he sees Harriet as wholly incapable, rejecting the place in which society has told her to stay. Instead she is her own animal, but in
Arnold’s opinion a vulgar one. It is ironic that Arnold should condescendingly remark that the cat is an animal “craving for publicity” since his own greed for public recognition fuels his evil schemes.

Perhaps Arnold’s distaste at Harriet’s relationship with nature is also an instructive realization that it is her source of power—a power that is not dependent on him. Jane Marcus argues that the British fantasy novel depicts nature for the woman as “a wilderness of one’s own, away from family control of domestic space and male control of public space. Central to the concept of female wilderness is the rejection of heterosexuality. In the dream of freedom, one’s womb is one’s own only in the wilderness” (Wilderness 136). It is in this wilderness that women can develop away from the outside pressures of society. Here they may return to the ancient goddess tradition. Among nature, they are earth mothers, sorceresses, and huntresses. These antique powers are again realized and “the actors return to the prelapsarian world of preclassical feminine power” (136).

How does Harriet’s preoccupation with music figure into her world of “preclassical feminine power?” Harriet is able to tame the abilities of natural concordance. Music is an ancient art, and Harriet finds comfort and expression through it. Before he begins to resent Harriet, Arnold observes her relationship with her piano: “Rather it was as if some inhabiting spirit of the instrument had resolved no longer to tolerate the age-old conditions by which human virtuosity steals all the credit of its tunefulness, and was essaysing to make its music by itself, and found its new art difficult” (West 34). Harriet brings life to the piano. It is as if she feels its need for self-expression and she kindly lends it that ability. The instrument feels Harriet’s capacity for accommodation and decides finally to make music by itself. Again, Harriet has a special relationship with spirits, here those of music and harmony. The passage shows that Harriet understands what it feels like to have been caged and contained. It is as if Harriet encourages a situation where the piano can free itself as her music has freed her. In essence, the two have freed each other.

Harriet seems to follow in the tradition of the blind soothsayer, suffering “from a disorder of sight” (16). Her vision “fluctuates with her strength” so that glasses cannot correct the problem (16). When she reads her newspapers she must lean very close to them on the floor in order to see them (17). She cannot predict the future with her mind-reading abilities, but she is a person whose weak physical sight is greatly compensated for by her capacity for psychological sight. Harriet finds that she can read Arnold Condorex’s mind. She relates her gift to him in disbelief: “I was in your mind. And because I was in your mind I knew what your body was doing. You were pulling back the curtains—” (27). This is the supernatural gift of Rebecca West’s fantasy heroine, and this is what gives Harriet her strength, not simply her power over Arnold. When she can see inside Arnold’s mind, she can understand him. The connection between Harriet and Arnold is an intricate one, as they embody West’s view of the essential masculine and feminine. While Arnold has the capacity to read Harriet’s mind as well, he does not realize it until the very end of the novel. He lives in denial of this power, while Harriet embraces her gift.

In Western cultural tradition, masculinity accounts for the intellectual, reasonable part of the balance, while femininity accounts for intuition. Thus, while Arnold actively resists his queer ability to read Harriet’s mind, she passively comprehends the gift. In this way, Harriet is able to truly understand the motives of Arnold as she sees his hidden agendas. She is observant, while Arnold resists understanding the situation. Their relationship, which should be an understanding balance of power, is instead a struggle. Arnold feels threatened by Harriet’s faculty, and takes drastic measures to end her life. He is not comfortable with Harriet having a glimpse into his thoughts and the power this vision entails.

This power struggle can be applied to the general situation in inter-war England. During the war, women had a glimpse into the lives of men. They were able to enter the workplace and were empowered by this contribution to the war effort. Finally, they knew how it felt to be public, active members of society. But after the war, they were encouraged to go back home. Men were perhaps threatened by the women’s experience in the workplace. Therefore, society shoved women back into the private, household orbit. The men refused to yield their “power,” and women were instead forced to adjust to the changes in their lives. The dislocation resulted in angst that the literary fantasy heroine embodies. Rebecca West’s vision of this power struggle has been seen by some critics as ultimately unhopeful for women: “West’s dualistic vision assumes that male jealousy of women’s power can never be overcome and that she is doomed eternally to know and to forgive” (Marcus, Wilderness 157). Thus, if the fate of women is to follow the model presented by Harriet Hume, then they will remain locked in her universe of essentialist femininity, where women are the passive creators who must accept and accommodate. This is not the only possible view of the ending, however. Harriet and Arnold stand together as the story closes and Arnold comments that this is his “proper place” (288). Arnold has realized the potential of their relationship. Before, Arnold viewed the relationship as a battle for power. He struggled to control Harriet by reducing her to something he could disparage. The fact that Harriet possessed so much insight into his character threatened him. However, by standing side by side, they reflect a final scene of harmony. Arnold finally understands that their relationship need not be a power struggle; instead, it can be a balance. Women are not locked into a particular role; instead, both men and women are able to share energies to create a powerful equilibrium. In this hopeful reading of the novel’s ending, Arnold understands his role in the equilibrium.

The stories that Harriet tells Arnold throughout the novel shed light on the message of its ending. These stories are related to the relationship of Harriet and Arnold as well as the exchange
between men and women. Harriet’s story of the Ladies Frances, Georgina, and Arabella Dudley combines elements of nature, mysticism, sisterly bonds, and a power struggle. The myth of the three sisters begins when they are infants and are lost and found on the lawn asleep “linked by a thick cable of flowers” (West 40). Beginning in this innocent, infantile stage as the children find their solace in mother earth and each other, Harriet demonstrates the magical comfort found in nature as well as femininity. As the tale progresses, the sisters are forced to separate in order to marry, and their beauty falter: “They were still handsome; but they were not, as they had often seemed when they trod the sward parks in their floral panoply, immortal goddesses” (43). The expected domestic role for women has interfered with their connection to nature, sisterhood, and themselves. As these women grow further from their life source, natural femininity, they lose their strength and spirituality. No longer connected by their flower rope, their inner sense of connection is severed as well. Finally, when the women reunite and secure themselves together with flowers, they are “in greater beauty then they had been for many years” (45). They are transformed back into their goddess form and proceed to escape from the confines of being household objects as wives. They are no longer the possession of their husbands, but they posses themselves through their relationship with nature and each other. They proceed to parade down the streets of London to the garden where “the earth about them trembled, to a degree it swallowed them” (47). In this mystical stage, they are ultimately taken back into their mother earth and become trees, permanent reminders of this bond. They have successfully retreated from a dominating patriarchal society to an eternally safe escape into the mystical womb of nature.

Jane Marcus calls the fairy tale a “modern version of the myth of sisterhood” and a “classical invocation of the female spirits in triumph over patriarchal rape and marriage” (Wilderness 143). Indeed, the story creates a space where the feminine presence can escape restrictive social bonds. It is a fable with the moral preaching the importance of life as a goddess. In this life, a woman can find her identity in an ancient realm of wilderness and feminine bonds. Yet it is beneficial to examine the parallels between the tale of the three sisters and the plot of Harriet Hume itself. Harriet attempts to free herself from the shackles of interwar Britain’s masculine notion of the domestic woman. As the three women permanently return to the earth, Harriet remains in her natural sphere even in her death. Arnold is drawn to her garden home where the two are able to stand peacefully together at the end of the story. The Ladies Frances, Georgina, and Arabella Dudley are the heroines of a fairy tale where they are able to escape to the comforting arms of mother earth. Similarly, Harriet finds a permanent refuge in the same garden as these trees. Although Arnold comes to the garden with evil intentions, his perception of Harriet changes. At the end, he finally understands that their relationship is should not be a struggle, but a delicate balance. Harriet is able to remain in her home where Arnold joins her. The ending suggests that there are solutions to the contemporary power struggle to be found in nature, a comfort to be found in a return to the organic world. While the sisters find a refuge free of masculine presence, Harriet’s refuge accommodates both the female and male being. Harriet forgives Arnold, and as a spirit, carries on as the cheerful hostess to Arnold and the policemen. It is the female spirit that accommodates the male spirit, and Marcus harshly criticizes West’s closing. “Her ending suggests that both women and men are at fault in the battle of the sexes and that women will always surrender” (Marcus, Wilderness 146). But this is assuming that the struggle for power must be won.

Thus, the ending of Harriet Hume may not be such a negative one after all. Harriet does forgive her transgressor. But does she surrender to him? Are forgiveness and accommodation of another being so retrogressive? After all, the garden in which Harriet finds endless comfort and power can be seen as accommodating. In the same way, Harriet can accommodate Arnold. Harriet is not interested in winning a battle. Although Arnold has blatantly caused Harriet’s physical end, it is Harriet who must finally explain to Arnold the phenomenon in the garden, for it is she who holds the knowledge. She comforts Arnold when he sees the “sweetness dripping from the stars,” hears the noise of grass growing, “rain hissing upwards,” and the “kissing” noise of the buds in the garden opening (West 279). Arnold is finally forced to experience fully Harriet’s world. Here, she has the power and is the expert and Arnold must ask the questions. In this reading, the fantasy heroine has not triumphed over the masculine forces of her world, but instead has revealed her own power within her own setting. Furthermore, the ending presents what West perhaps thought to be a solution to uneven gender roles. After all, it is not only Harriet who has surrendered in the ending; Arnold accepts Harriet’s offer to stand by her side, suggesting they finally stand on equal ground. As he finally accepts Harriet’s natural world, the two are able to exchange equally and are finally balanced. Rebecca West was perhaps not suggesting that the perfect scenario was one where one group won the battle of the sexes. Instead, she suggests that an ideal universe contains man and woman in perfect proportion where they can understand each other and share power fairly.

Although mind-reading and a supernatural connection with nature were not fully realistic options for the displaced women of this period, readers of novels like Harriet Hume would nevertheless have found comfort in its parables of female power. In any case, Harriet offers a fully feminine character mighty in her own right. She never submits to domestic “bliss,” but finds her own way through music, magic, and her garden. She is a likable character who in the end does not fold in the face of male domination. Arnold can take her life, but her spirit remains in the garden where he must now learn and understand. The trees of Ladies Frances, Georgina and Arabella become “full and well-fleshed, and a cable of foliage to which the moonlight gave an appearance of flowers” (West 280). These arboreal spirits seem to nod their head in approval as they remind Harriet of her bond.
with their flower cable. Opting out of the competitiveness of male power structures, Harriet is now eternally secure in her garden. Harriet's "little garden of her own" has indeed become her ancient wilderness to which she may now introduce to the masculine presence of Arnold Condorex.

Chapter Three:

That's why we become witches: to show scorn of pretending life's a safe business, to satisfy our passion for adventure.—Laura Willowes, from Lolly Willowes

Mary Butts's novel Ash of Rings depicts a woman who is encouraged from birth to connect freely with nature. In Harriet Hume by Rebecca West, the heroine experiences the joys of nature through her garden at will. More importantly, both seem to have the mystical connection to power without struggle. However, Sylvia Townsend Warner's novel, Lolly Willowes or the Loving Huntsman, features a feminine character who is unable at first to freely find her place within nature, and she must tear away from the constraints of her family to finally experience a mystical connection with the English countryside. Laura Willowes is the heroine of the story, a woman who remains unmarried and is, for much of the novel, controlled by her family. In her introduction to the recent reissue of the novel, Alison Lurie discusses the relevance of Lolly Willowes to the unmarried British women of the inter-war period. She explains that during this period there was an excess of single women due to the fact that many young men ventured into the various colonies of the British empire. The horrendous mortality rate of the First World War also lessened these women's chances of finding a husband (vii-viii). It is important to remember that in a society so concerned with having women in the home as housewife and mother, this was a considerable problem. Women who did not marry and have children were looked at as being unfortunate creatures. Warner presents Laura Willowes as being pitied by her family as a hopeless, single spinster and passed around to be "Aunt Lolly" to the children.

Lolly Willowes is not the tale of a woman who remains trapped in the confines of society. Lurie says that the novel "was, in fact, a subtle demand for women's right to privacy and independence from their families, for power over their own lives— even if they had to make a pact with Satan to get it" (vii). Laura does indeed make a literal pact with the devil in the final step to gain complete freedom from her family. In this way, Lolly Willowes makes a statement concerning women's role in society at this time. Warner implies that women needed a place to be empowered, whether it be a quiet place in the country or by interaction with the devil. On the other hand, the novel could be demonstrating society's view of women if they do stake their claim on a place of their own. Even though Laura never demonstrates any pure evil, she is still a witch. As Lurie comments, "The implication, of course, is that a woman who refuses the "Aunt Lolly" role is, in the view of conventional society, a kind of witch, even if she does no evil" (xiii). Whatever the case, Lolly Willowes is the tale of a woman who finds the power of nature and makes herself mystical in her own right.

Sylvia Townsend Warner presents the story of Laura Willowes in three sections. In the first section, the reader is introduced to Laura's background. Laura, born in 1874, is the youngest of the Willowes children. Her two older brothers, Henry and James, both use their baby sister as a prop for their games as children: "They performed the brotherly office of teaching her to throw and to catch; and when they played at Knights or Red Indians, Laura was dutifully cast for some passive female part" (16). Laura's mother grows ill after her daughter's birth and dies when Laura is still a young girl. These circumstances contribute to the strong bond that develops between Laura and her father, Everard Willowes. Laura grows to womanhood while living very contentedly with her father at their home, Lady Place. At his death, Laura's brother James and his family take over the estate. The second section of the novel concerns Laura's move from Lady Place to London where she lives with her other brother Henry and his family. There, she is shoved into identities created for her by her family: "One was Aunt Lolly, a middle-aging lady, lightfooted upon stairs, and indispensable for Christmas Eve and birthday preparation" (Warner 57). Laura has been pulled from her life in the country, and she physically shows the negative symptoms of this displacement. Jane Marcus says, "This ten-year limbo is rendered with chilling eloquence. London is colder than the country. The water is hard. Laura's hands roughen, and her face hardens. Patriarchal time is shown as oppressive, like Woolf's depiction of Big Ben in Mrs. Dalloway" (Wilderness 150). Despite this repression, Laura eventually experiences a sort of spiritual awakening and plans to move to a small village named Great Mop. The final part of the novel covers the rediscovery of Laura's identity.

During her childhood, Laura is able to develop a sense of connection with the country. However, even at Lady Place, she is sometimes prevented from experiencing nature on her own terms. A scene depicts the contentment that Laura found in nature while also demonstrating the restraints that her brothers placed on her. Once as Henry and James played their outdoor games, they tie Laura to a tree as a captive princess, and when they played at Knights or Red Indians, Laura was dutifully cast for some passive female part. However, this situation foreshadows Laura's future as the "captive" of her brothers and their families. Although at this point, Laura is satisfied to be a passive participant in their games, she eventually finds that she must connect with nature in her own way instead of being tied to it by her brothers.
Furthermore, Laura’s parents have carefully told their boys to make sure their young daughter does not fall into the pond when they play outside. Bruce Knoll points out that “the proscription against falling into the pond can also be read as a proscription against immersing herself in nature. Already as a young girl, Laura is steered away from that force which is most important to her feminine character.” Knoll goes on to discuss that these games with her brothers teach Laura the role that she was expected to assume as she grew older, that of a passive female shuffled around according to the inclinations of her brothers (2). However, she is happy on her own and no one stops her from pursuing her own inclinations. Despite the fact that she does go along with her brothers’ game, Laura does not seem changed by it. Even when she is bound to the tree, Laura seems to have a connection to the forest that transcends the role of passive female. She may seem to be submissive to her family, but it seems as though Laura has already discovered that there is a curious empowerment to be found in nature.

For some time, Laura remains in this passive role within her family, although she is able to shrug off the then-encouraged role of wife and mother. Her family does attempt to find her a husband. When Laura is still at Lady Place, family members such as her Aunt Emmy try to convince her that it is time for courtship. But these attempts are unsuccessful, and Laura’s father believes that “his relief at seeing Laura’s budding suitors nipped in their bud was due to the conviction that not one of them was good enough for her” (28). Everard never feels the need to find a husband for Laura, and he seems to understand her need for freedom at Lady Place. Furthermore, Laura feels no need for a suitor, for she prefers the company of her father over any young man (27). As the mistress of Lady Place due to her mother’s death, Laura is content to read books from the library and pursue her interests in “botany and brewery” (31). At Lady Place, Laura often roams the countryside looking for herbs, and this interest reveals her early connection to nature (and, potentially, to witchcraft) that is not then allowed to flourish. Already, Laura is discovering a way to make brews and herbal potions with “magical” abilities to affect people’s state of being out of natural ingredients. Laura has begun to develop a special relationship with nature where she has the knowledge and power to mystically manipulate natural components into something beneficial.

After Everard’s death, Laura is sent to London where her family continues to try to find her a suitor. Henry and Caroline attempt to make a match between Laura and Mr. Arbuthnot, a stiff lawyer who works with Henry. Although his conversation somewhat appeals to her, Laura feels that “nothing would have induced her to marry him.” However, she finally rid herself of this suitor by commenting to him that he resembles a werewolf, and Henry and Caroline end all of their attempts. Laura does not necessarily make this comment in order to bother Mr. Arbuthnot, although it greatly bothers Henry and Caroline. She actually amuses herself with thoughts of the man devouring lambs as a werewolf, and it seems as though Laura believes that her potential suitor may be a werewolf because “lots of people are without knowing” (53). Laura’s statement may have appalled everyone else, but Laura actually felt as though Mr. Arbuthnot could be that dark, mysterious creature. This foreshadowing demonstrates Laura’s interest in mysterious creatures and her realization that an “ordinary” person might actually be one.

Despite her important role as mistress of Lady Place before her father’s death, Laura simply has no interest in marriage. Caroline finds Laura’s situation unfortunate and feels “a kind of pity for the unused virgin.” She represents traditional society, convinced that the only satisfying role for a woman was in the domestic setting. Caroline feels both sorry for Laura and “emotionally plumper” herself due to her own wifehood and maternity (55). For her, a woman who does not realize an opportunity to be a successful wife and mother, such as Laura, is to be pitied. However, Laura herself begins to yearn for freedom away from the city, and a mystical escape from the oppression of the patriarchal society of London.

Laura is able to avoid becoming a wife or a mother, but she is forced to play the somewhat maternal role of “Aunt Lolly” to her nieces and nephews. Laura’s identity is further displaced when Henry’s eldest daughter, Fanny, is unable to pronounce Laura’s name as a child and calls her Lolly. Even Laura’s name has been taken away from her in her brother’s household and “Laura was put away” (57). Her identity has been shelved. Although her relationship with the children is not a close one, they nonetheless look to their aunt as a guardian, if only in the utilitarian sense. She is appreciated by her family only for her practical contributions, and she is unfulfilled. During summer holidays near the sea, Laura “would have liked to go by herself for long walks inland and find strange herbs, but she was too useful to be allowed to stray” (61). This again alludes to her need to connect to nature (and, potentially, magic) through her botanical interests. Laura may be “useful” to the family, but she is otherwise unhappy. She wants to make her own way through nature, but her familial role of “Aunt Lolly” prevents her from having the opportunity. This instance parallels her brothers’ childhood games that placed Laura in the passive female role; she is tied to her identity as “Aunt Lolly” and unable to explore the wilderness for herself.

The world of London, to Laura, seems coldly structured. Things in Henry’s household are methodical. “Even Laura, introduced as a sort of extra wheel, soon found herself part of the mechanism, and, interworking with the other wheels, went round as busily as they” (44). Life there is as strictly organized as Caroline’s clothing drawer “where nightgowns and chemises lay folded exactly upon each other in a purity that disdained even lavender” (48). But Laura craves a release from this structured existence. As the years pass by and the children grow, Laura begins to daydream that she is “in the country, at dusk, and alone, and strangely at peace” (73). Her longings for solitude in nature and freedom from the mechanical lifestyle at her brother’s home
are fully awakened when she enters a little florist and greengrocer shop by chance on a walk back home. She finds the shop extremely comforting in its disarray. “The aspect of the shop pleased her greatly. It was small and homely. Fruit and flowers and vegetables were crowded together in countrified disorder” (79). The shop also has “the apples and pears, the eggs, the disordered nuts overflowing from their compartments.” The little shop is bursting with greenery, and Laura gets lost in its state of natural disorder. She finds a sort of peace there, as “she forgot that she was in London, she forgot the whole of her London life. She seemed to be standing alone in a darkening orchard…” (80). This incident leads to Laura’s discovery of her future residence, Great Mop. After buying chrysanthemums and discovering their origins in the Chilterns, she buys a guidebook and decides to move there.

Back at her home, Laura reads the guidebook “curled askew on the bed” (83). Again, Sylvia Townsend Warner refers to disarrangement. This reflects Laura’s longings to be where things do not function as a machine, and everything does not have its own tidy place in the home, including herself. The wilderness offers the perfection of disarray, where leaves fall where they please to the forest floor. She announces to her family that she will move there, but they believe the plan to be in jest. Finally, Henry realizes that she is serious, and pleads with her to stay, telling her that her plan is “absurd.” Her nephew Titus is the only family member who is impressed by her plans. Laura reveals that “it’s only my own way, Henry” (94). Although Henry has lost much of her money in bad investments, she is still able to tear away from London. Despite her lack of funds, she is able to rent a room in Great Mop. Besides, Laura feels as though “it is best as one grows older to strip oneself of possessions, to shed oneself downward like a tree, and be almost wholly earth before one dies” (98). In this unstructured life, there is a feeling of belonging where Laura is able to find her own place in relation to the earth.

Jane Marcus says that “Great Mop’s name is obviously a play on a witch’s broomstick, the phallic object of domestic service which links the repeated acts of bringing order and cleanliness with the power to fly” (Wilderness 152). Furthering this idea, the broomstick could be seen as actually transcending domestic order as it enables one to fly wildly about the wind, and it also rejects its original purpose in the home and becomes a magical tool. Laura, too, rejects her place in London as “Aunt Lolly” when she moves to Great Mop and becomes a witch. In leaving the familial sphere, she places herself in a world where she finally has the chance to find her own role. Much like the broomstick, a woman’s place was then thought to be in the home, but both broomstick and Laura find that they are able to exceed their original purpose and find a mystical place in the world.

Laura’s magic began with her earlier experiments with herbs. Her magic develops in Great Mop as she shapes the townspeople in dough and bakes them, making grotesque figures: “Laura felt slightly ashamed of her freak. It was unkind to play these tricks with her neighbor’s bodies” (130). She slowly finds her way around Great Mop and realizes the secret of both herself and the town. The “mystery” of Great Mop is the witchcraft that takes place there, but Laura begins to find her own magic before she recognizes this secret. As an apprentice to a local man who raises birds, she feels a special connection to nature as a henwife. Laura makes the transformation “from Foolish Virgin to Wise Virgin, as potent as the henwife in fairy tales who ‘hatched the future in her apron...’ Henwifery transforms her, rides her of her anger against her family for keeping her from the world of women’s natural magic” (Marcus, Wilderness 154). She has finally found a world of comfort in Great Mop, and discovers her own role. If her role of henwife does transform her, then it is into a woman who is able to understand her miserable past and her mystical future.

However, her little universe is threatened when her grown nephew, Titus, decides to join Laura in Great Mop, a town that he finds to be charming. Bruce Knoll comments that “the threat [of Titus] is more dangerous because of its subtility, since to Laura he does not appear as a remnant of the masculine culture that she has fled” (9). However, Titus becomes much more than a “subtle” threat to Laura in Great Mop. Before, Laura had felt a sense of fond connection to Titus, but when he moves to the village, she becomes aggravated by his infringement on her privacy: “Even when she felt pretty sure that she had escaped she could not profit by her solitude, for Titus’s voice still jangled on her nerves” (143). Laura realizes his smothering masculine presence as she sees his pipe and tobacco pouch on the mantelpiece, “they lay there like the orb and scepter of an usurping monarch” (Warner 145). Titus represents to Laura the repressive society that she has come to Great Mop to escape, and she becomes desperate to regain her private sense of identity in the town. Titus has come to Great Mop to write a book, and enjoys the area with “a possessive and masculine love” and “loved the countryside as though it were a body” (147). If Titus loves Great Mop as though it were a woman, then Laura feels a woman to woman connection with the area. The countryside seems like a feminine presence that welcomes her to come and get aquainted. Laura’s love for Great Mop is a growing one that she develops through her trust for it. She has struggled to come there, unlike Titus, and now struggles to learn to love it.

Laura grows angrier as Titus further “root[s] downward in the soil” of the village (147). The novel almost suggests that there is a battle between Laura and Titus for Great Mop. But Laura is disturbed because a masculine figure has again obstructed her freedom. Not only has he invaded her love for the countryside, he also expects Laura to be “Aunt Lolly” to him again. She deeply resents him for his destruction of her newfound character and his reduction of her back to her familial role; “In vain she had tried to escape, transient and delusive had been her ecstasies of relief. She had thrown away twenty years of her life like a handful of old rags, but the wind had blown them back again, and dressed...
her in her old uniform” (150). While Laura understands that her	nephew has “a kind heart” and he means her “nothing but good,”
she nevertheless is bitter towards him because he does not
understand that she simply wants to be left alone. It is not his
actual character but what he represents that she begins to hate.
Laura imagines that Titus has opened the door for the rest of her
relatives to reduce her cruelly back to the role of “Aunt Lolly.”
In terror, Laura pictures her family coming to her to welcome her
back as they “seize on her soul” (151). In desperation, Laura cries
out for help from her nightmare. Although the woods are silent
to her plea, she nonetheless feels as though she has made a pledge
that Satan—or something—has heard her pleas for help, and she
makes a compact with the Devil “sealed with the round red seal
of her blood” (155). With Satan’s help, she magically casts Titus
out of the village and is again free to make her own discoveries
in the wilderness.

What are the traditional implications of witchcraft, and
how does this concern Laura and her own relationship with the
devil? The Standard Dictionary of Folklore, Mythology, and
Legend includes in the definition of a witch as “one having
supernatural powers in the natural world, especially to work evil,
and usually by association with evil spirits or the Devil” with
“knowledge of drugs to produce love, fertility, death, etc....”
(179). This definition implies negative connotations for
witchcraft, and that its sole purpose may be magically to perform
evil acts. However, Laura discovers her own form of witchcraft
as she establishes a relationship with the Devil. The freedom she
finds in her communications with Satan transcends traditionally
evil connections. Satan is not an evil entity in Lolly Willowes;
instead, he is a caring gatherer of desperate spirits that have been
forgotten by the God of a cold society. Marcus explains, “the
novel does not only dignifies spinsterhood. It mocks the patriarchal
God whom Virginia Woolf called ‘Milton’s bohey,’ and allies
Milton’s Satan with women and the old natural magic of the
religion of outcasts in the forest” (Wilderness 155). But perhaps
the Satan in Lolly Willowes does not so much “mock” God as
play the “Loving Huntsman” for people who cannot find the
connection they need in a rigid, demanding society. Laura seems
to discover her own version of Satan, who offers her protection.

Indeed, there are two versions of witchery in Great Mop.
The scene of the “Witches’ Sabbath” in the novel exemplifies
these two types. Mrs. Leak, whom Laura rents a room from in
Great Mop, invites Laura to go on a walk with her. In reality, the
walk is a trip to the Witches’ Sabbath, the great secret of the
village. “Mrs. Leak was a witch too; a matronly witch like Agnes
Sampson, she would be Laura’s chaperone” (170). As Laura
comes to the outdoor ceremony, she hopes that the celebration
will be a “different and more exhilarating affair” than the social
activities that she had been uncomfortable with in London. But
domineering old man from the village sternly stares at the two
women before he allows them to go through the gate to the
ceremony showing that the stiff patriarchy is present even at this
Witches’ Sabbath (171). Nevertheless, the ceremony is fascinating
with candles in the ditch, paper garlands in the trees, and
spinning dancers (172). However, Laura becomes disheartened:
“Even as a witch, it seemed, she was doomed to social failure,
and her first Sabbath was not going to open livelier vista than
were opened by her first ball.” Laura identifies this relationship
with the Devil, no matter how lively, with the oppressive social
scene of her life before Great Mop. She cannot become excited
at this occasion because it represents to her meaningless social
interaction, as reflecting the monotony of dancing with the same
partners and making pointless conversation at balls in London
(174).

The Sabbath becomes even more discouraging as Laura
meets a young man who she later discovers had sold his soul to
the devil in order to be the most important person at the party on
a weekly basis. He seems to be representing himself as Satan, and
Laura becomes infuriated by the whole ordeal when he smiles
lifelessly to her and licks her cheek with his serpent-like tongue
(182). She is disgusted, and leaves the Sabbath. At this point,
Laura is upset that the Prince of Darkness is such a vulgar and
intrusive character. He is a disgusting masculine presence who
treats her with disrespect by licking her face and smiling eerily.
Certainly, this could not be the Satan with whom she made a
contract. She wanders into the night, and settles into the woods
where she finally watches the sun rise. Here a very gentle man
with a walking stick stumbles across Laura, the true Satan. The
man, who is dressed as a gamekeeper, knows Laura before she
knows him. The two carry on a peaceful conversation in the
morning light, and he tells her to ask him if she ever needs any
help before he wanders back off into the woods (186). This is
the figure who truly protects Laura. Laura’s Satan does not need to
hide in the darkness of night, nor does he come to Laura as part
of a demonic source. Instead, he is a gentle man who finds her
alone in the sunlight, and offers his help to her with kindness.
She finally discovers that “this was the real Satan. And as for the
other, whom her spirit had so impetuously disowned, she had
done well to disown him, for he was nothing but an impostor, a
charlatan, a dummy” (188). Laura has found her real protector,
a kind man who has gathered her from the lifeless souls of
society.

Satan is not presented as evil in Lolly Willowes. Instead, he
is very much the “Loving Huntsman” of the title. Laura’s
witchcraft is not negative; instead, its purpose is to establish her
own protected place in the woods. In another discussion that
Laura has with the Devil, she comes to realize that witchcraft is
an alternative to settling for dullness in domestic society. She
sees potential witches all over England, “child-rearing, house-
keeping, hanging washed dishcloths on current bushes; and for
diversion each other’s silly conversation, and listening to men
talking together in the way that men talk and women listen”
(212). However, witchcraft has offered Laura a way out of all of
this. In her witchery, Laura magically connects with the
wilderness, and gather her herbs and make potions as she had
always longed to do freely. The Devil is the leader of a group of restless souls who have come to him so that they might find their place within the wilderness. He has lovingly gathered them, yet allows them to do as they choose. Laura is not required to follow a schedule of witchcraft, nor must she attend the Sabbath. Satan is not like the orderly Christ who Carolyn said folded his gravo clothes in the tomb (48). Instead, the Devil lets his followers know that he is there whenever they might come to him. He does not require their structured praise or their rigid observances. He simply enables them to become empowered through their own magical connection to nature.

_Lolly Willowes_ is a satirical attack on the traditional notions of God and Satan. It explores what society views as good and evil, and why individuals choose to see things in this way. In the novel, Laura discovers that the Devil is the enemy of souls not because, as society says, he is the “embodiment of all evil,” but because “his memory was too long, too retentive; there was no appeasing its witness, no hoodwinking it with the present” (221). Satan remembers everything, particularly those like Laura who are shoved aside and forgotten. Satan is not presented as the Prince of Darkness; instead, he is the opposite of the patriarchal God of a patriarchal society. Laura’s world before Great Mop was one where the powerful felt they could dominate whomever they chose. Laura’s family bound her to domestic suffocation where she was never granted the opportunity to make her own choices. But Laura does find a world of her own; and a leader who lends her his power to make her own strength. Through the Devil’s magic, Laura finds her own mystical place in the wilderness where she can sleep undisturbed under his “satisfied but profoundly indifferent ownership” (222).

Were Laura’s only choices, however, “Aunt Lolly” or witch? Or is Sylvia Townsend Warner simply showing what the society of that time believed the options to be? If women were not wives or mothers, then they were shunted with negative connotations. The identity of witch might seem to be an evil one, but Sylvia Townsend Warner presents witchcraft as an alternative for women to settling down into dullness in their lives. Although the devil is represented as a man in the novel, he is not presented as a macho presence; instead he is liberating. Yet it is curious that he must be portrayed as a dominant male figure. Whatever the case, “the devil is the emancipator of women” in the novel (Marcus, Wilderness 156). Unlike the patriarchy, and her family, he is not controlling: rather, he provides the tools for Laura’s freedom. Laura sums up her feelings on her escape from domestic imprisonment at the end of the novel in one of her conversations with the devil: “One doesn’t become a witch to run round being harmful, or to run round being helpful either, a district visitor on a broomstick. It’s to escape all that—to have a life of one’s own, not an existence doled out to you by others” (215). _Lolly Willowes_ demonstrates the length to which women were willing to go to escape the patriarchal confines of the inter-war period.

**Conclusion:**

A writer’s country is a territory within his own brain; and we run the risk of disillusionment if we try to turn such phantom cities into tangible brick and mortar.—Virginia Woolf, from “Literary Geography”

In 1918, photographer Horace W. Nicholls captured a woman farm worker feeding poultry near Langstock, in Hampshire. Surrounded by hens and geese, the smiling woman stands in the center of the picture with her hand in a basket of feed. The background is filled with trees and overhanging, and a small cottage is tucked into the shrubbery. The photograph “manages to create the fairy-tale world which illustrators... so memorably evoked out of such elements as the cottage secretively hidden by overhanging hedgerows” (Condell 147). Indeed, there is an element of magic in the picture. It seems as though anything could happen in the mystical world of this photograph. The woman is engulfed in the countryside, surrounded by her hens and the forest. The picture is reminiscent of Laura’s short time as a henwife at Great Mop in _Lolly Willowes_. While working with the birds, she related to the henwife in the fairy tales and “she understood now why kings and queens resorted to the henwife in their difficulties. The henwife held their destinies in the crook of her arm, and hatched the future in her apron.” Laura ponders further that the henwife “practiced her art under cover of henwifery” (133). The women of these fairy tales were magical, and Laura’s short time as a henwife makes her feel “wise and potent” (133). But the smiling woman in the picture could be any of the heroines of _Ashe of Rings, Harriet Hume_, or _Lolly Willowes_. All of these women find their strength amid nature where the restrictions of inter-war society are not present. They have a complex relationship with the natural that is related to their mystical connection to power. They find this within nature, as Laura Willowes did as she became comfortable with the birds and felt empowered by connection to fairy-tale magic. The characters of these fantasy novels all find a unique alternative to the roles traditionally offered to them during the inter-war period. Many women were encouraged to return to the home to find their strength as mothers and wives, and many resisted this pressure and fought to remain in the workplace among men. However, Vanna Ashe, _Harriet Hume_, and Laura Willowes find their own roles of empowerment amid the magic of nature.

While each of these women find similar connections to the magic of nature, there are differences to be examined in these fantasy novels such as treatment of men, degrees of familial relationships, and the presence of World War I. In discussing the various treatment of men in these novels, it is important here to establish what is meant by gender roles. Bonnie Kime Scott says that “gender is a category constructed through cultural and social systems. Unlike sex, it is not a biological fact determined at conception.” Therefore, gender roles are what traits a society matches up with particular sexes; thus “in history, across cultures, and in the lifetime development of the individual, there are
variations in what it means to be masculine, or feminine” (Scott, Gender 2). Societal definitions of gender roles had undergone change, and they vary according to several factors such as locale, time, or situation. During the inter-war period, gender roles were changing, much to the dismay of many: “By 1918, the restrictive Victorian image of womanhood—physically frail, sheltered, leisureed, private—had been undermined by the wartime experience of both sexes” (Condell 157). Women had stepped into masculine roles during the war, proving themselves to be outgoing, public, active, physically brave, and responsible. Their own expectations were altered, even as the war ended and society demanded that they resume their passive role in the home. In the novels discussed, gender roles are modified and traditionally feminine characteristics are praised as powerful and wise.

In Ashe of Rings, Butts presents Serge as a man in constant struggle with himself and the world around him. He struggles to understand the power of the Rings, but is ultimately unable to do so. He suffers as he concludes that he cannot determine what is real. He flees from the actual battles of war, yet is always being drawn to Judy, the personification of war itself. Serge lives his life in a tragic disillusion. While he may feel as though he has escaped the power of war, in reality he is unable to leave it behind. Even as Vanna tries to introduce the protective powers of the Rings to him, he still sees it as an “abominable gallery in the dark” because there is a “blaze over [his] eyes” (225). Because the pain and suffering of the war is the one thing that Serge has felt and therefore concludes are real, he cannot accept the good that Vanna offers him. As with the war-torn society of England, Serge has felt the pain of loss and destruction for so long that he cannot trust what is authentic. Serge thinks to himself as he sees Vanna, “Women to run the world. Men to lie about in quiet and think. Not necessarily of anything” (93). Serge is a passive presence who is tossed about by two strong feminine forces.

He is a deep thinker whose soul is constantly torn between good and evil, but he never actually takes any significant action in the novel. In fact, his only actions are withdrawals from situations, his flights from war and from the Rings. Serge is submissive to both Judy and Vanna. His great decision in Ashe of Rings seems to be which force he will eventually obey. This is an interesting reversal of traditional gender roles. Serge plays the passive, feminine role, while the women of the novel are the active, masculine powers.

By contrast, Arnold Condorex in Harriet Hume is a domineering man who struggles to contain Harriet. During one of their conversations, Harriet “hears” him thinking that “a man must rise in the world! Dear God, did she not understand?” (56). Indeed, this is Arnold’s primary goal, to move up the social ranks to a position of domination. However, the novel celebrates passive, understanding feminine traits, and Arnold Condorex, the threatening male presence, even comes to honor them. During the final fantasy episode, Arnold must abandon his previous motives. “True, I was an excellent administrator, but all the same I feel guilty beside you and your life spent in contemplation of the eternal beauties” (267). Arnold acknowledges that there is also power to be found in the passive understanding of ancient powers. Arnold eventually learns that there is power in the passive, feminine forces of life. He finally sees that their is knowledge to be gained in observation of nature, and he looks to Harriet to teach him these things.

Bruce Knoll has written of gender roles in relation to Lolly Willowes saying that, “London society is centered on the masculine ideal, which is portrayed as an aggressive, destructive force. Such an arrangement allows only a passive role for the female characters of the novel” (1). He says that the novel is the story of Laura’s flight from the domination of the city to the freedom of the country where she can find an active role without “resorting to the traditional male responses of control, domination, and aggressiveness” (13). Obviously, Laura does make the transformation from passivity to power, but there is also the question of the force that helps her to make this change. The Devil is portrayed (as usual) as a man in the novel. What implications does this have? Is Laura again submitting to a masculine force as she makes a contract with him? Since the Devil is presented as an alternative to traditional patriarchal powers, it is hardly believable to think that Laura is subservient to him. Satan in Lolly Willowes does not have the masculine traits of desire for domination and control that the other men of the novel demonstrate. The novel is a satire on the traditional, patriarchal God, and the Devil is a caring, accommodating persona. Laura does not submit herself to the Devil, rather she willingly makes a pact with him. While the men of her family tried to manipulate Laura, Satan allows Laura to make her own decisions and follow her own path. Laura and Satan converse on the same level, and he offers her help if she needs it. Sylvia Townsend Warner offers a solution to the oppressive powers of Laura’s society.

The novels also present family as one of these “oppressive powers.” There are three degrees of family interaction from Vanna’s place in the family chain of protectors of the Rings to Harriet’s seemingly complete lack of family association. These degrees parallel the heroine’s rejection of patriarchy. While Vanna is taught to be powerful by her father, Laura’s father is a more passive man and her brothers later oppress her and Harriet’s father is only a bearded portrait, a past she has put behind her. Vanna Ashe’s familial identity can be seen as the center of the novel. She is born heir to the power of the Rings, and there is the implication that she, too, must have a child who will inherit the same role. Anthony Ashe raises his daughter to understand the mystical. Vanna is protected, empowered, and comforted by her family role at Rings. Even at the end of the novel, she sleeps in peace at Rings as the “sea wind pour[s] in over her” (232). The clash between her and her mother, Melita, is resolved, and she has a special bond with her brother, Valentine. Her father was a source of wisdom and magic when she was a child, and the
struggle in the story is for her rightful succession. Vanna rejects not familial connection or patriarchal oppression; but, symbolically, war, a destructive force that is typically associated with the masculine.

While Vanna is empowered by her familial connections and never rejects interaction with others, Laura Willowe's finds her power after disassociating herself from her family and living alone in the wilderness. Laura is raised in a family whose primary concern for her well-being is her marriage to a respectable man. Her mother is a frail woman and her father, although loving, is content to allow Laura to be the mistress at Lady Place. Vanna has never had to struggle to overcome a passive existence in the house, but Laura becomes empowered as she eventually overcomes the “dullness” of a domestic realm (212). Vanna is empowered as the heir to a powerful force, but Laura’s strength comes as she discovers her own connection to a natural force, that of Satan’s magic. Although her father never attempts to dominate over Laura, her brothers are the (almost comic) controllers. From Henry and James tying her to a tree to Henry foolishly squandering her inheritance, they are satiric characters who have power but never use it productively or wisely. At the end of the novel, Laura seems to reject human interaction completely, choosing to make her own path alone, with the exception of the occasional discussion with the ever-caring Devil.

Harriet Hume’s family is briefly mentioned as Arnold examines some of her old photographs. Arnold sees the image of Harriet’s father as a “bearded creature pretentiously austere, overblown with patriarchy, as avid for opportunities to raise a hand to heaven to bless or curse his children as a prima donna for arias” (19). When Harriet explains her father to Arnold, she comments that he “breaks the silence of our hills with a terrorsomeness that has something of their own air of enduring forever” (30). It becomes obvious that she has left her father’s smothering dominance in her past in order to construct her own world outside of this patriarchy. Her tale of the Ladies Frances, Georgina and Arabella Dudley further demonstrates Harriet’s views on familial constraint. When the sisters marry and separate, they loose their beauty, and presumably their happiness. Only after reuniting and praying to Mother Earth are they swallowed by the ground and immortalized into a celebration of natural beauty as trees. Their flight from male domination suggests that patriarchy is ruinous for the women. They marry as is expected of them, but there is no self-fulfillment in this role for the sisters. The women finally find peace in the earth, a haven of nature within the city of London. The underlying message regarding marriage here is not that marriage itself is a destructive union. Instead, Harriet Hume sees the problem as marriage for the wrong reasons, such as self-satisfied tradition or monetary gain. Arnold Condorex abuses marriage by marrying a woman for profitable advancements. But marriage for these reasons is enforced by the expectations of the London patriarchy. Indeed, Jane Marcus argues that London is the “capital of Patriarchy” and “was, and is, unmistakably male.” She mentions a witticism from Natalie Barney that said that “nothing in London was made for women, not even the men” (Wilderness 139). But, like Laura Willowe’s, the sisters Dudley escape this patriarchy in nature. To Harriet as well, nature is a cocoon of feminine comfort. She does not reject the patriarchy of London by fleeing the city, but creates her own space in the city where she is in control.

The war is a source of great stress and displacement among the women (and men) in these novels. For example, in Lolly Willowe’s, Laura’s niece Fancy loses her new husband and decides to go to France to drive motor lorries. However, the war had no such excitement for Laura. Four times a week she went to a depot and did up parcels (63). At the end of World War I, Laura goes up to her room and faints (65). Laura reflects that “there was no difference between her and Henry and Caroline in their resumption of peace. But they, she thought, had done with the war, whereas she had only shelved it, and that by an accident of consciousness” (66). The war is not mentioned again in the novel, but its presence does serve a part in Laura’s life. Laura has put the trauma of the war in the back of her mind, and its destruction stays with her. Although she helps in the war effort, she never becomes excited or even feels it gives her a sense of purpose. She rejects the “cheap symbolism” of the recruiting posters in her work room, and therefore rejects the propaganda view of the war as an opportunity for the country to pull together. Instead, she feels that “blood was being shed for her” (64). While her family finds great pride in their country at war, Laura rejects these patriotic emotions. As she later rejects her family hierarchy and the organization of the Witches’ Sabbath, she also dismisses the propaganda of camaraderie connected to the war.

Though its phrasing is often 18th-century and Arnold’s initial maneuverings are reminiscent of the Victorian era of empire-building, most of Harriet Hume takes place after the Great War. Arnold is caught up in postwar politics in his rise to power. Harriet wonders if politicians are not “all occupied in finding a form of government which shall allow that invisible thing, the will of the people, to express its sense of the need for its own preservation... and which shall not be deflected from this by the personal interests of any group” (142). Arnold reflects later that “it is strange that this fundamental stuff of politics has never interested me. 'Tis the negotiation that has ever charmed me, and the struggle for eminence” (143). This exchange reflects their differing attitudes towards war, as well as Arnold’s, where skillful negotiation is like a war. While Harriet is concerned with a government that provides freedom for the people to do as they choose, Arnold is concerned with control over others. Judy Marston personifies this control and destructiveness of the war in Ashe of Rings. The novel is directly affected by the war, and Vanna fights her own battle at the Rings. Serge is a victim of war in general. He has fled war in his own country, and flees the war in England. But the destructiveness of war as displayed in Judy emotionally tears him apart and numbs his senses. Even as Vanna has fought for his soul in a symbolic battle, he has still
been overcome by war. At the end of the novel, although Vanna has protected the Rings for the presence, she still can only "Pray for the peace" (231).

War affects the characters in different degrees; however, Vanna, Harriet, and Laura all must confront the destruction the war has wrought. Its consequences shape the world of these characters, and it ties the novels together. During this period, England was at a time where its social norms were being questioned and traditions are being shattered. The country struggled to return to a familiar, prewar state. But things had changed for women, and although they were being pulled back into private life, they had already found opportunities to have a very public voice. The characters of the novels discussed are unique to this period because they find their voice in their own realm. Vanna Ashe fights her battle at the Rings, Harriet Hume expresses herself through her music and her garden, and Laura Willowes escapes to Great Mop. As they reject traditional paths to power, neither settles for a domestic "power" nor chooses to battle men on their own turf. Jane Marcus argues that "the feminist fantasy novel of the twenties is a response to realism's failure to make permanent female space in the citadels of male power" (Wilderness 141). The authors of these novels have all created this space in nature for their characters.

Many public figures of the inter-war period used essentialist arguments to try to force women back into the home. Verbal abuse, unequal pay, acts of government, media images, and domesticated female education were all factors that worked to encourage women to return to the familial setting after the First World War (Beddoe 4). They made use of the argument that women belonged back in their "natural" setting, the home, where they were nurturing individuals fulfilled by doing their inherent duty as wives and mothers. Since women are physically able to give birth, then it follows they must have the natural inclination for a life in the home as nourishing protectors of their offspring. Inter-war Britain used this argument in an attempt to eliminate women from the workplace with the belief that society should return to its previous patriarchal structure with women back home to restock the dwindled population. But the novels of Mary Butts, Rebecca West, and Sylvia Townsend Warner, all feminists, demonstrated an essentialist view of women as well. Bonnie Kime Scott says that Rebecca West believed that "while women as wives and mothers have remained in contact with primitive instincts, men have been put into conflict with their primitive selves" (Scott, Refiguring 168). The women in these novels experience many necessary steps in their private wilderness: they learn to think for themselves, they grow wiser, they practice their own crafts. Their "gardens" represent a world of wild understanding away from the confines of a restrictive society. The authors of these works make the connection between women and nature as if to say that the "natural" place for a woman to be is in a setting where she can be empowered through freely learning what her contribution to the world will be. Vanna, Harriet, and Laura all find scope for their primal instincts in nature.

These women writers used essentialist notions of the feminine to demonstrate that women's essential nature was best served by not having to follow a strictly determined path. For instance, Vanna Ashe is nurturing as she saves Serge from starvation and attempts to protect him from the evil powers of Judy Marston. As he lays ill in his apartment, Vanna forces her way in and tells him, "I will light your fire. You are not to fight me. Then I will go and come back with everything you want" (89). She feeds him, washes him, and cleans his dirty home. "She bent over the long broom like an oarsman, and the dust-pan followed the rubbish like a hunter's knife... the dust was afraid of her" (90). Just as Harriet Hume dismisses a weed from service in her flower bed, Vanna has full command of the dust in the room. Ironically, as she completes domestic tasks, she is described in aggressive, masculine terms. Rowing and hunting are traditionally men's duties, yet Butts applies them to female service. Vanna nurtures Serge, but does it powerfully.

As Vanna nurses Serge back to health, she also tries to show him what is right in the world. "Her care for him quieted her sensuality. She worked to restore his beauty, as to bring out the grain in wood. She held up a glass while he brushed his hair like dark feathers" (94). If Vanna is attracted to Serge, it is calmed by her sense of duty to help him. She struggles to save what has been dulled by war and Judy. Vanna Ashe's feminine nurturing goes far beyond the domestic: she is a priestess who combines knowledge of her ancient past with the present power of the Rings to transcend the limitations of what society defines as fully feminine. Foy says that "Butts's goddess heroine attempts to use her ancient, secret knowledge in a society usually controlled by male hegemony and moves in a sphere all her own" (32). Vanna, in her mystical womanhood, proves strong enough to be protector of the Rings and thereby nurture the cosmos as well.

Harriet Hume's femininity is also powerful, and it is enough to threaten the patriarchal world of Arnold Condorex. Harriet is a creator of both musical art and life in her flower beds. As a mother gives birth and then strives to properly rear her child, Harriet is concerned with the development of her music and garden. She carefully keeps her garden as Arnold observes it to be "less an exhibition of flowers than a green sanctuary" (74). Her garden is a sanctuary to Harriet, a place where she feels safe and protected. But it is also her private home where she performs domestic duties. At the end of the novel, Harriet displays nurturing feminine attributes as she plays the good hostess to the ghost policemen: "...the French windows swung very wide, and a silly tinkling voice cried, 'Gentlemen! Gentlemen!' and the two less than dove-sized hands held out a tray with two glasses on it in the beam of light" (284). Harriet does embody essential feminine traits, but, like Vanna, she is also a mystical woman with supernatural defense against the aggressive masculine forces of London. Harriet's powers over Arnold threaten him,
and he often reduces Harriet Hume to purely physical terms, perhaps to regain control over her. Jane Marcus says that “Arnold is afraid of the garden and its lush fertility as he fears Harriet’s sexuality and calls her slut, jade, wench, trollo…” (Wilderness 143). He oscillates between dismissing her sexuality with vulgar terms to crudely enjoying her beauty, as when he thinks, as he sees Harriet’s cloak slip from her shoulders, that “he was therefore able to enjoy that sense of being at an advantage which he always derived from admiring a woman’s beauty in detail” (183). Arnold imagines himself to be more powerful when he can either categorize Harriet in degrading terms or deem himself worthy of “appreciating” her beauty. His masculine gaze helps him to regain the power he has lost as Harriet enters his thoughts. Both Harriet’s straightforward sexuality and her supernatural power scare him, although he comes to understand them on a deeper level at the end of the novel.

Both Mary Butts and Rebecca West use their heroines to redefine what inter-war society defined as properly feminine. In the same manner, Sylvia Townsend Warner finds the essence of the feminine in a role with traditionally negative connotations: “There is no more exquisitely powerful evocation of the joys of spinsterhood in print,” boasts Jane Marcus. Lolly Willowes celebrates the joys of women’s solitude, the satisfaction to be found in introspection away from the stressful influence of human interaction. Although Laura Willowes rejects men, she never dismisses her own womanhood. Rather, she despises the domestic role that society has forced women to accept. “It sounds very petty to complain about, but I tell you, that sort of thing settles down on one like a fine dust, and by and by the dust is age, settling down” (Wilderness 212). Her flight into the wilderness is her resistance to this “settling down.” Whereas spinsterhood was seen as unfortunate and pathetic, Lolly Willowes redefines it as a rejection of the mundane. Although she feels worthwhile as she raises the hens, she feels used and worthless as Aunt Lolly. But her role as henwife and then witch is her own path, one that her family has not chosen. Laura does not give in to the pressure of any community, whether it be London, the Witches’ Sabbath, or the Great Mop village. Her rejection of society is a celebration of herself just as she is, without the embellishment of others.

Perhaps what is essential to understand regarding these novels is that they reflect women’s need for their own space in inter-war Britain, a place where they might meditate on what was positive in their lives away from the harsh realities of social pressure, lost loved ones, and a confusing political situation. These fantasy novels offer this space to their characters: an ancient rock formation by the sea, a small patch of nature within a harsh city, or a dark wilderness. The stories allow female readers to imagine what might be possible if they could find a place where they could fully explore their ancient femininity as a priestess, a resourceful artist, or a witch. The magic and myth of these tales offer a hope in fantasy that could also be applied on a realistic level. Although the supernatural traits could not have helped the real women of this period to find their own stable place in society, readers of novels such as these would nevertheless have found comfort in examples of female power.

Ashe of Rings, Harriet Hume, and Lolly Willowes all explore the possibility of women not limited by societal expectations. While it is difficult in any society for an individual to find a private world where she can become a goddess, these novels explore what happens when fantasy and circumstance allow it to happen. Perhaps this concept is discouraging if the only way that woman can find a “permanent female space” is in a fairy-tale. But these novels offer more than a magical solution. They examine and question societal standards of their time while demonstrating that women can be both powerful and essentially feminine and that such an ideal is not a fantasy at all.

Works Cited:
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Faculty Comments:

Ms. Holub's faculty mentor, Debra Rae Cohen, makes the following comments about her work:

Ms. Holub's thesis project derives from her deep interest in both British culture and women's issues; it involves an investigation into sources of empowerment for women writers during the period between the World Wars, usually considered a time of feminist retrenchment. Examining works by three understudied women writers of the inter-war period, Ms. Holub contends that their works represent by way of mythology and folkways an alternative pathway to female power. She focuses on novels by Mary Butts, Rebecca West, and Sylvia Townsend Warner, all of which feature heroines who find nature and mysticism, rather than the patriarchal public sphere, appropriate loci for effecting change.

Ms. Holub's work has important implications for illuminating a complex period often too easily and reductively categorized as one of Modernist experimentalism and conservative domesticity. While post-Great War anxieties did result in a resurgence of the ideologies of home and hearth, this research makes clear that such constraints were far from monolithic. Ms. Holub's ambition and intelligence, and her potential for truly excellent scholarship, give her project enormous potential.

Two of Ms. Holub's thesis committee members, Mavourneen Dwyer and Susan M. Marren, also remarked on the quality of her work. Professor Dwyer said:

I first had the pleasure of meeting Ms. Holub when she took part in our UA London Study Abroad Trip in the summer of 2001. The course consists of a four-week study tour of London theatre and environs and is sponsored by the UA Department of Drama and the English department. It was easy to tell during our time in England that Ms. Holub was being deeply affected by everything she saw and experienced. Consequently, I was not surprised when she later chose a topic for her thesis, that would require a good deal of research into the social history of England. I have read the thesis and I find that it gives evidence of the same enthusiasm and thoughtful attention to detail that characterized her reviews, reports and essays during the London course last summer. Ms. Holub is obviously intrigued by her subject matter, and her thesis is thorough, fresh and perceptive in its insight.

And Professor Marren said:

I have known Ms. Holub for about a year. I first encountered her when she was an outstanding student in my senior research seminar on turn-of-the-twentieth-century American literature in the fall semester of 2001. In that course I was lucky enough to have several of the best students I have encountered in 12 years of teaching, and I found Ms. Holub to be one of the most thoughtful and intelligent among them. She makes perceptive observations in class discussion; she also listens carefully and responds to other students' and my own questions and comments thoughtfully. These qualities make her a very stimulating student.

Ms. Holub has often impressed me as unusually sophisticated in her thinking and surprisingly self-possessed for an undergraduate. In one instance, she gave an oral report on Charles Chesnutt's novel The Marrow of Tradition. As the assignment required, she chose several critical articles and succinctly summarized and evaluated them for the class. She managed to do so in a way that made Chesnutt come to life for the class, and led us to an excellent discussion. For that same course she wrote an essay comparing the fates — the progressions to eventual suicide — of the heroines in Kate Chopin's The Awakening and Edith Wharton's The House of Mirth. Ms. Holub's minute attention to detail and her speculations as to what those details might mean were fascinating; it became clear that she has the makings of a careful, imaginative and serious critic. In short, Ms. Holub writes clearly and gracefully, whether for an impromptu assignment or an original essay, and she is a first-rate researcher.
A DESIGN FOR A CHAPEL FOR SUBIACO ABBEY

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Abstract:

The Benedictine Abbey is a product of medieval thought and society; for centuries its architecture signified the cosmology and spirituality not only of the order but also of a widely accepted Christian worldview. Through the design of a Meditation Chapel for the Benedictine Abbey of Subiaco at Paris, Arkansas, this project explored the cultural meaning and technical execution of contemporary sacred architecture. Nearly a millennium ago, the skin and bones of Gothic structure and stained glass represented a sacred ideal. In the contemporary context, however, this research probed the possibilities of using systems (interior and exterior), particularly the design of the elevation and cladding of the chapel wall, to create a sacred space that could communicate spirituality through phenomenal and sensate qualities of site and materials in lieu of ancient symbol and sign.

The chapel represents the process of architectural design as a research inquiry through a series of interrelated investigations of site as a product of nature and time, of architectural expression as a poetic assemblage, of building skin and structure as biomorphic systems, and of building materials as conveyors of phenomenal qualities of space. These include (1.) generation of the cladding and structural systems through conceptual analog and analysis of an exemplary biological system, the beetle, through drawing exercises; (2.) directed study of Subiaco Abbey through phenomenological investigation documented in drawings; and (3.) comprehensive design of the chapel, including the integration of building systems. This comprehensive design in itself represents the cumulative discoveries of the research; together with the speculative process drawings through which the final design evolved, these design documents constitute the research program.

On a fundamental level this comprehensive project, executed as a capstone experience in the design studios that are integral to the professional program in architecture, demonstrated the mutually reinforcing relationship of technical knowledge, design facility, critical inquiry, and creative insight required of the architectural designer. More particularly, through the exploration of spirituality and sacred space, the research focuses attention on those formal and sensual characteristics of architecture from which it derives its popular meaning.

Premise:

The architecture of religious institutions has developed in parallel with that of all other institutions. Consequently during the past century, it has been shaped by the prevailing architectural ideologies of the modern era; religious architecture shares the same conventions of construction and technology that characterize secular buildings. Further modern architecture is defined largely by the reductive force of functionalism and by diminished economic will. Although there are notable exceptions, including such great works as Frank Lloyd Wright’s Unity Temple and E. Fay Jones’s Thorncrow Chapel, contemporary churches and chapels are distinguished only by vestigial symbolic elements that denote their use but have little of the tectonic power or the material gravity of historic ecclesiastical architecture.

Founded in the early twentieth century long after the great age of monasticism passed, Subiaco is a unique institution. The traditional design of its original buildings establishes the identity of Subiaco, but my program assumes that great contemporary architecture has the ability to nurture and strengthen the aspirations, ideals, and presence of the monastic community. Therefore, while the architectural program for the new meditation chapel is relatively simple in its functional requirements, the challenge of its design is not simply the creation of space but rather to invest the space created with spiritual meaning. The experience of the space must emphasize quality over quantity and privilege spirit over function. Finally, the design of the chapel explores the potential of architecture to expand beyond the limits of reality, encouraging users, in turn, to expand their perceptions and appreciation of social space beyond traditional expectations of monastic architecture.

Concept:

Subiaco Abbey is the product of years of development within the American Catholic church. Located centrally within
Arkansas’s Ozark Mountains, the campus consists of the main abbey building and a few supporting structures around the perimeter of the site. In addition to traditional monastic buildings, the campus includes housing for Subiaco’s clerical brothers and for students who attend the abbey’s school. The image of Subiaco, characterized by Neo-Romanesque and Neo-Gothic structures, conveys a traditional sense of place enhanced by the beautiful natural landscape. Along the plateau on the north end of the property there is a grove of pine trees where the abbey was first established. Not far down the path outside the grove of trees, there is a cemetery that borders one end of the site.

This project offered the opportunity to add a new structure to this ensemble; this new building is to be a place of worship designed to satisfy the spiritual needs of both the discrete monastic community and a larger lay community. Thus, the investigation and definition of those sensual qualities and physical features that connote spirituality was central to the design process. The premise of the project assumed that design begins with the understanding of a pure, natural form that can be transformed and elaborated into an architectural expression. In this case, the beetle was presented as a generator for the design process. Through a series of analytical drawings of the beetle, spatial investigations were accomplished that provided departure points for the chapel design. (See figures 1 and 2.) In other words, the investigation of the beetle provided a bridge to the invention of an entirely new type of spiritual space.

Inventions vs. Intentions:

The essence of the project was the creation of the spaces and the manipulation of light, sound, and scenographic effects that contribute to the aesthetic of a sacred space. More specifically, the program required that the chapel include a meditation space for one person, a place for a gathering of 10 people, and a sanctuary for a congregation of 100 people. Equally important, the chapel was intended to engage the site and be modest in form so that it would harmonize with the existing campus without detracting from the primacy of the abbey. These creations of site, space, and form were all informed by the in-depth exploration of the beetle as a creature whose body created space. The form and structure of the beetle suggested strategies for the spatial explorations of the chapel, hence the use of tonal perspective drawings (figures 3, 4, and 5) and the use of newly developed field drawings (figures 9, 10, and 11.) The field drawings allowed the designer to segue to the reality of technology and the challenge of making an abstraction a reality in invention.

Technology:

The processes of transforming design phase concepts to the reality of the final building design involved the expression of the technology developed for the building. Structure, substructure, interior and exterior surface materials, heating and air-conditioning, and lighting are prominent among the technical components that make a building viable, safe, and effective for its occupants. Remarkably, creating these components appropriately is the most important factor in creating a spatial place. The use of sectional drawings and models (figures 8, 14, and 15) facilitated the in-depth exploration of these building systems. In the final investigative phase of the project, the systems axonometric drawing proved to be useful in the elaboration of the technology, giving the project a coherent transition from preliminary sketch to realistic comprehensive design (figure16.)

Design:

During the middle stages of project development, the chapel became a system of large monolithic elements. Those elements involved a transformation from interior space to exterior surface. The unit, as the system is called, modified itself from one large external element to nine smaller interior elements. This was the architectural tactic used for expressing spirituality. I believe that if a person loses his or her sense of scale within a space, then the space appears to be not of that place. In this way, the space would encompass the transcendental experience of traditional ecclesiastical architecture.

The way the light and sound dance in the chapel is also extremely important. Direct light enters the space only at sunrise at the time the monks have their first service. The use of ambient lighting provides a mysteriously calm mood at any other time of the day. Light reflected off the zinc interior panels creates illumination for those times when only a little light is needed. On the exterior the cold-rolled steel-plate system, allowed to take on a rusted patina, would interact with pre-existing buildings and suggest that the new chapel would stand the test of time in much the same manner as its predecessors, the existing abbey buildings.

Faculty Comments:

Mr. Siebert’s mentor, Visiting Professor David Murphree had left before this journal was formatted for publication and was unavailable for comment; however, one of Mr. Siebert’s other fifth year design critics, Tim de Noble, was extremely complimentary about the project. He said:

I had the opportunity to work with Mr. Siebert in my capacities as one of the Comprehensive Studio critics and as one of the faculty teaching the requisite technology course. The Comprehensive Studio was not divided into separate sections for purposes other than administration, allowing the three faculty members to ‘float’ to critique all students on a periodic basis. In this capacity I was able to observe Josh’s effort at approaching and developing his design for the project, a chapel at the Academy in Subiaco, Arkansas. At all times throughout the semester Josh exhibited unparalleled intensity, creativity, inventiveness, skill and craft. His work reflects his
individual ability and intuition and is not the result of ‘heavy-handedness’ on the part of his critics. In fact his work, because of his skill and well-developed intuition, is the most independent of all the students’ design solutions for the project. Additionally, I worked with Josh as his professor in the Technology class. This class was organized to advance the application of technological issues in service of the Comprehensive Studio through concurrent and apropos assignments. In these endeavors Josh continually set the standard by his proactive, timely, and inventive efforts. Finally, though not the primary thrust of this award, I wish to recognize Josh for his willingness to help less capable students. Josh possesses a wonderful, selfless nature and subsequently has contributed to the wonderful esprit de core exhibited by this fifth year class.
Editors: Inquiry: The University of Arkansas Undergraduate Research Journal

ARCHITECTURE: Josh Siebert A Chapel for Subiaco

Figure 3

Figure 4
Editor's note: Figures 5, 6, and 7 are omitted due to space restrictions.
Figure 8.
THE EFFECTS OF ENCLOSURE TYPE ON AGGRESSIVE BEHAVIOR IN CAPTIVE CHIMPANZEES

Erica Renee Findley
Department of Anthropology

Faculty Mentor: Peter S. Ungar
Department of Anthropology

Abstract:

Male chimpanzees are known to be aggressively territorial in the wild. It is expected that the limited enclosures of captivity would make them more aggressive. The Kansas City Zoo was chosen as an ideal venue to explore this assumption because it boasts the largest outdoor enclosure of its kind, and the apes alternate between this and much smaller indoor housing.

Quantitative data on crowd size, time of day, temperature, and frequency/intensity/duration of aggressive behaviors were recorded for the alpha male in both enclosures during 300 hours of observation. These data were used to test the following hypotheses: as measured by frequency/intensity/duration, aggression will increase 1) inside relative to outside, 2) with number of visitors, 3) with temperature, and 4) during the middle of the day.

Statistical tests and graphical analysis showed that the frequency and duration of aggressive events did, in fact, increase inside, while the intensity decreased. The number of visitors, temperature, and time of day showed little relationship to aggressive interaction, although a few patterns were seen. These conclusions not only aid in the understanding of captive chimpanzee aggression, but also can be used to improve conditions for chimpanzees in zoological parks throughout the United States.

Introduction:

Endangered species are kept in captivity for research and protection from extinction. To maintain animal well-being, zoological facilities must design living areas to limit possible sources of stress. Stress leads to aggression, which can have serious health consequences for the animals. This project examines the effects of enclosure size and type on aggressive behavior in chimpanzees at the Kansas City Zoological Park.

The Problem:

Aggression is a normal part of life for wild chimpanzees. Such behaviors are associated mostly with social excitement, territorial defense, competition for food and mates, and the protection of offspring and social allies (Goodall, 1986). Still, levels of hostility between group members may well be higher in captivity than in the wild, especially where movement is restricted, and crowding a problem. A study by Boyce and coauthors (1998) provides a good example of this for other primates. These authors found that rhesus macaques crowded together showed a five-fold increase in severe injuries. Other researchers echo these concerns, noting that abnormally high levels of aggression occur among animals reared in grossly restricted environments (Davenport and Berkson, 1963; and particularly Walsh et al., 1982). Given the severe consequences of increased levels of aggression, it is important to see whether these patterns hold true for other primates, such as Pan troglodytes, the common chimpanzees. The Kansas City Zoo is an ideal place to test these assumptions. The outdoor enclosure is the largest of its kind in the United States (approximately 3.5 acres in size), and the apes alternate between this and smaller indoor housing.

Justification For Research:

This study concentrates on levels of aggression—the primary concern when dealing with animal welfare. According to Hinde (1983), studies of animal and human behavior have shown that opportunities to behave aggressively may make further aggression more likely on a future occasion. Further, if aggression is to be controlled, the external conditions responsible must be removed. Unfortunately, the principles involved in the evolution of increasingly complex behavior and the role that it has played in shaping the direction of evolution are still not well understood (Martin and Bateson, 1993; and Wrangham et al., 1994). Therefore, this project has considerable implications for understanding the effects of enclosure size, crowd size, temperature extremes, and time of day on levels of chimpanzee aggression. Results could therefore help define better management techniques for captive chimpanzees. Finally, by contributing to a growing body of information, these data can be used to reconstruct captive environments and improve conditions for chimpanzees in zoological parks throughout the United States.
Model:

This study predicts that in a larger, outdoor habitat, less aggression will be seen. However, as disturbances and discomforts occur, such as increased visitor number and temperature, the frequency/intensity/duration of aggressive events will increase. The following hypotheses were generated to test these assumptions.

Hypothesis 1:
Aggression will be greater in the more restrictive indoor enclosure, as opposed to the larger outdoor enclosure, as measured by frequency, intensity, and duration.

Hypothesis 2:
Aggression will increase with number of visitors as measured by frequency, intensity, and duration.

Hypothesis 3:
Aggression will increase with temperature as measured by frequency, intensity, and duration.

Hypothesis 4:
Aggression will increase midday (time of day 2) as measured by frequency, intensity, and duration.

Materials and Methods:

Housing:

The two habitats have very different immediate physical environments. The outdoor enclosure spreads across 3.5 acres of land, is scattered with trees, and presents little visual or auditory contact with visitors to the zoo. Visitors are enclosed in a small building, separated from the chimpanzees by large, tinted panes of glass. Three small, open-aired, viewing areas exist, but are separated from the enclosure by a walled ditch approximately six meters deep and three meters across.

The indoor enclosure, on the other hand, consists of five pairs of adjacent cages, each approximately five meters long and three meters wide, separated from one another by a long hallway. Each cage consists of three concrete walls and one wall of bars facing the hallway. Although much smaller than the outdoor habitat, the indoor enclosures are isolated entirely from visitors and have a much more controlled environment, with temperatures averaging between 75 and 80 degrees Fahrenheit year-round.

Subjects:

The subjects of this study included 12 chimpanzees total: three adult males, four adult females, one adolescent male, two adolescent females, and two baby females.

As numerous studies have shown that the 'alpha' male is most prone to engage in aggressive interactions (Martin and Bateson, 1993), this project focused on Joshua. Using the focal animal sampling technique, data on other individuals were not recorded.

Observations:

Data on aggressive and affiliative behaviors were recorded in both enclosures, between 8:30 AM and 4:45 PM, five days a week, for eight weeks and approximately three hundred hours of observation. Daily observations at both sites began with a record of temperature (temp). Subsequent temperature readings were taken every hour throughout the day. Outside, the number of visitors was recorded every ten minutes and during aggressive and affiliative encounters. Time of day (time) was subdivided into three categories: 8:30 to 11:15 for Time of Day 1, 11:16 to 2:00 for Time of Day 2, and 2:01 to 4:45 for Time of Day 3. Using the definitions below, the following data were collected:

Time was recorded at the beginning (begin) and end (end) of each behavior.

Total duration (total) was calculated by subtracting 'begin' from 'end'.

Location (in/out) was labeled 'I' for indoor enclosure and 'O' for outdoor enclosure.

Contact type was subdivided into two categories—affiliative (af) and aggressive (A).

Affiliative behaviors were divided into four categories: grooming (G), being groomed (GB), playing (P), and copulation (C)

Aggressive and affiliative activities were divided into two categories—vocalizations (voc) and facial expressions (face). The vocalizations recorded were pant hoot (ph), loud bark (lb), scream (s), and chirp (ch), while facial expressions included play face (pf), display face (df), and compressed lips (cl).

Aggressive actions (action) were subdivided into the following categories: bite (b), hair bristle (hb), throw (th), chase (ch), rock (r), sway (s), jump (j), take (t), foot stomp (st), displacement (d), bang (bg), drag (dr), and bipedalism (bp).

Measurements Made After Data Collection:

Frequency of aggressive and affiliative behaviors was compiled both overall and during each time of day interval.

Intensity of each aggressive behavior was subdivided into three categories: Intensity 1 consisting of one or two behaviors (vocalizations, facial expressions, or actions); Intensity 2, either three behaviors or just a bite, hit, drag, throw, or bipedal movement; and Intensity 3, more than three behaviors, or any one behavior combined with bite, hit, drag, throw, or bipedal movement.
Duration of each behavior (both aggressive and affiliative) was subdivided into three categories: Duration 1 defining behaviors with total time between 1-10 seconds; Duration 2, 11 seconds to 1 minute; and Duration 3, greater than 1 minute.

Discussion:

This study examines how possible sources of stress in relation to enclosure type affect levels of aggression in captive chimpanzees. By looking at the frequency, intensity, and duration of aggressive behaviors demonstrated by the focal animal in each enclosure, and comparing these to the possible sources of stress, baseline was established and variables isolated for significance.

Enclosure Type (Outside vs. Inside):

Results of this study indicated that there was a significant difference between the frequency, intensity, and duration of aggressive interactions outside as compared to those inside (Table 3). As shown by Figure 1, aggressive interactions were significantly more frequent inside than outside. As shown by Figure 2, events of each intensity were also more frequent in the smaller enclosure. When comparing the ratios of intensities in each enclosure, however, the majority of events inside were of intensity 1, while the majority of events outside were of intensity 3. Thus, although the frequency of aggressive interactions of each intensity was significantly greater inside, the overall intensity actually increased outside. This inconsistency can be explained in terms of boredom. Because the chimpanzees had less to do in the smaller, more restrictive enclosure, they tended to become bored and picked small fights with one another (de Waal, 1989). By allowing the chimpanzees more space, the small skirmishes of boredom were eliminated, and only those interactions necessary to maintain social structure were displayed. It follows that these interactions would be of greater intensity considering they were acts of dominance and display. Thus, the majority of aggressive interactions outside would be of greater intensity.

Aggressive events of the highest duration, on the other hand, increased inside relative to outside. As shown by Figure 3, the majority of events outside were of durations 1 and 2. Therefore, only a small minority of events lasted longer than a minute (duration 3). Inside, on the other hand, almost a third of aggressive events were of the longest duration. Once again, this can be explained in terms of boredom. Because Joshua had less to occupy his time inside, his displays tended to increase in duration. The superior acoustics inside also allowed a much louder, intimidating display, which caused the other chimpanzees to become more excited, further encouraging Joshua’s prolonged aggression. In the open-air enclosure, however, displays could not be as spectacular and would therefore not last as long.

Visitors Number:

Results of this study showed that crowd size had no significant impact on frequency or duration of aggressive events, but did have an effect on the intensity of aggressive interaction (Table 4). It follows that the frequency and duration of aggressive behaviors would not be impacted by crowd size because the layout of the outdoor enclosure minimizes both visual and auditory interaction. However, Figure 4 shows that intensity level did increase significantly with visitor number. This suggests that although the glass limited noise, large numbers of visitors created enough disturbance to instigate displays of greater intensity.

Temperature:

Temperature had no significant impact on frequency, intensity, or duration of aggressive behavior in either enclosure (Table 6). This was expected for the indoor enclosure, considering temperature was controlled, but because outside temperature remained mild throughout the course of this study, it follows that little impact would be seen in either enclosure.

Time of Day:

Time of day had no significant impact on duration of aggressive interactions outside or frequency/intensity of aggressive behavior in either enclosure (Table 7). The duration of events inside, however, did show a significant difference in relation to time of day, although it did not increase midday as predicted. Instead, events of duration 1 decreased in frequency throughout the day, and events of longer duration decreased in frequency midday (Figure 5). This decrease could be related to the fact that the keepers left for an extended period of time midday. Thus, the disturbances created by the keepers in the morning and late afternoon could have influenced Joshua to display for prolonged periods of time in comparison to those while the keepers were away.

Implications:

In short, a large, outdoor enclosure can effectively reduce abnormal levels of aggression if the visitors are sufficiently separated from the animals and the temperatures remain mild. It is important for zoological facilities to keep these parameters in mind when designing captive environments. For example, visitors should always be separated by glass—not only to keep them from throwing foreign objects into the enclosure, but also to minimize pheromone disturbance and visual/auditory contact. Secondly, climate must be considered. Enclosures built in areas with highly variable weather patterns should either contain transfers between indoor and outdoor facilities, or indoor housing that maximizes vertical and horizontal space.
Works Cited:

RESULTS

Definitions (Tables 1 and 2)

<table>
<thead>
<tr>
<th>Intensity 1</th>
<th>1 or 2 behaviors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity 2</td>
<td>3 behaviors, or just b, h, dr, th, or bp</td>
</tr>
<tr>
<td>Intensity 3</td>
<td>&gt; 3 behaviors, or 1 behavior and b, h, dr, th, bp</td>
</tr>
</tbody>
</table>

Enclosure Type (Outside vs. Inside)

Hypothesis 1: Aggression will be greater inside than outside as measured by frequency, intensity, and duration.

Table 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Chi-Square</th>
<th>Degrees of Freedom</th>
<th>Probability</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) Frequency</td>
<td>51.212</td>
<td>1</td>
<td>0.000</td>
<td>Yes</td>
</tr>
<tr>
<td>B) Intensity</td>
<td>8.589</td>
<td>2</td>
<td>0.014</td>
<td>Yes</td>
</tr>
<tr>
<td>C) Duration</td>
<td>16.987</td>
<td>2</td>
<td>0.000</td>
<td>Yes</td>
</tr>
</tbody>
</table>

A) Frequency

This figure shows that aggressive relative to affiliative events were more frequent inside than outside. Because the frequency of affiliative events does not differ greatly between the two enclosure types, it is the difference in aggressive events that drives the significant
B) Intensity

This figure shows that although events of each intensity were more frequent inside than outside, the majority of events outside were of higher intensity. Note also that the number of events was compiled for one-hundred-and-twenty hours of observation in each enclosure.

C) Duration

This figure shows that aggressive interactions of each duration were more frequent inside than outside. Inside, events of duration 1 and 3 happened at relatively the same frequency, while outside, events of duration 3 occurred much less than those of duration 1. This suggests that overall duration did increase inside relative to outside.

Visitor Number (Outside Only)

Hypothesis 2: Aggression will increase with number of visitors as measured by frequency, intensity, and duration.

Table 4

<table>
<thead>
<tr>
<th>Variable</th>
<th>MWU or KW Test Statistic</th>
<th>Degrees of Freedom</th>
<th>Probability</th>
<th>Significance</th>
</tr>
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<tr>
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<td>4518</td>
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<td>B) Intensity</td>
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<td>0.005</td>
<td>Yes</td>
</tr>
<tr>
<td>C) Duration</td>
<td>1.898</td>
<td>2</td>
<td>0.387</td>
<td>No</td>
</tr>
</tbody>
</table>

Figure 4 – Intensity of Aggressive Events in Relation to Visitor #

Note: On this boxplot and subsequent—the median for each dataset is indicated by the center line, the first and third quartiles are the edges of the box, which is known as the inter-quartile range (IQR), and the extreme values are the ends of the extended lines. Points at a greater distance from the median than 1.5 times the IQR are plotted individually as circles and asterisks. These points represent outside values and far outside values.

This figure shows that intensity level did increase with visitor number, as shown by the increase in visitor mean and median from intensity 1 to intensity 3.
Table 5

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Minimum Vis</th>
<th>Maximum Vis</th>
<th>Mean Vis</th>
<th>Median Vis</th>
<th>St Deviation</th>
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<tr>
<td>1</td>
<td>0</td>
<td>50</td>
<td>7.139</td>
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<td>12.079</td>
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<tr>
<td>2</td>
<td>0</td>
<td>45</td>
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<td>10</td>
<td>15.180</td>
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<tr>
<td>3</td>
<td>0</td>
<td>70</td>
<td>20.578</td>
<td>13</td>
<td>21.165</td>
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</tbody>
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Temperature (Outside vs. Inside)

**Hypothesis 3:** Aggression will increase with temperature as measured by frequency, duration, and intensity.

Table 6

<table>
<thead>
<tr>
<th>Variable</th>
<th>Enclosure</th>
<th>MWU or KW</th>
<th>DF</th>
<th>Probability</th>
<th>Significance</th>
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</thead>
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<tr>
<td></td>
<td>Inside</td>
<td>9226.5</td>
<td>1</td>
<td>0.301</td>
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<tr>
<td>B) Intensity</td>
<td>Outside</td>
<td>0.234</td>
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<td>0.89</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Inside</td>
<td>0.697</td>
<td>2</td>
<td>0.706</td>
<td>No</td>
</tr>
<tr>
<td>C) Duration</td>
<td>Outside</td>
<td>1.775</td>
<td>2</td>
<td>0.412</td>
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<td></td>
<td>Inside</td>
<td>2.901</td>
<td>2</td>
<td>0.234</td>
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</tr>
</tbody>
</table>

Time of Day (Outside vs. Inside)

**Hypothesis 4:** Aggression outside will increase midday (time of day 2) as measured by frequency, intensity, and duration.

Table 7

<table>
<thead>
<tr>
<th>Variable</th>
<th>Enclosure</th>
<th>Chi-Square</th>
<th>DF</th>
<th>Probability</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) Frequency</td>
<td>Outside</td>
<td>2.997</td>
<td>2</td>
<td>0.223</td>
<td>No</td>
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<td></td>
<td>Inside</td>
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<td>2</td>
<td>0.668</td>
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<tr>
<td>B) Intensity</td>
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<td>4</td>
<td>0.274</td>
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<tr>
<td></td>
<td>Inside</td>
<td>2.163</td>
<td>4</td>
<td>0.706</td>
<td>No</td>
</tr>
<tr>
<td>C) Duration</td>
<td>Outside</td>
<td>5.599</td>
<td>4</td>
<td>0.231</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Inside</td>
<td>17.922</td>
<td>4</td>
<td>0.001</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**C) Duration**

Figure 5: Inside

![Duration of Aggressive Events in Relation to Time of Day](image)

This figure shows that the duration of events did not increase midday as predicted. Instead, events of duration 1 decreased in frequency throughout the day, and events of longer duration decreased in frequency midday.
Facility Comments:

Ms. Findley's faculty mentor had the following things to say about her:

Ms. Findley is among the best and brightest students I have known during my six years at the University of Arkansas. She maintains an extremely high GPA, is active in student organizations, and has real-life experience working in the field of study she wishes to pursue. Ms. Findley is a senior and took my course in Primate Adaptation and Evolution last year. Despite the fact that this was a graduate level course, Ms. Findley held her own with M.A.- and Ph.D.-level students very well, and received a grade of A. Ms. Findley's academic goal is to earn a doctorate with a focus in primate behavioral ecology. She takes this goal very seriously. For example, she has supplemented her education here at the University of Arkansas with courses in animal ecology at the University of Canterbury, New Zealand.

Her research aptitude is evidenced both by her class work and by her work done at the Kansas City Zoological Park. There, she served as researcher, interpreter and educator. She has excelled in classes emphasizing research and quantitative methods. Also, she won a prestigious State of Arkansas Information Liaison Office undergraduate grant under my direction to conduct behavioral research on captive chimpanzee aggression, which is the subject of this article.

Ms. Findley is also very well rounded. She is a motivated, enthusiastic student leader. She serves as Vice President of the Outdoor Recreational Club for the University, is Treasurer for the Student Sierra Coalition, and recently raised funds as Financial Coordinator to send University of Arkansas students to the Environmental Conference at the University of Pennsylvania. She clearly has the ability to meet her goals and commitments. In sum, Erica Findley is a bright, energetic, enthusiastic, motivated student who has clearly defined goals and a great future in academia ahead of her.

Another of her teachers, Loredana Lanzani, is very complimentary about Ms. Findley's abilities. She says:

I have known Ms. Findley since August 2000, when she enrolled in my advanced undergraduate mathematics course on Differential Equations. Even though all the 37 students in the class had been carefully selected and proved to be very talented and dedicated, it became clear from the very beginning that Ms. Findley would define the top of the class. I like to compare my teaching experience at the University of Arkansas with my experience as an instructor at Purdue University, where I have had extensive contacts with many science or engineering majors. None of the students I had known at Purdue could even remotely compare to Ms. Findley in terms of mathematical ability and rigor, intuition, enthusiasm and curiosity. Not one lecture went by without Erica being with me or, more often, ahead of me in the presentation. She showed equal enthusiasm both for the theoretical aspects of the subject (in fact, I could tell from her remarks that she was able all the time to pin down the details that I had left out in the proofs) and for the many applications to biology, physics and engineering that were covered throughout the course. It goes without saying that Ms. Findley's written work was spotless, and she earned the best score in the class (and I should add that, based on my five-year experience at the University of Arkansas, the number of talented students in Ms. Findley's class was unusually large). In short, she was, by all measures, the very best in the class. There is no doubt that Erica R. Findley ranks in the top 1% among the over 400 students I have so far had the good fortune to assist in their intellectual growth. I am confident that she will succeed in any career she may choose to pursue.

Although I am best qualified to assess Erica R. Findley's scientific merits, I feel that her deep and genuine concern for the social issues that are related to her research area, primate evolutionary biology, cannot be left unmentioned. She is perfectly aware that her research in the branch of primatology dealing with dysfunctional behavior of captive primates may bring invaluable insight to the analysis of human behavior under stressful circumstances. This awareness has fueled Ms. Findley's exceptional drive in pursuing exhausting fieldwork as a zoo intern, and has given her a crystal-clear vision of her goals and long-term objectives. Indeed, the continuity between her current and past research and her plans for future work are one of the aspects of her personality that I find most appealing; I feel that the determination and consistency she has demonstrated so far show a great deal of promise toward the successful completion of her ultimate goal, to become a professor of primate evolutionary biology. To find an individual, in particular a woman, that is as clearly driven, talented and motivated at such a young age is so rare an event that all efforts must be made to support that individual in the pursuit of her career plans and her vision.
RESULTS OF A "STAGES OF CHANGE" PILOT SURVEY FROM AN OSTEOPOROSIS PREVENTION OUTREACH PROGRAM

Amy S. Gray
Department of Health Science

Faculty Mentor: Lori Turner
Department of Health Science

Abstract:

Osteoporosis is a serious health problem and crippling condition that often results in premature mortality and significant morbidity that may be manifested in the form of fractures, bone deformation, and pain. Osteoporosis affects almost 44 million people in the United States, 80% of whom are women. The purpose of this project was to test a pilot survey using the Stages of Change Model for health behaviors that may affect the risk of osteoporosis. Current research is lacking in use of the Stages of Change model for studying behaviors related to the prevention and reducing the risk of osteoporosis.

Three surveys were developed to evaluate behavior changes and behavior change intentions resulting from an osteoporosis outreach program for middle-aged women. The program provided educational classes, screening, individual counseling, and referral for 342 women in Northwest Arkansas. The program took place from January to October 2001. The stages surveyed included contemplation, preparation, and action stages, referring to them respectively, as thinking, planning, and have made changes. Subjects were surveyed regarding behavior changes and behavior change intentions that occurred as a direct result of the program.

Behaviors surveyed included nutritional habits, specifically calcium intake, level of physical activity, and other behaviors, such as hormone replacement therapy, smoking, and prescription drug use, which affect osteoporosis. Results regarding nutrition variables concluded, 60% started consuming more dairy products, 29% started eating more calcium-rich vegetables, 42% began consuming calcium fortified products such as orange juice, 39% started taking a calcium supplement, 28% started eating more calcium-rich vegetables, and 25% modified their food preparation techniques to include more calcium. Reports of physical activity changes include, 20% started yard work, 19% began walking and 14% began a weight-training program, 13% reported making some other change in their activity level that was not listed on the survey. Regarding other behavior changes, 7% started on hormone replacement therapy and 26% reported some other behavior change that was not listed on the survey. An osteoporosis outreach program may be instrumental in facilitating behavior changes that support bone health and, therefore, osteoporosis prevention.

Introduction:

Osteoporosis:

Osteoporosis is also known as the silent thief because it often goes undetected. It is a crippling condition that often results in premature mortality and significant morbidity that may be manifested in the form of fractures, bone deformation, and pain (1). Osteoporosis and low bone mass are serious public health problems in the United States that affect 44 million men and women aged 50 and older in the United States, 80% of whom are women (2). Estimates indicate that osteoporosis is responsible for more than 1.5 million fractures annually (2). It also contributes to 90 percent of hip fractures in women and 80 percent of hip fractures in men (3,4). According to the National Osteoporosis Foundation, the estimated national direct expenditures, which include hospitals and nursing homes, for osteoporosis and associated fractures was $17 billion ($47 million daily). Considering the growth of the elderly population most affected by this disease, the cost is estimated to triple by the year 2040 (4). Research indicates that an increase in bone mass density by five percent may decrease the risk of fractures by up to 25 percent (4, 5). Physical activity, nutrition, hormone replacement therapy, and other behaviors may contribute to an increase in BMD, therefore, these behaviors were surveyed in this project (6).

Osteoporosis is a multifaceted disease, which is characterized by low BMD, and is usually found in postmenopausal women (6). During the immediate years following the termination of menses there is an express loss of bone density associated with decreased levels of estrogen in the body (6). Women lose up to one percent of bone mass per year after age 35; by age 50 that figure rises three to five percent per year and continues to rise for about 15 years (7).
There are also other significant risk factors including race, lifestyle, diet, and physical inactivity (8). Non-Hispanic white women are disproportionately afflicted with this disease, although the rate of osteoporosis in other races and ethnic groups is also significant (2). People who smoke or consume alcohol in excess are also considered to be more at risk for osteoporosis as well as people with a diet low in calcium (9). Sedentary lifestyle and lack of weight bearing physical activity are also risk factors for low bone mass and osteoporosis (6, 7, 10, 11). It is often recommended that women include a regimen of hormone replacement therapy, along with an adequate calcium intake and weight-bearing exercise to prevent osteoporosis.

The federal government has noted in its Health Objectives for the Nation an urgent need to reduce deaths due to falls, reduce the incidence of hip fractures, and increase the amount of women educated about osteoporosis (12). Goals for osteoporosis in the Healthy People 2010 report include a 20% reduction of the proportion of adults with osteoporosis (4). The major risk factor for hip fracture is osteoporosis. Practically all persons with a hip fracture require hospitalizing for treatment, and 20% of persons who fracture a hip die within a year (4, 13).

**Health Behavior Theory:**

Health behavior theory can provide the foundation for various stages of planning, implementing, and evaluating a program (14). Theories are guides to help health educators answer why a population is not following public health and medical advice, what needs to be done to reach a population, and how to get a population to change their behavior. Theories can provide insight for shaping programs and strategies to reach specific populations (14).

The Stages of Change model (developed by Prochaska) is based on a theory used to describe the motivational readiness of a person to change a health behavior (15). The model suggests that people move through five stages to achieve behavior change: precontemplation, contemplation, preparation, action, and maintenance. Each stage translates, respectively, to the following: not intending to change, intending to change within 6 months, actively planning to change, overtly making changes in their behavior, and changing behavior for six months with increased confidence and self-efficacy (16). The Stages of Change model is a spiral in which people may move forward and backward along the continuum at anytime.

The Stages of Change model is appealing because it helps identify the type of intervention that is most effective in each of the stages (17). Healthful dietary and physical activity changes are different than smoking and other addictive behaviors (17). A person may be in the maintenance stage of meeting requirements of five servings of fruits and vegetables a day, but in the contemplation stages of increasing their dairy intake to meet daily calcium requirements. Ideally, the health educator should first assess each individual behavior (dietary, physical activity, hormone replacement therapy, smoking, etc.) that affects osteoporosis, and map out a well-defined sequence of interventions to help move the client through each stage for each behavior (see Table 1).

Since many things affect bone health, there are multiple behaviors that can be changed to reduce the risk of osteoporosis. This is why the Osteoporosis Prevention Outreach Program focused on behavior change and the reason for using the Stages of Change model for the surveys.

**Purpose:**

The purpose of this project was to pilot test a survey using the Stages of Change model for health behaviors that may affect the risk of osteoporosis. Documentation of the model for addictive behaviors, such as smoking, is abundant, but research on the model for behaviors such as exercise and dietary change is limited (17, 18, 19, 20). Current research is lacking in use of the Stages of Change model for studying behaviors related to the prevention and reduction of osteoporosis risk.

**Methods:**

Subjects for the pilot study were participants in the Osteoporosis Prevention Outreach Program conducted in Northwest Arkansas by Lori Turner, Ph.D. from January 2001-December 2001. They were surveyed regarding behavior changes and behavior change intentions that occurred as a direct result of the program.

Participants were recruited from the University of Arkansas, Fayetteville; Springdale school district, Tyson Foods, the Jones Center for Families, and local beauty and tanning salons. Different types of media were utilized for recruitment including the University of Arkansas’s daily e-mail announcements, the Jones Center for Families’ television network, and flyers including a photograph of a woman with osteoporosis characteristics. This resulted in the 392 women from Northwest Arkansas registering for the Osteoporosis Prevention Outreach Program. Three hundred and forty-two participants completed the program. Program components included four educational classes, screening, and individual consultation.

Each participant attended one class per month from February to May 2001. Participants were responsible for making their own appointments for the classes and bone scan. No reminders were provided in between classes or before bone density scan appointments. The last class was held in May 2001, and some participants from that class were not scanned until October 2001. The final 342 women who had been through the educational classes were then scanned using duel energy x-ray absorptiometry (DEXA). These subjects were also administered the three stages of change tests. As the participants came in for their bone scan they were given instructions on how to fill out the surveys. There was a least one person available at all times so participants could ask questions.
The principle investigator, Lori Turner, developed the Stages of Change questionnaires. Questions were tested for face validity. The survey format was multiple choice, and participants were asked to circle all of the changes that applied. Participants were asked about the same behavior change on each stage. Only three of the five stages of the model were evaluated: contemplation, preparation, and action, referring to them as thinking, planning, and making changes, respectively. Subjects were surveyed regarding behavior changes and behavior change intentions that occurred as a direct result of the program. Dietary habits, physical activity, and other behaviors that relate to osteoporosis were surveyed.

The dietary behaviors surveyed on the nutrition questionnaire asked participants if they had added, were planning to add, or were thinking about adding the following: servings of milk or other dairy products, soy milk, tofu, calcium-rich vegetables, calcium-fortified food products, calcium supplements, and/or food preparation techniques to include more calcium.

The physical activity behaviors questionnaire asked subjects if they were making, were planning to make, or were thinking about making changes in the following activities: walking a mile or more without stopping, jogging or running, riding a bike (or exercise bike), swimming, aerobicics, calisthenics or yoga, gardening or yard work, and weight training.

Participants were also asked if they were making, were planning to make, or were thinking about making any other changes in the following: taking hormone replacement therapy, quitting smoking, taking Fosamax or another prescription drug for osteoporosis. Each survey included an opportunity for participants to list any other change not mentioned in the survey and included a no-change option.

Results:

Retention rate was 87 percent for the entire program. Subjects were mostly middle-aged, married, Caucasian women, with moderate levels of education. The level of education varied greatly, from nine to twenty-six years, with a mean of 16 years. See Table 2 for more demographic information.

Ninety-nine percent of participants responded to the activity change and other behavior change surveys, and 95 percent responded to the nutritional change survey. A reason for 95% response on the nutrition survey could be due to an open-ended questionnaire before it, which may have led participants to think it was the last page. Large changes in behavior were reported regarding nutrition and activity variables. See Table 3 for nutrition variable results and Table 4 for activity variable results.

Regarding other behavior changes, 7% started on hormone replacement therapy and 26% reported some other behavior change that was not listed on the survey. Five percent reported planning on hormone replacement therapy, 4% are planning to quit smoking, 11% are planning to start another behavior not listed. Eight percent are thinking about taking hormone replacement therapy, 4% are thinking about taking a prescription drug for osteoporosis, and 7% are thinking about another change not listed on the survey.

Discussion:

A large percentage of participants reported changes in their dietary habits. This is probably due to the behavior requiring a small amount of effort to change for most people. Most women go to the grocery store and prepare the meals for their families; therefore, it is easy for them to purchase calcium-rich foods and change their food preparation techniques to increase calcium intake.

Changes in physical activity were not as common. It takes a great amount of effort to make changes in physical activity levels for some people. Finding time and finding an activity that is enjoyable is necessary for long-term results but is often difficult for people who are busy with multifaceted lives (4).

The figures for the other behaviors are low. Many women may not be thinking about hormone replacement therapy yet because they are not postmenopausal. Few participants were smokers so they would not be thinking about quitting. Most women at the time the survey was given did not know their bone density so they would not be considering taking any prescription drug to combat the effects of osteoporosis.

Some limitations of this study include self-reported data and no pre-test data. Self-reported data can be inaccurate, for example, consider a report by Green and colleagues in which self-rated low-fat diet was compared with actual nutrient intake (30% or less calories from fat) (21). Sixty percent reported themselves in the action or maintenance stages, while nutrient intake only classified 20% in either of those stages.

Participants were not pre-tested based on the Stages of Change model. In future studies pre-testing needs to be done to find any significant differences in self-reporting behavior change. Future research needs to address this by being more accurate when assessing participant behavior change based on classes in an osteoporosis prevention outreach program. Monitoring actual nutrient intake, amount of physical activity, and other behaviors over the course of the classes would be an ideal way to collect accurate data. Participants could keep a log of diet, physical activity, and other behaviors or meet with nutritionists, personal trainers, and doctors to keep reporting as accurate as possible and to measure consistency between self-report and true measurement.

The results from this study imply that an osteoporosis outreach program may be instrumental in facilitating behavior changes that support bone health and, therefore, osteoporosis prevention. The combination of educational classes and screening may have been effective in launching behavior change. There are plans to do a follow-up survey in 18-24 months to see how long
the behavior changes have lasted or if there has been an increase in participants moving to action stages.

References:


Table 1: The Stages of Change Model

<table>
<thead>
<tr>
<th>Stage</th>
<th>Behavior-increasing daily calcium intake through foods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precontemplation</td>
<td>Not aware of the necessity of consuming daily requirements of calcium</td>
</tr>
<tr>
<td>Contemplation</td>
<td>Thinking about consuming more dairy products to increase calcium intake, gets information on daily requirements of calcium and calcium-rich foods</td>
</tr>
<tr>
<td>Preparation</td>
<td>Purchases milk, cheese, yogurt, and calcium-rich vegetables</td>
</tr>
<tr>
<td>Action</td>
<td>Prepares food with milk, eats yogurt for breakfast, eats at least two servings of calcium-rich vegetables a day</td>
</tr>
<tr>
<td>Maintenance</td>
<td>Keeps a log of calcium-rich foods consumed over a period of 6 months</td>
</tr>
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</table>
Table 2: Demographics of Participants N=342

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Ethnicity/Race/</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>93.0</td>
</tr>
<tr>
<td>Asian</td>
<td>2.5</td>
</tr>
<tr>
<td>Latino</td>
<td>1.5</td>
</tr>
<tr>
<td>Other</td>
<td>3.0</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>30-39</td>
<td>10.0</td>
</tr>
<tr>
<td>40-49</td>
<td>41.1</td>
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<tr>
<td>50-59</td>
<td>41.3</td>
</tr>
<tr>
<td>60-64</td>
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</tr>
<tr>
<td>Marital Status</td>
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<tr>
<td>Married</td>
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</tr>
<tr>
<td>Divorced</td>
<td>13.0</td>
</tr>
<tr>
<td>Single</td>
<td>11.0</td>
</tr>
<tr>
<td>Widowed</td>
<td>3.0</td>
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</table>
### Table 3: Reported Nutritional Behavior Change N=326

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Action (making)</th>
<th>Preparation (planning)</th>
<th>Contemplation (thinking)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 or more servings of milk or other dairy products each day</td>
<td>60%</td>
<td>12%</td>
<td>5%</td>
</tr>
<tr>
<td>Calcium fortified products, such as orange juice each day</td>
<td>42%</td>
<td>20%</td>
<td>11%</td>
</tr>
<tr>
<td>Calcium supplement</td>
<td>39%</td>
<td>19%</td>
<td>10%</td>
</tr>
<tr>
<td>Calcium-rich vegetables</td>
<td>28%</td>
<td>29%</td>
<td>15%</td>
</tr>
<tr>
<td>Modified food preparation techniques to include calcium</td>
<td>25%</td>
<td>16%</td>
<td></td>
</tr>
<tr>
<td>Tofu to diet</td>
<td>6%</td>
<td>7%</td>
<td>13%</td>
</tr>
<tr>
<td>Soy milk to diet</td>
<td>11%</td>
<td>7%</td>
<td>9%</td>
</tr>
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</table>

### Table 4: Reported Physical Activity Changes

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Action (making)</th>
<th>Preparation (planning)</th>
<th>Contemplation (thinking)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yard work or gardening</td>
<td>20%</td>
<td>10%</td>
<td>4%</td>
</tr>
<tr>
<td>Walking</td>
<td>19%</td>
<td>29%</td>
<td>15%</td>
</tr>
<tr>
<td>Weight training</td>
<td>14%</td>
<td>27%</td>
<td>17%</td>
</tr>
<tr>
<td>Yoga</td>
<td>5%</td>
<td>16%</td>
<td>10%</td>
</tr>
</tbody>
</table>
Faculty Comments:

Ms. Gray’s faculty mentor, Lori Turner, made the following remarks about Ms. Gray’s work:

I have known Ms. Gray for 2 years, as she has been a student in our Health Science Program. I have been impressed with her academic skills and keen mind. Ms. Gray displays high intellectual ability, breadth and depth of knowledge, and outstanding presentation ability, both orally and in written form. Last year Ms. Gray received our Outstanding Undergraduate Student Award in Health Science.

Over the past year I have supervised Ms. Gray’s internship activities. She has proven to be a valuable assistant for the implementation of my Osteoporosis Outreach Program that served 392 women. She assisted with the implementation of a variety of classes as well as facilitated data collection of survey data and body composition measures. Ms. Gray was also involved in data entry, data analysis of the Stages of Change behavior intentions survey and other various surveys. She was responsible for the development of an impressive poster presentation for our state AHPERD conference last November. I have been impressed with her enthusiasm, creativity, and ability to work independently. In addition, Ms. Gray has demonstrated responsibility, initiative, reliability and dependability.

Ms. Gray is self-motivated and shows great promise as a health professional. While she is self-directed and able to work independently, she also works well with others. Her interpersonal skills are strong, and she demonstrates a high level of emotional maturity. She communicates well and has a pleasant disposition.

Another of her Health Science faculty members, Michael Young, had much to say about Ms. Gray’s abilities. He wrote:

Ms. Gray is an undergraduate student in our community health program. She is on-track to graduate in May 2002 with a GPA just short of 4.0. Last spring we named her our undergraduate major of the year. It was no contest. All faculty agreed that she was the clear choice for the award. In fact, we wanted to give her the award this year too, but thought it unfair to other students. This spring she will represent the University of Arkansas in San Diego as our AAHE undergraduate major of the year.

Ms. Gray has been the recipient of several scholarships, including a scholarship from our state AHPERD organization and the Brandon Burlsworth Scholarship. These are both noteworthy because of the competitive nature of the scholarships. The AHPERD scholarship places emphasis on academic achievement and character. This is indeed a young woman who makes good grades, but there is far more to Amy Gray than a great GPA. Ms. Gray has helped provide her own financial support for herself while pursuing her education. She worked as a customer service clerk in the HPER service center, and did so well she was moved to the position of building supervisor. The building is the recreation center for the campus, with basketball courts, Olympic swimming pool, racquetball courts, and a fitness center. There are also classrooms, faculty offices, and much equipment. During the shifts for which Ms. Gray is responsible, she supervises at least seven employees, and addresses any problems that arise with the facility or with “customers” (students, faculty, dependents, and guests) using the facility.

Ms. Gray has also been involved in professional development service activities. She has worked extensively with one of the most productive young faculty members in the country, Lori Turner. Dr. Turner is currently leading a project designed to reduce risk of osteoporosis. This involves several components including bone scans and a series of educational classes. Ms. Gray has played an important role in the project. She has assisted in facilitating some of the classes and has been actively involved in data collection and data entry. She is the co-author in a recent presentation from the project and co-author on a paper. She also was responsible for a poster presentation at this year’s state AAHPERD conference. In addition, she has served as the Chair of the University Health Center’s Advisory Board. This is the type of involvement that we like to see, but do not often receive, from our graduate students. In my over 20 years at the University of Arkansas, I have seen few, if any, undergraduate students who have evidenced Ms. Gray’s degree of commitment and professional involvement. In addition to her professional involvement, she has found time to volunteer in other areas on campus and in the community. On campus, she has been involved with the Office of Student Involvement and Leadership and has worked with new student orientation. In the community, she has been a volunteer for Habitat for Humanity.

Ms. Gray’s submission to Inquiry is based on important work relative to osteoporosis prevention, which she has undertaken with Dr. Lori Turner. Dr. Turner’s work in this area has received more publicity than any other recent research conducted at the University of Arkansas. Ms. Gray has played a significant role in this overall work that Dr. Turner is leading. Ms. Gray’s manuscript deals with an aspect of the project, “stages of change” in which she has taken a leading role. The stages of change model is an important model to explain health behavior and readiness to adopt a change in behavior. The model has been applied to numerous health concerns, but there is relatively little in the literature related to behavior change specific to osteoporosis prevention. Thus, her work is significant, not only because it is research conducted by an undergraduate student, it is research that can make a contribution to the professional literature.
GLYCOGEN RESYNTHESIS AND RECOVERY FROM EXERCISE: EFFECTS OF VERY-LONG-CHAIN ACYL-COENZYME, A DEHYDROGENASE DEFICIENCY

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Human Performance Laboratory
Department of Health Science, Kinesiology, Recreation and Dance

Abstract:
Disorders of fatty acid metabolism pose a variety of problems including hypoglycemia, muscle weakness, muscle cramping, rhabdomyolysis, and, in the most severe cases, death. Individuals with a deficiency of very-long-chain acyl-CoA dehydrogenase (VLCAD), the enzyme initiating the oxidation of very-long-chain fatty acids, are particularly prone to exercised-induced declines in muscle function because of the importance of very-long-chain fatty acids to energy metabolism in exercise. The purpose of this project was to study muscle glycogen resynthesis in the recovery from high intensity exercise in VLCAD deficient mice. Recovery of skeletal muscle function (gastrocnemius) following exercise is not as rapid in VLCAD deficient mice as recovery in non-deficient mice. Based upon previous reports, recovery of muscle from exercise is, at least in part, related to glycogen resynthesis. It was hypothesized that muscle glycogen levels as well as liver glycogen levels would be significantly depressed 24 hours after exercise in VLCAD deficient exercised mice compared to non-exercised VLCAD deficient and exercised non-exercised VLCAD deficient mice. Twenty VLCAD deficient mice (knockout mice) and 20 non-deficient 129 Sv/C57BL6 mice were used in this project. Ten from each group exercised on a motor-driven treadmill to exhaustion using high intensity interval exercise while an additional 10 animals from each group served as non-exercised controls. The exercised mice were allowed to recover for 24 hours and then anesthetized using sodium pentobarbital (80 mg·Kg⁻¹). The right and left gastrocnemius muscles, the vastus lateralis muscle, the heart, and the liver were removed and frozen and the glycogen content subsequently determined. The resynthesis of glycogen was only significantly (p < 0.05) effected in the vastus lateralis muscle and the liver. Resynthesis was significantly reduced in the vastus muscle of VLCAD deficient mice and enhanced in the vastus muscle of non-deficient mice. It was concluded that VLCAD deficiency does delay recovery of muscle glycogen after exercise and that the delay may be affected by muscle fiber type or patterns of recruitment.

Introduction:
Glycogen utilization, both oxidatively and non-oxidatively begins after the first 30 seconds of exercise. For the first 10 to 15 minutes of prolonged exercise, muscle glycogen is the primary, but not exclusive-energy source. As exercise proceeds, fatty acids become energetically more important and muscle glycogen and blood glucose less important to total energy production. In an animal with an inability to utilize very-long-chain fatty acids, muscle glycogen and blood glucose likely remain primary energy sources. Indeed, it is not uncommon to observe muscle and liver glycogen depletion after both short-term high intensity and prolonged exercise even in animals capable of using fatty acids.

Muscle glycogen depletion parallels the perception of fatigue and significant stores of muscle glycogen are needed for optimal performance (1). At the conclusion of exercise, muscle, heart and liver glycogen stores must be resynthesized. The depletion of muscle glycogen provides a strong drive for its own resynthesis and skeletal muscle glycogen resynthesis takes precedence metabolically over liver glycogen resynthesis. Glycogen resynthesis is assumed to be under negative feedback control (9). When muscle glycogen stores are depleted, there is an increase in the rate of blood glucose transport into the muscle and an increased capacity to dispose of glucose by converting it to glycogen (3). An inverse relationship exists between serum free fatty acid (FFA) concentration and muscle glycogen content. A low glycogen content stimulates glycogen synthase activity, and high levels of FFA regulate the amount of glycogen in the muscle when glycogen stores are filling (7).

Very-long-chain fatty acids, particularly palmitic acid, are a primary energy source for skeletal muscle and cardiac tissue especially during fasting and prolonged exercise (2). They are metabolized in the mitochondria through a process requiring activation of acyl coenzyme A (acyl-CoA) esters followed by transport across the mitochondrial membrane. The oxidation of activated fatty acids is carried out through the sequential activities of the enzymes of b-oxidation, the Krebs cycle, and the respiratory chain to generate ATP. The first step in b oxidation is catalyzed by long-chain acyl-CoA dehydrogenase (LACAD), the enzyme initiating the oxidation of very-long-chain fatty acids.
by one of four chain length specific acyl-CoA dehydrogenase enzymes. For very-long-chain fatty acyl-CoA, the first step is catalyzed by very-long-chain acyl-CoA dehydrogenase (VLCAD) (4). A genetically linked deficiency or absence of this enzyme is associated with exercise intolerance characterized by cardiac and skeletal muscle myopathy, exercise-induced muscle pain, muscle weakness, hypoglycemia, myoglobinuria, fatigue, and recurrent episodes of rhabdomyolysis (4-6; 8). Although little is known about the responses of enzyme deficient humans or mice, it is likely that the inability to use very-long-chain fatty acids results in the increased utilization of carbohydrate to supply the energy demands of exercise.

Previous observations (unpublished) in our laboratory have determined that mice with this deficiency do not completely recover muscle function 24 hours following high intensity exercise, while non-deficient mice fully recover. These observations sparked interest in studying the muscle, liver, and cardiac glycogen content of the deficient and non-deficient mice twenty-four hours post-exercise. We hypothesized that the VLCAD deficient mice would not resynthesize glycogen following high intensity exercise as completely as non-VLCAD deficient mice.

Methods and Procedures:

Animals. Twenty-one VLCAD deficient mice (knockout mice) and 20 non-deficient 129 Sv/C57BL6 mice obtained from the Jackson Laboratories weighing between 10 - 25 g were housed 5 to 10 per cage in a room on a 12-hour light/dark cycle. All mice were fed solid food pellets and water *ad libitum*. All experiments were approved by the University of Arkansas Institutional Animal Care and Use Committee.

Procedures. Ten mice from each group were exercised on a motor driven treadmill to exhaustion using high intensity interval exercise while the additional animals from each group served as non-exercised controls. The groups were designated as VLCAD deficient, exercised (DE); VLCAD deficient, non-exercised (DNE); non-deficient, exercised (NDE); and non-deficient, non-exercised (NDNE). Each exercising mouse then ran at an initial speed of 16 m/min and 0% grade. The speed and grade were then increased at 6-min intervals to 24 m/min and 0% grade, 30 m/min at 2% grade, 35.5 m/min at 4% grade, 41 m/min at 6% grade, and 47 m/min at 8% grade. Exercise was terminated when the mouse could no longer keep up with the treadmill and when removed from the treadmill showed little movement. The mice were allowed to recover for 24 hours. At the end of the 24 hour recovery period, the mice were anesthetized using sodium pentobarbital (80mg/kg). The gastrocnemius muscles, the vastus lateralis muscles, the heart, and the liver were removed and immediately frozen using liquid nitrogen.

Serum and Tissue Analyses. The glycogen content of each of the tissues was determined using the phenol-sulfuric acid procedure. At the time of the surgical procedures, a blood sample was taken and the serum analyzed using o-toluidine to determine glucose concentration.

Statistical Analysis. The results were analyzed using ANOVA of data in a 2x2 factorial design. The Tukey procedure was used for post-hoc comparisons when indicated by a significant Fratio. All comparisons were made at the 0.05 level of significance.

Results:

Descriptive Statistics. The body weight, run times to exhaustion, and serum glucose concentrations are shown in Table 1. The body weight of the mice in the NDE group was significantly (p < 0.05) greater than that of the mice in the other three groups. The non-deficient mice ran approximately six minutes longer than the VLCAD deficient mice. However, the difference was not statistically significant. It was anticipated that the enzyme deficient mice, particularly the exercised mice, would have significantly lower serum glucose concentrations. Despite the highly elevated glucose concentration of the NDE mice, the difference was not significant.

Tissue Analysis. No differences were found in glycogen concentration in either the gastrocnemius muscle (Figure 1) or the heart (Figure 2). The ANOVA for the vastus lateralis muscle indicated a significant exercise state and deficiency interaction. The post hoc analysis showed that exercise caused a significant (p < 0.05) reduction in glycogen in VLCAD deficient mice but a significant (p < 0.05) increase in the non-deficient mice (Figure 3). In the liver (Figure 4), the only significant (p < 0.05) effect was that of exercise. Exercise caused an increase in liver glycogen but, as seen in Figure 4, the difference was largely due to the effects of exercise in the non-deficient mice.

Discussion and Conclusion:

Interestingly, all glycogen levels except in the liver for DE mice were lower in concentration than DNE mice. However, the only statistically significant difference observed was in the vastus lateralis muscle. One reason for this may be the possibility that the vastus lateralis muscle is used more predominantly than the gastrocnemius muscle by mice while running. Another possibility is a fiber type effect on muscle glycogen recovery in the deficient animals. The vastus lateralis muscle has a distinct white component and a distinct red component. The gastrocnemius muscle on the other hand is a muscle of mixed fiber types. After exercise, one expects to see a supercompensation of muscle glycogen, but this was only seen in the NDE mice in the vastus lateralis muscle and the liver but not in the gastrocnemius. Therefore, the fact that the DE mice did not show supercompensation and did not even recover to the DNE resting levels of glycogen becomes striking and perhaps very important for future experiments.
Both DE and NDE mice showed an exercise effect on their liver glycogen levels, having a higher concentration than their non-exercised counterparts; however, the deficient mice did not show as large an exercise effect as the non-deficient mice. This is perhaps due to the prolonged recovery period of the vastus lateralis muscle of the DE. It is interesting that the glycogen concentrations of the mice in this group show an exercise effect since muscle glycogen resynthesis takes precedence metabolically. A possibility is that the vastus lateralis glycogen levels were far more depressed following exercise than the other tissues and therefore needed more time to recover and delayed the recovery of liver glycogen. This certainly is an area for further study specifically investigating the glycogen levels of DE mice 48 hours after exercise.

Since there was no significant difference in serum glucose levels between DE and NDE mice, one might expect complete recovery of glycogen in all tissues in both groups. However, this did not occur, indicating that glucose is not the only rate-limiting factor for glycogen resynthesis in the enzyme deficient mice. The serum glucose values in this study, however, must be viewed with some caution. The values were much higher for all groups in this study than have been observed in previous studies in our laboratory (unpublished).

In conclusion, VLCAD deficiency does delay exercise recovery in some tissues, particularly the vastus lateralis muscle, as evidenced by the decreased rate of glycogen resynthesis. Whether the reduced rate of resynthesis translates into a functional difference in the muscle is not clear. We have previously observed that the functional capacity of the gastrocnemius muscle is depressed 24 hours after this same exercise bout. Clearly, in that muscle it is unlikely that delayed glycogen resynthesis is the reason for the depressed function since this investigation confirms that glycogen resynthesis in the gastrocnemius muscle is not delayed by the VLCAD deficiency. Further research needs to be explored to determine the effect of muscle fiber type on both the resynthesis of muscle glycogen post-exercise and muscle function post-exercise. Similarly, blood glucose utilization in VLCAD deficient mice needs further study.

References:

Table 1. Descriptive characteristics of VLCAD deficient and non-deficient mice.
Exercise consisted of high intensity intervals on a motor driven treadmill.

<table>
<thead>
<tr>
<th></th>
<th>Non-deficient</th>
<th></th>
<th>VLCAD deficient</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exercised</td>
<td>Not exercised</td>
<td>Exercised</td>
<td>Not exercised</td>
</tr>
<tr>
<td>Body Weight (g)</td>
<td>18.5 ± 1.6</td>
<td>14.8 ± 0.7</td>
<td>12.7 ± 0.3</td>
<td>13.2 ± 0.5</td>
</tr>
<tr>
<td>Run Time (min)</td>
<td>23.5 ± 2.7</td>
<td>17.7 ± 1.5</td>
<td></td>
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<tr>
<td>Glucose (mg%)</td>
<td>324 ± 36</td>
<td>228 ± 31</td>
<td>240 ± 9</td>
<td>241 ± 26</td>
</tr>
</tbody>
</table>

^ Significantly (p < 0.05) from all other groups.
Figure 1. Glycogen concentration of the gastrocnemius muscle twenty-four hours following high intensity exercise to exhaustion in VLCAD deficient and non-deficient mice.

Figure 2. Cardiac glycogen content twenty-four hours after exercise in VLCAD deficient and non-deficient mice.

Figure 3. Vastus lateralis muscle glycogen from VLCAD deficient and non-deficient mice twenty-four hours after high intensity exercise.

A Significantly (p < 0.05) different from the non-exercised VLCAD deficient mice.
B Significantly (p < 0.05) different from the non-exercised, non-deficient mice.
C Significantly (p < 0.05) different from the non-exercised VLCAD deficient mice.

Figure 4. Liver glycogen twenty-fours after high intensity exercise in VLCAD deficient and non-deficient mice.
Faculty Comments:

Mr. Johnson’s faculty mentor, Charles Riggs, has many positive remarks about Mr. Johnson’s research. He writes:

I am pleased to have had the opportunity to work closely with Mr. Kyle Johnson over the past two and one half years both in the classroom and in the laboratory. During that time, I have had numerous opportunities to evaluate his academic performance and his professional promise. Needless to say, I have been very impressed with his capabilities and talents and, particularly, his willingness to work. That willingness is evidenced by his voluntary participation in research in my laboratory. The data utilized in the paper published here is a part of a larger project working with me and other students examining the consequences and potential benefits of exercise for people with genetically linked metabolic disorders. His project has focused on muscle glycogen resynthesis after exercise, a factor important in recovery from exercise and possibly important in preventing some of the negative effects of exercise in enzyme deficient individuals.

Mr. Johnson is clearly one of the top two or three undergraduate students I have had the opportunity to work with during my 24 years in higher education. He is highly motivated, self-disciplined, and very intelligent. He approaches his work with enthusiasm and perseverance and continually strives for excellence. Mr. Johnson has excelled in a rigorous course of study despite a busy extracurricular schedule. He has been a University cheerleader for the past three and one half years. He has been involved in several campus organizations and community charities. Even with all of these responsibilities, Mr. Johnson still volunteered to participate in my research. He is involved in data collection and analysis, but more importantly, he serves as a mentor to other graduate and undergraduate students participating in our research. I have been highly impressed by Kyle Johnson. He sets high goals and is very focused in his pursuit of those goals. His has been a quality project that provides important clues to the mystery of the muscular responses of enzyme deficient individuals to exercise while at the same time provoking several additional research questions.

Two other of Mr. Johnson’s professors are equally enthusiastic about his work. Inza Fort writes:

I have had the privilege of teaching Mr. Johnson in one of my classes, KINS 3353, Mechanics of Human Movement, which is a detailed analysis of osteology, arthrology, and musculature as it pertains to movement and skill analysis. Out of a class of 30 students, Mr. Johnson achieved the highest point total. He is one of those students you love to have in class—attentive, questioning, analytical. Mr. Johnson has maintained a 3.94 GPA in Exercise Science. He became interested in research after taking Exercise Physiology and has spent many hours in the Human Performance Lab learning detailed research techniques under the mentorship of Dr. Charles Riggs. Assay analysis consumes many hours, and Mr. Johnson has dedicated himself to being thorough in his procedures. His protocol involved surgical procedures in mice so that muscle glycogen content could be analyzed after intense exercise. These procedures involved a high degree of precision in data collection. This project is important research with application for individuals with metabolism disorders, and the implications may include alterations in conventional thought about efficient exercise for those with enzyme deficiencies and metabolic disorders.

And Barry Brown says:

I have known Mr. Johnson for approximately 2 years, both as a student in two upper-level, science-based classes and as an undergraduate student involved in sophisticated research. Mr. Johnson has been working with Dr. Charles Riggs on a research project to determine the alteration in glycogen resynthesis in mice who demonstrate a deficiency in a very-long-chain fatty acid coenzyme (VLCAD). This is significant in that individuals deficient in this particular coenzyme A dehydrogenase may exhibit a reduction in muscle function especially at a young age. He has demonstrated a particular interest and affinity for this research methodology and has been a key player in the analytical process. The purpose of Mr. Johnson’s research is to determine the role of glycogen resynthesis in muscle during the recovery from intense exercise in mice deficient in VLCAD. Little research has appeared in this area in our professional literature. With the knowledge obtained through Mr. Johnson’s project, we may be able to explain hypoglycemic responses, muscle weakness, cramping, rhabdomyolysis and even death in individuals who suffer from this deficiency.
PROMOTING FOREIGN DIRECT INVESTMENT IN BULGARIA

Vessela Kapoulian
Sam M. Walton College of Business
Faculty Mentor: Dr. Wayne Y. Lee
Alice L. Walton Chair in Finance

Abstract:

This paper examines the factors that favor Foreign Direct Investment (FDI) in Bulgaria and recommends public policy changes that will enhance the international competitiveness of Bulgaria.

Four factors that favor an increase in FDI in Bulgaria are clearly identified. First, although the economic environment is considered challenging compared to other countries in the region, Bulgaria enjoys political stability. Second, Bulgaria offers a low-cost, well-educated labor pool, although additional public investments in foreign language training may be needed. Third, there are investment opportunities for infrastructure development through privatization in the areas of energy, information technology (IT), and transportation. Bulgaria is searching for significant partners in the renovation of the nuclear power plant in Kozlodui, IT improvements, and Build Operate Transfer (BOT) projects for the reconstruction of major highways and transportation hubs. Last but not least, Bulgaria is geographically well situated and has the potential to be an attractive tourist destination.

I. Introduction:

FDI into Bulgaria has increased considerably – from $34.4 million in 1992 to $1.1 billion in 2000 (Table 1) and comes predominantly from European Union (EU) countries, Russia, and the United States (www.bfia.org). Moreover, the percentage of FDI in the form of investment inflows (Greenfield investments, reinvestments, joint ventures, additional investments in companies with foreign participation) versus privatization is increasing, with the industrial, finance, and trade sectors attracting considerably more FDI than agriculture, construction, telecommunications, tourism, and transportation.

Nonetheless, FDI in Bulgaria is relatively low compared to other countries in Eastern Europe. In a Multilateral Investment Guarantee Agency (MIGA) survey published in January 2002, Bulgaria ranks 10th among Eastern European countries in FDI in the manufacturing and service sectors. In addition, it ranks last among Eastern European countries preferred for FDI by firms from Western Europe, North America, and the Asia/Pacific Rim.

The low FDI investment is not surprising. On one hand, Bulgaria has a stable political and legal environment, a low-cost highly skilled labor force, and strategic geographic location — factors that are important to foreign investors. On the other hand, access to consumers, a well-developed transportation, energy, and IT infrastructure, as well as a dynamic market economy are even more critical factors in the location decisions of foreign investors. With a population of approximately 8 million, Bulgaria does not represent a large consumer market. The transportation network is in need of substantial renovation. The possibility of closing the country’s nuclear power plant creates uncertainty about the availability of energy. And even though Bulgaria has made significant progress in its economic development, it is still considered weak compared to other countries in the region.

II. Political and Legal Climate:

Located on the Balkan Peninsula, Bulgaria is in a region that is well known for intense political and ethnic conflicts. Proximity notwithstanding, Bulgaria has not only managed to maintain its independence and avoid entanglement in these conflicts over the years but has contributed to their early resolution — for example, the recent Kosovo and Serbia crisis. In 1994 Bulgaria joined the NATO Partnership for Peace and is actively pursuing membership in NATO and the European Union (EU).

Bulgaria is a Parliamentary Republic and conforms to the Constitution of the Republic passed by the National Assembly in 1991. The Constitution provides for a separation of powers in the executive (14 Ministries), legislative (Parliament), and judicial (Supreme Courts) branches with a system of checks and balances. The President is the Head of State, and is elected once every 5 years for no more than two terms. He represents the Republic of Bulgaria in its international relations and embodies the unity of the country.
In addition, Bulgaria has well-defined foreign investment and trade laws. Foreign investment legislation covers legal and international guarantees for foreign investors, protection against expropriation, and provides for profit and capital repatriation. Two government entities oversee the FDI process. The Bulgarian Foreign Investment Agency (BFIA) coordinates the activities of state institutions in the field of foreign investment. The Advisory Council on Foreign Investment and Financing, comprised of representatives from the largest foreign investors, promulgates and adopts measures for improving the FDI climate in Bulgaria.

III. Economic Climate:

Bulgaria progressed in its economic development since the crisis of 1996-1997. Inflation decreased to a single digit rate of 4.8% in 2001 (Figure 1). Instead of shrinking, GDP grew to a positive 4.7% as of third quarter of 2001 (Figure 2). And in December 1999, Bulgaria was invited to start negotiations for accession to the European Union – an acknowledgment of the positive economic and political changes taking place.

The economic stabilization was made possible with the assistance of international financial institutions like the IMF and the World Bank, but more importantly, by the creation of a Currency Board. The Board supervises the Bulgarian National Bank (BNB), is responsible for determining the bank’s aggregate amount of monetary liabilities, and ensures that the bank extends no loans to the Government or any Government agency. The financial discipline that the Board imposes on the Bulgarian Government encourages improvements in tax collection and restrains fiscal spending.

With the establishment of the Board, the Bulgarian currency (Lev), initially pegged to the German Mark (DEM), is now pegged to the Euro. Constraints on currency trading are removed. The long-term effects on the Bulgarian economy of fixing the Lev to the Euro are still unknown. However, the World Bank representative for Bulgaria, Thomas O’Brien, notes that the Euro will help bring about price transparency and lower transaction costs as well as put pressure on local producers to improve productivity levels to keep prices competitive.

A survey of 36 foreign businesses in Bulgaria indicates that only two out of five invested with the purpose of exporting their products. Three out of five companies serve the domestic market. This is puzzling because the size of the Bulgarian market is small and easily saturated. Foreign companies in Bulgaria should really direct their focus toward exports.

Bulgaria’s strategic geographic location, participation in free trade agreements, established partnerships and cultural similarities with Eastern European countries provide access to a contiguous market area with over 600 million consumers (www.bfia.org). Since 1998, exports of industrial goods with Bulgarian origin into the EU are duty-free. Bulgaria is a member of the World Trade Organization (WTO) and a signatory to the Central European Free Trade Agreement (CEFTA). Bulgaria also has trade agreements with Macedonia, Turkey, and the European Free Trade Association (EFTA). As a former Soviet Bloc member country, it still maintains strong economic relations with Russia and other Eastern European countries (Dobosiewicz, 64).

IV. Human Capital:

In a recent book, Michael Porter argues that a major determinant of a country’s competitive advantage is the availability of favorable factor conditions (Porter 71). Bulgaria is not rich in natural resources and raw materials. Its competitive advantage lies in a low-cost (Table 2) and well-educated labor force.

The average salary in Bulgaria of $100-150 per month translates to a wage rate between $0.63 and $0.93 per hour based on a 40-hour workweek. The workforce of approximately 4.5 million has a 98 percent literacy rate with 63.1 percent of the workforce completing secondary, technical, or vocational education. 26.1 percent of Bulgarians choose to pursue higher education in engineering, economics, medicine, and science at one of the many colleges and universities in the country (Table 3). Furthermore, many of the European languages (English, German, French, Spanish, Italian) are taught from an early age, and there are specialized language high schools throughout the country that emphasize foreign-language education.

Foreign investors do face some challenges. First, many college graduates come directly out of the universities with little or no experience. Middle and senior management lack Western experience. Second, partial or fully foreign-owned companies are not exempt from the requirements of the Bulgarian Labor Code that governs employer-employee contracts and provides excessive protections for workers. Trade unions exert important influence on employees. Working closely with the union is critical to labor productivity. Last, but not least, foreign investors may have to contend with bribery practices. Although government employees are generally paid more than their private sector counterparts, approximately $150 versus $135, respectively, these wages are low. Adding to this, the lack of promotion opportunities in the government sector creates conditions for corruption. To limit corruption, the Bulgarian government has adopted a national anti-corruption strategy that includes a reform of the administrative system and several newly adopted laws and regulations. The improved rating of Bulgaria in the comparative index of Transparency International is evidence that corrupt practices are under control. In 1998, Bulgaria was ranked 66th (out of 99), and in 2001, ranked 47th, approaching the status of other Central European countries.

The preceding discussion suggests that the following government actions will enhance the labor market conditions in Bulgaria – increased investment in foreign language training.
changes in the hiring process, and implementation of foreign exchange and government-sponsored educational and training programs.

Even though Bulgarians are required to study a foreign language in school, not all choose to exploit this opportunity to their advantage. A significant number of university graduates and managers do not speak a foreign language. To make Bulgarian cadres more attractive to foreign investors, the government needs to ensure better implementation of current foreign language training initiatives. It can call for foreign language exams upon high-school graduation, foreign language courses and graduation exams in college, and foreign language requirements for managers. People with foreign language(s) skills should have priority when promotion opportunities arise.

As an incentive for Bulgarians to study a foreign language, the government can also create more foreign exchange programs with high schools and universities abroad. Exchange programs will make Bulgarians more accustomed to Western lifestyle and corporate culture. Government scholarships for higher education abroad are another way to make Bulgarians highly competitive. Bulgarians awarded these scholarships will bring back the knowledge and Western corporate culture with them. This will facilitate the restructuring process for foreign investors interested in acquiring Bulgarian companies.

V. Infrastructure and Information Technology Development:

About 52.5% of the assets of state-owned enterprises have been privatized, but numerous investment opportunities through privatization still exist. This paper focuses on opportunities in three sectors—energy, information technology, and transportation.

A. Energy:

Bulgaria’s power generation comes from nuclear, coal-fired, and hydroelectric power stations. The only important domestic energy resource is low quality, high sulfur content, lignite coal. Bulgaria relies on fuels (oil, natural gas, good quality coal and nuclear fuel) imported mainly from Russia. The country imports more than 70% of its primary energy resources compared to 40% for EU countries.

A primary source of power in Bulgaria is the nuclear power plant in Kozlodui (over 40%); the other is the coal-fired plant Maritza-East (30%). In 1993, the European Bank for Reconstruction and Development (EBRD) donated 24 million euro to support the decommissioning of the two oldest nuclear reactors in Kozlodui that were subsequently closed in 1999. The EU postponed its decision on the closure of two other reactors until 2006. Bulgaria’s admission to the EU is, however, contingent upon the closing of these reactors. Renovation of these two reactors that currently remain in operation is yet to be addressed.

Two other factors make changes in the energy sector urgent—the high cost (approximately $0.05/kWh) and consumption of electrical power. The total energy consumption of an average Bulgarian household is three times higher than that of other countries in the region because alternative sources for heating are lacking (there is no household gas system in place), and the industrial equipment used is old.

All three facts suggest the following recommendations—finding a foreign partner for the total renovation of the nuclear power plant and developing alternative sources of energy.

The Bulgarian government has limited financial ability to renovate its nuclear plant and relies heavily on foreign assistance. In 2000 Citibank provided a $77 million loan to help Bulgaria partially modernize the other two reactors. In addition to seeking more financial aid, the government needs to provide a legal and regulatory framework that will facilitate the flow of foreign capital into the energy sector.

The development of alternative sources of energy is another option the government needs to explore. Bulgaria has a well-developed network for electrical power transfer inside the country and to neighboring countries. There are hydroelectric and coal-fired power plants in Bulgaria. Currently 22 of the 77 hydroelectric power plants are open for privatization. The participation of US-based corporations Entergy and AES in the modernization of the Maritza-East coal-fired power plant is an example of profitable opportunities in the energy sector that foreign investors could explore.

Apart from the thermo-, hydro-, and nuclear power plants, Bulgaria has gas transfer and gas-distribution network developed for industrial consumers. Bulgaria is located at a key crossroad in Europe—the transit gas pipelines from Russia to the South pass through Bulgaria. These pipelines connect Greece and Turkey to the European network. According to the Bulgarian Department of Energy, if future gas pipelines are built from Central Asia to Bulgaria and onward to Central Europe, the country may become an alternative East-West corridor. This will diversify the dependence of Western Europe on imported natural gas. Thus foreign investors in the energy sector can benefit from Bulgaria’s favorable geographic location.

B. Technology and Telecommunications:

The reliability and quality of IT is another important FDI location factor. The IT sector in Bulgaria is still in its infancy. But to develop a strong IT sector and make it attractive to foreign investors, the government needs to initiate changes in education, regulation, law enforcement, and business incentives.

If Bulgaria is to have a well-developed IT industry, the government needs to invest in technology education to ensure the development of IT specialists. The current computer classes offered during the senior year in high schools are not sufficient.
It classes have to be available earlier and offered more frequently. For that purpose, the government needs to sponsor or find sponsors to provide computer labs in the high schools and universities. Offering higher salaries and/or university scholarships for IT professionals and specialists in the fields of mathematics and sciences is another way to encourage the development of young IT specialists.

Bulgaria has a well-defined copyright and intellectual property law. Intellectual property legislation has been amended and modernized. In April 1999, the U.S. Trade Representative removed Bulgaria from the Special 301 Watch List. However, the reality is different. Even though copyright violation was reduced from 90% to 76%, the government still needs to ensure the proper enforcement of existing copyright and intellectual property laws. Imposing larger fees and monetary punishment for violators is a possible solution. High software and technology acquisition costs contribute to the problem too. With low monthly incomes, the average Bulgarian cannot afford to pay a high price. The Bulgarian International Business Association (BIBA) suggests accelerated depreciation of technology and technical equipment that will reduce the costs of investment in new technologies, enable businesses to charge lower prices, and mitigate the incentives to violate copyright. The Bulgarian government has already taken actions in this direction. Starting on January 1, 2003, it plans to exempt the import of high tech equipment from duties, taxes, and charges.11

C. Transportation:

Bulgaria’s strategic geographic location presents many opportunities for foreign investors in the transportation sector. A network of highways crosses the country and offers connections to Central and Western Europe, Russia, Asia, the Adriatic, the Aegean and Black Seas. The Pan European Transport Corridors crossing Bulgaria are Corridor IV, VII, VIII, IX, and X. With its geographic location and transportation network Bulgaria has the potential to become a transportation hub. Bulgaria’s road infrastructure is reasonably well developed but not well maintained due to insufficient funding. However, with the financial support of the European Investment Bank (EIB) and the EBRD, essential maintenance was carried out on Corridors IV, VIII, and IX. In addition, there are development projects under the Stability Pact of South-Eastern Europe that include the construction of a road and a rail bridge over the Danube River, the renovation of ports along the Danube River, and the construction of a new terminal and a runway at Sofia Airport.9

Road reconstruction grants and loans mostly cover the main road networks. However, these roads represent only 10% of the country’s road network.10 Second- and third-grade roads comprise the rest and are in poor condition. This fact suggests that the Bulgarian government should find a significant partner for road reconstruction and offer Build Operate Transfer (BOT) projects. BOT projects entitle a company that invests in road renovation to participate in the revenues generated from payments by road users. Examples of successfully introduced user-pay approaches are the highways in Serbia and Macedonia. They are in a much better condition than those in Bulgaria because they collect toll fees. According to BIBA, a well-developed toll road policy for Bulgaria could contribute $25 million — $200 million to government coffers.

The added revenue from road transport operations can also help the government increase railway subsidies to an acceptable level. Bulgarian State Railways are among the least subsidised railways in Europe and in the region.11 Under the program for infrastructure development 2001-2005 the Ministry of Transport and Communications plans to offer railway passenger and freight services for privatization.9 Even though the state retains infrastructure ownership, foreign investors that participate in the railway reconstruction effort can still benefit from the increase in railway traffic.

Apart from its road and railway networks, Bulgaria has two major ports — Bourgas and Varna. Both have direct connections with road and railway networks. The ports, which are being renovated, will help increase cargo capacity and provide opportunities for oil transfer from the Central Asian Republics to Europe.

Bourgas is also one of the six duty-free zones in Bulgaria. The other zones are Plovdiv, Viden (close to Romania), Rousse (close to Romania), Dragoman (close to Serbia), and Svilengrad (close to Turkey). These duty-free zones in conjunction with Bulgaria’s roads, railway network, and ports present the opportunity for turning Bulgaria into a transportation and trade hub of the Balkans and South-Eastern Europe.

VI. Geographic Location - Tourism:

The favorable geographic location and mild climate also favor the development of tourism in Bulgaria. Bulgaria is situated in the southeastern part of the Balkan Peninsula. The country has a coastline that offers recreation at the beach and surfing. The winter is cold, with significant snowfalls — a favorable condition for the development of ski resorts in Bulgaria. The mountains in Bulgaria occupy about 50% of the country.12 This factor favors hiking and mountain tourism. In addition, Bulgaria has about 500 deposits of mineral waters with over 1,600 water sources — a natural resource that allows for the development of spa resorts.12 The low value of the Bulgarian Lev compared to other currencies makes it generally less expensive for foreigners to spend time in Bulgaria.

The government must renovate many of the existing hotels in the country, however, to take advantage of these favorable conditions. Hotels and resort facilities need to be constructed according to European standards. For example, most of the old hotels are not air-conditioned. Partnering with the government on the hotel renovation could be a profitable opportunity for a
foreign investor. For example two of the biggest resorts in the country — Golden Sands and Pamporovo AD — have been privatized. In addition, the EBRD has provided loans varying from $100,000 to $1 million for the medium-sized and small tourist enterprises. This fact along with a 17.06% increase in tourists in 2001 (Table 4) suggests that there are still opportunities to explore.

For the successful exploitation of opportunities in tourism, the government needs to take several other action steps. First, it needs to design an effective marketing and advertising plan to promote Bulgaria as a tourist location internationally. Second, as noted previously, foreign language training is critical. Given that the majority of tourists visiting Bulgaria are from Eastern Europe, Russia, Germany, and the United Kingdom (Table 4), Russian, German, and English should have priority over other foreign languages. Last but not least, it has to ensure a low crime rate and high-quality customer service.

VII. Conclusion:

To summarize, if the Bulgarian government wants to attract FDI, it has to enhance Bulgaria's competitive advantage — its human capital, infrastructure and technology development opportunities, and strategic geographic location. In the area of human capital, the government needs to invest in and ensure a high quality of foreign language training. Foreign exchange programs, graduation exams, and a job and promotion selection process that rewards foreign language skills are ways by which these objectives may be accomplished. In the energy sector, finding a significant partner for the reconstruction of the Kozlodui power plant as well as exploring the alternative sources of energy (coal-fired and hydro-electric stations) available is critical. Developing IT professionals is important for starting a successful technology sector. The government needs to identify a sponsor to provide computer labs in schools and start IT education earlier. Subsidies, tax breaks, and elimination of import fees for companies involved in technology are other ways to encourage their development. BOT projects based on the user-pay approach for infrastructure construction are possible solutions for the transportation sector. Finally, Bulgaria's strategic location opens investment opportunities in the tourism sector. But to entice foreign investors, the government needs to ensure customer service and foreign language training, hotel renovation, and a low crime rate.

It is clear that the steps taken to promote FDI are interrelated. By improving the foreign language skills of the population the government will make Bulgaria's labor pool attractive to foreign investors. However, by doing so, it will also facilitate the communication process with tourists and make Bulgaria an attractive travel location. By refining the road and railway network, the government will not only help turn Bulgaria into a transportation hub, but will also facilitate tourist traveling. Adopting modern technologies and equipment, will bring improvements in the IT sector and will also result in less consumption of energy and energy resources.

Understanding that these interrelated factors affect FDI will help the Bulgarian government create strategies that make the country internationally competitive.

Endnotes:
1 www.miga.org.
2 www.nsi.bg.
4 www.bfia.org.
5 www.transparency.org
6 www.priv.government.bg
7 www.doe.bg.
8 www.nek.bg.
10 www.mtc.government.bg.

Books:

FDI Survey Questionnaire:
1. How did you hear about investment opportunities in Bulgaria?
2. Why did you choose Bulgaria?
3. What other countries did you consider investing in (apart from Bulgaria)?
4. Do you invest in Bulgaria with the purpose to export your products and services or do you invest in order to serve the Bulgarian market?
5. If so, what percentage of your products do you export and what percentage do you offer to the Bulgarian market?
Figures and Tables

Table 1: Foreign Direct Investment by Years

<table>
<thead>
<tr>
<th>Year</th>
<th>Privatization</th>
<th>Other</th>
<th>Cumulative Total</th>
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<td>1992</td>
<td>34.4</td>
<td>34.4</td>
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<td></td>
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<tr>
<td>1993</td>
<td>22.0</td>
<td>80.4</td>
<td>102.4</td>
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<tr>
<td>1994</td>
<td>134.2</td>
<td>76.7</td>
<td>210.9</td>
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<tr>
<td>1995</td>
<td>26.0</td>
<td>136.6</td>
<td>162.6</td>
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</tr>
<tr>
<td>1996</td>
<td>76.4</td>
<td>180.0</td>
<td>256.4</td>
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<tr>
<td>1997</td>
<td>421.4</td>
<td>214.8</td>
<td>636.2</td>
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<td>1998</td>
<td>155.8</td>
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<td>305.7</td>
<td>500.4</td>
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<td>2000</td>
<td>366.0</td>
<td>635.5</td>
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<td>Total</td>
<td>1,507.5</td>
<td>2,323.0</td>
<td>3,830.5</td>
<td>32,881</td>
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Source: www.bfia.org

Figure 1:

Source: www.bfia.org

Table 2: Labor Cost Per Worker 1995-1999

<table>
<thead>
<tr>
<th>Country</th>
<th>Annual Labor Cost (USD) per Worker in Manufacturing</th>
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<tbody>
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<td>Bulgaria</td>
<td>1,179</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>1,876</td>
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<td>Greece</td>
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<td>Hungary</td>
<td>2,777</td>
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<td>Romania</td>
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<td>Slovenia</td>
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<td>Turkey</td>
<td>7,958</td>
</tr>
<tr>
<td>Austria</td>
<td>28,342</td>
</tr>
<tr>
<td>Germany</td>
<td>33,226</td>
</tr>
<tr>
<td>Italy</td>
<td>34,859</td>
</tr>
<tr>
<td>Spain</td>
<td>19,329</td>
</tr>
<tr>
<td>UK</td>
<td>23,843</td>
</tr>
</tbody>
</table>

Source: www.worldbank.org

Figure 2:

Source: www.bfia.org

Table 3: Percentage of population in respective age category attending school

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Kindergarten</td>
<td>66.2</td>
<td>62.1</td>
<td>65.2</td>
<td>66.4</td>
</tr>
<tr>
<td>Elementary</td>
<td>95.5</td>
<td>96.0</td>
<td>96.8</td>
<td>96.4</td>
</tr>
<tr>
<td>Junior High School</td>
<td>78.4</td>
<td>79.1</td>
<td>80.2</td>
<td>81.4</td>
</tr>
<tr>
<td>High School</td>
<td>61.5</td>
<td>61.3</td>
<td>62.1</td>
<td>63.1</td>
</tr>
<tr>
<td>Associate Degree</td>
<td>+0.5</td>
<td>0.7</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>College</td>
<td>3.0</td>
<td>3.0</td>
<td>2.7</td>
<td>2.3</td>
</tr>
<tr>
<td>University</td>
<td>21.4</td>
<td>21.6</td>
<td>22.6</td>
<td>23.2</td>
</tr>
</tbody>
</table>

Source: www.msi.bg

Table 4: Arrivals with Purpose of Visit “Tourism” (January-December 2001)

<table>
<thead>
<tr>
<th>No.</th>
<th>Country</th>
<th>Tourists</th>
<th>% Change 2000-2001</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Macedonia</td>
<td>643,106</td>
<td>-2.32%</td>
</tr>
<tr>
<td>2</td>
<td>Germany</td>
<td>374,323</td>
<td>42.31%</td>
</tr>
<tr>
<td>3</td>
<td>Yugoslavia</td>
<td>359,467</td>
<td>64.59%</td>
</tr>
<tr>
<td>4</td>
<td>Greece</td>
<td>344,677</td>
<td>7.16%</td>
</tr>
<tr>
<td>5</td>
<td>Romania</td>
<td>227,286</td>
<td>11.43%</td>
</tr>
<tr>
<td>6</td>
<td>Russia</td>
<td>130,886</td>
<td>23.92%</td>
</tr>
<tr>
<td>7</td>
<td>Ukraine</td>
<td>70,168</td>
<td>18.37%</td>
</tr>
<tr>
<td>8</td>
<td>UK</td>
<td>69,202</td>
<td>33.15%</td>
</tr>
<tr>
<td>9</td>
<td>Israel</td>
<td>50,366</td>
<td>62.95%</td>
</tr>
<tr>
<td>10</td>
<td>Sweden</td>
<td>24,343</td>
<td>20.18%</td>
</tr>
<tr>
<td>11</td>
<td>Turkey</td>
<td>24,343</td>
<td>53.70%</td>
</tr>
<tr>
<td>12</td>
<td>Czech Rep.</td>
<td>26,760</td>
<td>27.57%</td>
</tr>
<tr>
<td>13</td>
<td>Poland</td>
<td>31,492</td>
<td>66.03%</td>
</tr>
<tr>
<td>14</td>
<td>Slovakia</td>
<td>30,999</td>
<td>65.43%</td>
</tr>
<tr>
<td>15</td>
<td>Finland</td>
<td>29,178</td>
<td>51.38%</td>
</tr>
<tr>
<td>16</td>
<td>France</td>
<td>27,105</td>
<td>27.50%</td>
</tr>
<tr>
<td>17</td>
<td>Austria</td>
<td>27,253</td>
<td>225.14%</td>
</tr>
<tr>
<td>18</td>
<td>USA</td>
<td>25,560</td>
<td>21.89%</td>
</tr>
<tr>
<td>19</td>
<td>Belgium</td>
<td>24,946</td>
<td>42.56%</td>
</tr>
<tr>
<td>20</td>
<td>Denmark</td>
<td>19,372</td>
<td>28.85%</td>
</tr>
<tr>
<td>21</td>
<td>Italy</td>
<td>17,313</td>
<td>3.46%</td>
</tr>
<tr>
<td>22</td>
<td>Netherlands</td>
<td>16,422</td>
<td>8.66%</td>
</tr>
<tr>
<td>23</td>
<td>Belarus</td>
<td>15,386</td>
<td>-1.55%</td>
</tr>
<tr>
<td>24</td>
<td>Norway</td>
<td>10,485</td>
<td>4.01%</td>
</tr>
<tr>
<td>25</td>
<td>Hungary</td>
<td>6,818</td>
<td>28.23%</td>
</tr>
<tr>
<td>26</td>
<td>Switzerland</td>
<td>6,147</td>
<td>43.96%</td>
</tr>
<tr>
<td>27</td>
<td>Georgia</td>
<td>5,774</td>
<td>-10.22%</td>
</tr>
</tbody>
</table>

Source: www.mi政府.bg
Figure 3: Foreign Direct Investment by Countries in USD m (1992-2000)

<table>
<thead>
<tr>
<th>Country</th>
<th>USD m</th>
</tr>
</thead>
<tbody>
<tr>
<td>GERMANY</td>
<td>998</td>
</tr>
<tr>
<td>BELGIUM</td>
<td>413</td>
</tr>
<tr>
<td>ITALY</td>
<td>374</td>
</tr>
<tr>
<td>GREECE</td>
<td>328</td>
</tr>
<tr>
<td>CYPRUS</td>
<td>238</td>
</tr>
<tr>
<td>USA</td>
<td>236</td>
</tr>
<tr>
<td>AUSTRIA</td>
<td>214</td>
</tr>
<tr>
<td>RUSSIA</td>
<td>205</td>
</tr>
<tr>
<td>NETHERLANDS</td>
<td>183</td>
</tr>
<tr>
<td>UK</td>
<td>181</td>
</tr>
<tr>
<td>TURKEY</td>
<td>125</td>
</tr>
<tr>
<td>FRANCE</td>
<td>112</td>
</tr>
<tr>
<td>SPAIN</td>
<td>110</td>
</tr>
<tr>
<td>SWITZERLAND</td>
<td>104</td>
</tr>
<tr>
<td>KOREA</td>
<td>57</td>
</tr>
<tr>
<td>BAHAMAS</td>
<td>47</td>
</tr>
<tr>
<td>LUXEMBURG</td>
<td>40</td>
</tr>
<tr>
<td>IRELAND</td>
<td>29</td>
</tr>
<tr>
<td>ISRAEL</td>
<td>18</td>
</tr>
<tr>
<td>HUNGARY</td>
<td>17</td>
</tr>
<tr>
<td>CZECH</td>
<td>10</td>
</tr>
<tr>
<td>MALTA</td>
<td>10</td>
</tr>
<tr>
<td>LIECHTENSTEIN</td>
<td>9</td>
</tr>
<tr>
<td>SWEDEN</td>
<td>7</td>
</tr>
<tr>
<td>JAPAN</td>
<td>6</td>
</tr>
<tr>
<td>DENMARK</td>
<td>5</td>
</tr>
</tbody>
</table>

Source: www.btia.org

Faculty comments:

Ms. Kapoulian’s faculty mentor, Wayne Lee, made the following comments about her work:

In her thesis, Ms. Kapoulian identifies why foreign direct investment (FDI) in Bulgaria lags behind its neighbors and what specific actions the government can undertake to attract more investments. The amount of material that had to be read and analyzed was extensive. But she tackled the difficult and challenging task extremely well. For someone completely uninformed about Bulgaria (as I was), the thesis provides a nice, concise introduction to the opportunities and challenges of investing in Bulgaria. Though we met at least once every week, Ms. Kapoulian is very capable of independent work. I really enjoyed the interaction. It was a learning experience for me as well.

John Norwood, Director of Honors Studies for Walton College, also had complimentary things to say about Ms. Kapoulian’s work:

Ms. Kapoulian’s work represents a thorough investigation of what is needed to increase the amount of foreign investment in her home country of Bulgaria. The work should be of interest to anyone interested in promoting the economic development of Bulgaria.
THE ROLE OF WORLD TRADE ORGANIZATIONS IN SETTLING TRADE DISPUTES BETWEEN THE UNITED STATES AND THE EUROPEAN UNION

Erin Walker  
Department of Economics  
Faculty Mentor: Gary D. Ferrier  
Department of Economics

Abstract:

Description of Topic: In settling trade disputes, members of the World Trade Organization use a dispute settlement mechanism set forth in the Uruguay Round of trade negotiations. This multilateral system of settling disputes is implemented if a member believes other members are violating trade rules. Disputes arise when countries adopt policies that break the WTO agreements or that cause them to fail to fulfill obligations. Dispute settlement procedures have existed under many different trade agreements. While current processes are more effective than those of past agreements, they still lack credibility and effectiveness.

Research and Results: The United States and the European Union have used the WTO dispute settlement processes in settling many trade disputes over the past decade. Currently, the United States and European Union are involved in several disputes, including the trade of meat treated with growth hormones, the use of the U.S. Foreign Sales Corporation tax exemption, and state subsidization of the steel industry. Recent resolution of the long-standing dispute over the European Union banana regime is a positive indicator of progress in trade relations between the United States and the European Union. These cases will be used to illustrate the point that current WTO recommendations are not the most authoritative means of settling international trade disputes and to suggest improvements, such as increased use of negotiation and arbitration, to the dispute settlement process. The mutually benefiting trade relationships among independent nations can be greatly enhanced by cooperation in and resolution of trade issues. Improvements to the dispute settlement system would facilitate the edification of the global economic environment.

Summary of Problem:

The World Trade Organization (WTO) offers to its members a settlement mechanism for use in trade disputes. However, this mechanism is not as effective or credible as it could be, as it allows countries to delay in implementing WTO rulings. This is because the rulings do not specify what governments must do to comply with the decisions ("Monkey Business"). The mechanism looses efficiency because the process of settling trade disputes is too lengthy.

The Uruguay Round of trade negotiations, which ended in 1994, set forth a multilateral system of settling disputes for members of the WTO to use if they believe other members are violating trade rules. Disputes arise when countries adopt policies that break the WTO agreements or that cause them to fail to fulfill obligations. This dispute resolution system is based on a laissez-faire attitude under which quarreling countries are encouraged to settle disputes themselves through consultations and mediation.

The steps involved in settling disputes under the Uruguay Round agreement have target time periods to be carried out. The total amount of time spent on a case is set, but within that confine, the timeline for each stage is flexible. The Dispute Settlement Body oversees the case by establishing panels to consider the case, monitoring enforcement of the rulings, and allowing retaliation when rulings are not followed. The first stage of the process is consultation. Countries involved in the dispute must communicate in an effort to settle the problem themselves. If they are not successful, or if they simply do not want to communicate with one another, they may ask for mediation from the WTO director general. In the second stage, which occurs if consultation fails, the protesting nation may ask for a panel to be appointed to assist the Dispute Settlement Body in making a judgment on the case. This panel receives each side’s case in writing, and then, a preliminary hearing is held. At this hearing, the disputing countries and any other country with a declared interest in the controversy present their cases to the panel. Next, the countries involved present written rebuttals and make oral arguments at the second meeting of the panel. Throughout this entire process, the panel may consult outside experts if necessary. In the first draft of its report, the panel gives the factual and argument sections to the parties, who have two weeks to comment. The panel then submits an interim report including findings and conclusions to the two sides, allowing one week for review. A
final report is given to the disputing countries; three weeks later, the report is sent to all WTO members. The panel may make suggestions as to how to correct a trade policy that does not comply with WTO rules. Within sixty Ms. Kapouli and days of the final report being distributed, it becomes the Dispute Settlement Body’s ruling unless it is rejected by a consensus.

Either side can appeal the panel’s ruling based on a point of law, but evidence cannot be reexamined or introduced. The Dispute Settlement Body sets up a permanent seven-member Appellate Body that represents a diverse range of WTO member countries. The Appellate Body may uphold, modify, or reverse the panel’s ruling. Following the ruling of the Appellate Body, the Dispute Settlement Body has 30 days to accept or reject the appeals report, with a consensus required for rejection.

<table>
<thead>
<tr>
<th>Stage of Settlement Process:</th>
<th>Time Alotted:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consultations</td>
<td>60 days</td>
</tr>
<tr>
<td>Panel sets up and panelists' appointments</td>
<td>45 days</td>
</tr>
<tr>
<td>Final report to the parties</td>
<td>6 months</td>
</tr>
<tr>
<td>Final panel reports to WTO members</td>
<td>3 weeks</td>
</tr>
<tr>
<td>Dispute Settlement Body adopts report (if no appeal)</td>
<td>60 days</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1 year (without appeal)</strong></td>
</tr>
<tr>
<td>Appellate Body report</td>
<td>60-90 days</td>
</tr>
<tr>
<td>Dispute Settlement Body adopts Appellate Body Report</td>
<td>30 days</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1 year 3 months (with appeal)</strong></td>
</tr>
</tbody>
</table>

After the case has been decided, rapid action should be taken by the losing country to bring its trade policies into step with WTO rules. The “defendant” country must follow the recommendations of the panel or appellate body. It must state its intention to do so at the final meeting of the Dispute Settlement Body, where it can also make a case for acting within a reasonable amount of time instead of immediately. However, if corrective action is not taken within that time period, it has 20 days to negotiate with the other country and come up with a plan for compensation. If no agreement is reached within 20 days, the complaining country can ask the Dispute Settlement Body for permission to impose limited trade sanctions on the other country. This dispute settlement mechanism is widely used by member nations of the WTO. It has often been used by the United States and the European Union to settle trade disputes between the two. Trade relations between the United States and the European Union see few disputes, with only a little over 1% of transatlantic trade volume under formal dispute (Burghardt). However, the few problems that do exist between the groups have received an overwhelming amount of media attention. This attention far exceeds their economic importance (Wielard). The fact that the economies of the United States and the European Union are so closely tied contributes to the problems that exist. With such an extended relationship and sometimes differing ideas about economic circumstances, there are bound to be some disagreements; two of these disagreements will be used to illustrate the inefficiencies that exist in the WTO dispute resolution procedures.

Recently, trade disputes between the United States and the European Union have begun to move from traditional trade barrier issues to problems stemming from regulation, licensing, and standards relating to health, consumer, and environmental protection. The European Union has a $5 million banana market that was at the center of a major trade dispute for eight years. In 1993, the European Community adopted a Common Market Organization for bananas. The trade policies established under this system favored imports from former colonies of EU member nations. These policies restricted Latin American access to European banana markets. This regime was found to be illegal under WTO guidelines in 1997. In January 1999, a new import scheme was implemented. This was also declared illegal according to WTO standards because there was a quantity set aside specifically for ACP countries. The United States became involved in the case because two major American firms, Chiquita and Dole, market bananas produced in Latin America and were denied the opportunity to market their bananas to European nations. In April 1999, the United States was authorized by the WTO to place trade sanctions on the European Union for an annual value of $191 million (Bahree and Muffay). The United States imposed 100% duties on an equivalent amount of trade on March 3, 1999 (“New Developments Complicate Banana Dispute”).

The disputing parties eventually agreed on a solution based on a historical reference period. In 2006, trade policies for bananas imported into the European Union will transition to a tariff-only system. In the meantime, the European Union will establish quotas and an import licensing system based on historical trade shares that should increase prospects for Latin American banana imports. Imports from former colonies of EU member nations will still get preference.

Full implementation of this system depends on WTO members granting the European Union waivers of the GATT provisions that prohibit the preference of ACP countries’ banana imports. Beginning on July 1, 2001, the United States suspended the trade sanctions on the European Union that had been previously...
authorized by the WTO. The European Union was charged with the task of creating a proposal to the Council of Ministers that would adjust the quantities of bananas in the various quotas, increasing marketshare for Latin American producers and protecting access to the market for the ACP countries. The United States pledged to work to secure approval from the WTO for the European plan.

Some contention is based on the commercial interests of EU and U. S. business and government policies. The single biggest trade sanctions claim is seen in an EU complaint dealing with the U. S. Foreign Sales Corporation (FSQ-Winestock). This tax policy is provided for under the Deficit Reduction Act of 1984, which is the successor legislation to the Domestic International Sales Corporation of 1971 (Smith). An FSC is a separate foreign corporation that is incorporated in an approved jurisdiction outside the United States, electing with its shareholders’ consent to be treated as an FSC. This export tax benefit enables U.S. exporters to consider between 15% and 30% of their export income as tax exempt and is estimated to save U.S. companies approximately $4 billion annually (Eurecom). In an FSC, for each qualifying sale made by a firm to foreign customer, the firm pays a commission, determined by special transfer pricing rules, to the FSC. The commission is a tax-deductible expense to the firm. About one third of the commission income is taxable income to the FSC. The net income of the FSC is distributed back to the U. S. firm as a tax-exempt dividend (Smith). The European Union lodged a complaint in 1998 that the FSC provisions are an export subsidy and are a violation of WTO regulations (Dreazen and Rogers). However, the United States has questioned why the European Union waited 14 years to complain about the regulations. The United States believes that this challenge may be in response to U.S. challenges to EU import regimes regarding bananas and beef (Dougherty). The European Union has said that the challenge was intended to improve equality in trade relations, but there is little evidence that European companies were pushing for the change. Because some European firms benefit from the FSC tax policy, they are not fully encouraging of the EU claim (“The FSC Bomb”). The WTO ruled that the U.S. tax exemption was in violation of trade regulations. In November of 2000, Congress repealed the exemption and passed a fix-it bill that rewrote the legal basis for the program; the new law gives the same tax benefits as the original FSC program, but it extends the provision to a certain proportion of income from exporters’ foreign operations. It also eliminates the need for U.S. firms to set up an off-shore firm by simply exempting income earned overseas from U.S. taxes rather than having an exemption for exports only (Dreazen and Rogers). The new law broadened the scope of the beneficiaries for the FSC’s, with the hope that the WTO could be convinced that the policy was no longer dependent on exports. However, the WTO still felt that the policy was too export related and was contrary to the WTO agreement on subsidies (Smith). The new laws “provide illegal export subsidies, violate the trade body’s

agricultural agreement, and discriminate in favor of U.S. goods in breach of WTO rules” (Osborn and Denny). After the WTO ruling against the new FSC policy, the European Union requested the authority to impose $4 billion in retaliatory duties on U.S. goods. The United States filed an appeal in August of 2001 with the WTO, which was considered unlikely to be successful. However, the appeal allowed time for negotiations between the disputing sides to avoid punitive sanctions being placed on the United States (Alden). The European Union published an indicative list of retaliatory sanctions with 46 general categories; a detailed list with specific products would be compiled after the final WTO ruling. If the United States does not do away with the FSC tax policy, sanctions will be imposed in the form of 100% penalty tariffs on imports of U.S. goods up to the value allowed by the WTO (Winestock). The United States has said that if sanctions are imposed, it will respond by bringing cases against the European Union to the WTO (“The FSC Bomb”). The resulting escalation of the situation would be harmful to the trade relationship between the two sides. On January 14, 2002, the WTO threw out the U.S. appeal. At this point, both sides have 60 days to present arguments to a WTO arbitrator who will rule on any permitted retaliatory tariffs by the end of March (Mortished).

The resolution of both of these cases, the FSC tax exemption case in particular, hopefully will set a precedent for cooperation between disputing parties in settling trade conflicts. While neither dispute was settled through multilateral negotiations, and there was evidence of noncompliance with WTO rulings in both cases, both sides were willing to work together after the final rulings to avoid escalation of the situation into a full-blown trade war.

Conclusion Reached:

Through the illustrations provided by past trade disputes that have been resolved under the WTO’s dispute settlement mechanism, it is evident that some modifications must be implemented in the process. One option is to make the existing settlement process streamlined and efficient. A system involving permanent panelists and a speeding up of the process wherever possible will make the overall process more efficient. A permanent panel would create a team of experts who are familiar with the dispute settlement process and are readily available to review trade disputes. Because the panel would have experience in dealing with the procedures specified by the WTO, it would be able to move through those procedures quickly and arrive at rulings in an efficient manner. Clarification of the policies on the sequencing, arbitration procedure on the level of suspension of concessions, and the establishment of policies to lift suspension of concessions would also improve the overall processes. All parties involved in the dispute and its resolution would be aware of the actions involved in resolving a trade dispute. Another option is to place importance on negotiation with less emphasis on the procedural aspects of the settlement mechanism. Member nations, as well as the WTO itself, have expressed the view that
consultation is a more effective resolution tool than litigation. However, only 32 out of 203 cases had been settled outside of the WTO panel process for settling disputes by July 2000. Nations should clearly define their advocacy or independent of any official guidance from an outside authoritative body. Nations have differing opinions on which of these situations is more favorable to the settlement of disputes. The United States has proposed early, bilateral consultations before the disputing nations go to the WTO. It advocates the use of neutral, third party mediators or panels. The European Union has stated that mediation should occur within the parameters of the WTO because it believes that outside consultations have no enforcement capacity.

Value of Project:

Trade is a fundamental part of today's global economy. Healthy trade relationships allow countries to specialize in producing items best suited to their resources while acquiring other products that they do not produce from nations that specialize in those products. Trade also broadens the market that countries are able to target, resulting in greater and more comprehensive commerce. Aside form these theoretical benefits of trade, it is also advantageous for countries to form trade relationships because they can increase the income of those nations. For example, the Uruguay Round is estimated to increase the income of the United States by $42 billion each year, and NAFTA has produced gains of between $10 billion and $50 billion with tariff cuts of $14.2 billion (Office of the United States Trade Representative). The need for an efficient and effective means of settling trade conflicts is integral to maintaining healthy trade relationships and the economic advancements created through those relationships.

Works Cited:

Professor of Accounting Deborah W. Thomas sees Ms. Walker's work as affecting taxation. She says:

I first met Erin Walker when I served as a faculty sponsor for the first group of Bodenhamer Fellows on their trip to Washington, D.C. Ms. Walker's performance during the past four years has proved that her selection as a Bodenhamer Fellow was well deserved. She has tackled intellectual challenges by pursuing two majors in different colleges while maintaining high academic standards. Ms. Walker was a student in my Fundamentals of Taxation class last year, in which I was able to observe her work ethic and academic ability first hand. She has been a student leader, both within the Walton College as a Student Ambassador and for the University in student government. Ms. Walker has been a valuable member of the University of Arkansas community.

Ms. Walker's choice of thesis topic reflects her interest in both business and international relations. She is investigating the effectiveness of the World Trade Organization in dealing with international trade disputes. Ms. Walker focuses on recent cases, particularly the case between the United States and the European Union over banana trade, to evaluate the WTO procedures for resolving disagreements. This is an important topic as the global economy expands and more friction develops among the domestic interests of participating countries. In the area of taxation, this has been seen most recently with a WTO ruling requiring that the United States revise its international tax system.

Hoyt Purvis, Professor of International Relations, is also interested in Ms. Walker's work. He remarks:

I am pleased to be working with Erin Walker on her undergraduate honors research project on trade disputes between the United States and the European Union and the role of the World Trade Organization.

Ms. Walker has chosen a topic that in some respects brings together her unique combination of academic interests: accounting and international relations, with an additional concentration in European Studies.

She has identified and tackled a difficult but important topic and has gone about it in her usual thoughtful, serious, and persistent manner. I have been impressed by the way in which she has immersed herself in the complexity of regulatory issues involving the World Trade Organization and various issues in dispute between the United States and the European Union. She has been able to develop a good understanding of how these issues develop and what means there are for dealing with them as well as some of the implications for the future of U.S.-European economic relations.

Erin Walker has excelled as a student and has been willing to take on challenging subjects, as is evident from this research project. I am confident that the final product of Ms. Walker's work will represent a significant contribution to understanding what is involved in these trade disputes, the role of the WTO, and what all this could mean for the future of trade and economic relations between the United States and the European Union.
SECTION III: SCIENCES AND ENGINEERING
BIOLOGICAL SCIENCES; FOOD SCIENCE; CHEMISTRY AND BIOCHEMISTRY;
AGRICULTURAL, BIOLOGICAL, INDUSTRIAL, AND CHEMICAL ENGINEERING
EXTRACTION OF SILYMARIN COMPOUNDS FROM MILK THISTLE (SILYBUM MARIANUM) SEED USING HOT, LIQUID WATER AS THE SOLVENT

J.F Alvarez Barreto
Faculty Mentors: Dr. D.J Carrier
Department of Agricultural and Biological Engineering
and Dr. E.C. Clausen
Department of Chemical Engineering

Abstract:

High value specialty chemicals are usually obtained from natural products by extracting with generally regarded as safe (GRAS) solvents. Because organic solvents are quite often used, high operating and disposal costs often occur. When compared to traditional solvents, water can be viewed as an interesting alternative because of its low operating and disposal costs. Milk thistle contains compounds (taxifolin, silychristin, silydianin, silybinin A and silybinin B) that display hepatoprotective properties. This paper examines the batch extraction of silymarin compounds from milk thistle seed meal in 50°C, 70°C, 85°C and 100°C water as a function of time. For taxifolin, silychristin, silybinin A and silybinin B, extraction with 100°C water resulted in the highest yields. After 210 min of extraction at 100°C, the yield of taxifolin was 1.2 mg per g of seed, while the yields of silychristin, silybinin A and silybinin B were 5.0, 3.7 and 6.5 mg per g of seed, respectively. The overall diffusion coefficients for the four compounds increased with temperature and ranged from 0.14 x 10^-10 to 4 x 10^-10 m^2/sec, indicating that the diffusion coefficients could potentially be used for quantitative comparisons of extraction conditions. The ratios of the extracted compounds, and particularly the ratios at long extraction times, showed that the more polar compounds (taxifolin and silychristin) were preferentially extracted at 85°C, while the less polar silybinin was preferentially extracted at 100°C.

Introduction:

Milk thistle (Silybum marianum) is an annual or a biennial plant native to the Mediterranean and North Africa. It grows wild throughout Europe, North Africa, the Americas and Australia, but can also be cultivated (Hamid et al., 1983). The plants can reach a height of 10 feet with dark and shiny leaves, and purple to reddish flowers. Milk thistle has an indeterminate growth habit, resulting in staggered flowering and maturity (Carrier et al., 2002). The seeds of the plant contain a group of flavonoid compounds commonly named silymarin (Tittle and Wagner, 1978).

The dehydroflavonol, taxifolin, and the flavanolignans, silybinin, isosilybinin, silydianin and silychristin, are usually encompassed by the term silymarin (Figure 1). Some studies suggest that silybinin reduces the biliary cholesterol concentration (Duke, 1999). It has also been demonstrated that silybinin is useful in the intervention of hormone refractory human prostate cancer (Zl and Agarawal, 1999). Furthermore, the combination of silybinin and silychristin has been found helpful in decreasing the nephritic effects of chemical induced injury (Sonnenbichler et al., 1999).

The Deutsches Arzneibuch procedure for silymarin extraction is a two-step process in which seeds are first defatted in a Soxhlet extraction with petrol for 4 hr, followed by a second Soxhlet extraction with methanol for 5 hr. Using this procedure, reported silybinin yields were 11 mg of silybinin per g of seed (Benthin et al., 1999). Milk thistle was also extracted using pressurized liquid extraction techniques, in which 12 mg of silybinin per g of seed were obtained (Benthin et al., 1999). In extracting 0.4 mm milk thistle seed meal in a Soxhlet with petrol for 24 hr, followed by an ethanol Soxhlet for 4 hr, Wallace et al. (2002a) reported a silybinin yield of 22.2 mg per g of seed meal. The two-fold difference obtained by Wallace et al. (2002a) over Benthin et al. (1999) may not be significant, since the silybinin content of seed batches varies significantly (Carrier et al., 2002).

Wallace et al. (2002a) reported the analysis of three off-the-shelf milk thistle products, of which only two products contained silymarin compounds. Inconsistency between herbal supplement label and product content is not uncommon. For example, an analysis of ephedra products (Gurley et al., 2000) showed a broad range of ephedra alkaloid content, pointing most likely to manufacturing problems. The lack of consistency among products can be due in part to the extraction step, in which the desired molecules diffuse from the bulk herb to a solvent phase, usually ethanol, methanol, acetone, hexane or petroleum ether. To increase the quality of products, the extraction step should be well characterized, both in terms of rates and appropriate solvents.
The use of hot liquid water as an extraction solvent has recently caught the attention of some researchers (Basile et al., 1998; Kub-tov et al., 2001). Water is useful in extracting polar compounds, and may be useful in extracting polar compounds from plant material without prior defatting. In increasing the displays a dielectric constant compounds, and may be useful in extracting polar compounds at.,

of methanol, 33, and ethanol, 24. As a result, hot liquid (hot/liquid) water temperature up to its subcritical temperature, a decrease in (hot/liquid) water as the solvent, and consists anthracene, pyrene, chrysene, perylene and carbazole (Miller and 498 K (hot/liquid) water, where increases were observed as the search for milder and environmentally friendly solvents is intensified.

The purpose of this paper is to present results from the extraction of silymarin compounds from milk thistle seeds using (hot/liquid) water as the solvent, and consists of a first step toward process characterization. Silymarin compounds, ranging from highly polar (taxifolin) to less polar (silybinin), were extracted in 50-100°C water over 17 hr. From their concentrations, overall diffusion coefficients (D) were calculated and used as a parameter for evaluating the effectiveness of the extraction conditions.

Materials and Methods:

Extraction experiments
Milk thistle seeds were purchased from Frontier Herbs (Norway, IA) and ground with a coffee grinder to an average particle size of 0.4 mm. Extraction experiments were conducted at 50°C, 70°C, 85°C and 100°C, using 2 g of seed (contained in a cheesecloth bag) in 200 mL of deionized water. The leaching at 100°C was carried out in a 500-mL glass, round-bottom flask, fitted with a condenser for total reflux. The flask was heated in an electric mantel, and water was used to condense the vapor. The leaching experiments at 50°C, 70°C and 85°C were carried out in 500-mL bottles in a shaker water bath (Dubnoff Metabolic Shaking Incubator, Precision Scientific, Winchester, VA) set at 80 strokes per minute. Although the process conditions were slightly different when operating at or below 100°C, the long diffusion times observed in the experiments helped minimize the small differences in the systems.

Samples of extraction water were taken in triplicate every 30 min, including time zero, using a 1 mL pipette. Time zero was arbitrarily set as the time when the water started boiling (100°C), or when the temperature of the water in the bottles equilibrated with the set experimental temperature (50°C, 70°C, 85°C). To estimate the silymarin concentrations at time infinity, a final sample was taken after 300 min for the 100°C experiments and 1020 min for the 50°C, 70°C and 85°C experiments. The aliquots were placed in preweighed test tubes and weighed to determine aliquot weight. Subsequently, the aliquots were evaporated to dryness in a SpeedVac (Savant Instruments, Holbrook, NY). To the dried sample, 1 mL of methanol was added, after which they were vortexed and centrifuged (10 g). The supernatant was filtered and analyzed, as described below.

Chemical Analysis
The silymarin concentrations were determined by HPLC using a Waters system (Milford, MA) composed of an Alliance 2690 separations module and a 996 Photodiode Array, controlled with Millennium^ chromatography software. Separation of the silymarin compounds was obtained using a Symmetry E (Waters, Milford, MA) C 18 pre-column placed in series with a Symmetry E (Waters, Milford, MA) C 18 column (150 mm x 4.6 mm, 5 mm), both at 40°C. A 10 mL sample volume was injected. Solvent A was 20:80 methanol:water, while solvent B consisted of 80:20 methanol:water. The gradient program was initiated with 85:15 solvent A:solvent B flowing for 5 min; followed by a linear gradient of 45:55 solvent A:solvent B for 15 min. The proportions of 45:55 solvent A:solvent B were then held constant for 20 min, and brought back to 85:15 solvent A:solvent B over 10 min. The flow rate was 0.75 mL/min, and the silymarin compounds were monitored at 290 nm. Peak identification was confirmed by mass spectrometry (Pharmalytics, Saskatoon, Saskatchewan, Canada).

Calibration curves were prepared with silybinin from Sigma (St. Louis, MO), taxifolin from Extrasynthese (Lyon, France) and silychristin and silydianin from PhytoLab (Hamburg, Germany). No standard was available for isosilybinin, and thus this compound was excluded from the analysis. The silybinin standard obtained from Sigma contained two distinct peaks, which are further referred to as silybinin A (the first peak) and silybinin B (second peak). A sample chromatogram from the extraction of milk thistle seeds is shown in Figure 2. The HPLC procedure was previously described by Wallace et al., (2002a).

Mathematical Model
The overall diffusivities of the compounds (D) were determined using the method of Schwartzberg (1975), who presented a series solution to Fick’s second law of diffusion:

$$\frac{C(t)}{C_{\infty}} = \sum_{n=1}^{m} B_n \exp \left[ -\frac{q_n^2 D_s t}{a^2} \right]$$

In Equation (1), a is the particle size (mm), is the concentration of the selected silymarin at infinite time (mg/ml),
C is the concentration of the selected silymarin at any given time (mg/ml), B_0 and q_1 are parameters related to the geometry of the solid particles, D is the solute diffusivity (m^2/s) and t is the immersion time (min). Table 1 presents a summary of the constants used in the mathematical model, along with their corresponding values in these experiments.

The solution of Equation (1) may be approximated by the first term in the series for Dt/a^2 > 0.1. Thus, a plot of [X] vs. time yields a straight line with a slope that corresponds to the following expression:

\[-\frac{D}{2.303a^2}q_1^2\]

(2)

If the particle is considered a sphere, q_1 can be obtained by iteration through the following equation:

\[\tan q_1 = \frac{3q_1}{3 + \alpha q_1}\]

(3)

where \(\alpha\) is the stripping factor related to solid and liquid volume and equilibrium ratio. Thus, D is obtained by combining Equations (2) and (3) with the constants of Table 1.

Results and Discussions:

For all temperatures, three distinct experiments were conducted, of which three samples were taken per time point (total of nine samples per time point). Figure 3 demonstrates the reproducibility of the concentration-time data at each temperature by showing the silybinin B concentration in the extract water with time. The reproducibility of the data improved with increasing temperature as the concentration of the extracted compound increased.

Figure 4 shows typical results from the extraction of taxifolin, silychristin, silybinin A and silybinin B, presented as the yield of each compound (mg per g of seed) as a function of time and temperature. Each of the extracted compounds showed a consistent pattern of increasing yield with temperature and time. For each of the silymarin compounds, extraction with 100°C water produced the highest yield and concentration of compounds. After 210 min of extraction at 100°C, the yield of taxifolin was 1.2 mg per g of seed, while the yields of silychristin, silybinin A and silybinin B were 5.0, 3.7 and 6.5 mg per g of seed, respectively. After 300 min of extraction, the yields of taxifolin, silychristin, silybinin A and silybinin B were 0.92, 4.7, 3.7 and 6.7 mg per gram of seed, respectively (data not shown). A slight decrease in the yield of taxifolin was observed after 150 min, perhaps indicating the onset of decomposition. Water extraction at 100°C yielded about half of the amount of the silybinins obtained in the two-step Soxhlet extraction performed by Wallace et al. (2002a).

Table 2 shows overall diffusion coefficients for the compounds as a function of temperature. The diffusion coefficients increased with temperature, indicating that the diffusion coefficients could potentially be used for quantitative comparisons of extraction conditions, including various water temperatures and different solvents. The calculated coefficients for water extraction fall within the spectrum of diffusion coefficients obtained for the extraction of sucrose in water (Schwartzberg and Chao, 1982).

The ratios of the concentrations of taxifolin, silychristin and silybinin A to the concentration of silybinin B at 85 and 100°C as a function of time (sampling points) are shown in Figure 5. These temperatures were chosen because the flavanolignan concentrations were not as large at temperatures below 85°C. As is noted in Figure 5a, at 85°C the ratio of taxifolin to silybinin B increased rapidly to 0.35 g/g and then held constant at that level. At 100°C, the ratio reached a maximum of 0.32 g/g and then gradually fell with time to 0.18 g/g. This reduction in the ratio at 100°C shows that the taxifolin concentration reached its maximum faster than silybinin B. A similar behavior for the ratio of silychristin to silybinin B is noted in Figure 5b. At 85°C, the ratio rapidly rose to just above 0.9 g/g, and then gradually increased before leveling out at 1.1 g/g. At 100°C, the ratio increased to a maximum of 1.0 g/g, and then gradually fell to 0.78 g/g. The data of Figure 5c show that, excluding an initial sharp increase, the ratio of silybinin A to silybinin B at 85°C was constant at 0.65 g/g. At 100°C, the ratio was constant at about 0.6 g/g, again excluding the initial sharp period of increase.

These ratios, and particularly the ratios at long extraction times, show that the more polar compounds (taxifolin and silychristin) are preferentially extracted at 85°C, while the less polar compounds (silybinin A and B) are preferentially extracted at 100°C (see also the data of Table 3). The data reported by Wallace et al. (2002a) showed that the taxifolin to silybinin B, the silychristin to silybinin B and the silybinin A to silybinin B ratios were 0.02, 0.1 and 1.2. Thus, the ratios of extraction products using water at 100°C more closely resemble the Soxhlet extraction results than the water extractions at temperatures below 85°C. More dramatic differences in polar and nonpolar compound extraction with water are expected as the temperature of liquid water is further increased, thereby lowering the dielectric constant.

Although the yields of taxifolin, silychristin, silybinin A and silybinin B using water are half of what is reported in ethanol (Wallace et al., 2002a) this technology shows promise because of the omission of the defatting step. An oil removal step is necessary in the extraction procedures proposed by Kahol et al. (2001) and by Benthin et al. (1999). The work of Wallace et al. (2002b) will compare the extraction of non-defatted and defatted milk thistle seed meal using ethanol, as the solvent, will hopefully shed more light on this subject mater.
Conclusions:

Water is not only an interesting alternative solvent because of its low operating and disposal costs, but is also highly effective in extracting the silymarin compounds from milk thistle seed. For each of the compounds, extraction with 100°C water gave the highest yield and concentration. After 210 min of extraction at 100°C, the yield of taxifolin was 1.2 mg per g of seed, while the yields of silychristin, silybinin A and silybinin B were 5.0, 3.7 and 6.5 mg per g of seed, respectively. The overall diffusion coefficients for the four compounds increased with temperature and ranged from 0.1-4.0 x 10⁻¹⁰ m²/sec, indicating that the diffusion coefficients could potentially be used for quantitative comparisons of extraction conditions. The ratios of the extracted compounds, and particularly the ratios at long extraction times, showed that the more polar compounds (taxifolin and silychristin) were preferentially extracted at 85°C, while the less polar compounds (silybinin A and B) were preferentially extracted at 100°C.

Literature Cited:


Wallace S., D.J. Carrier, B. Beitle, E. Clausen and C. Griffith. 2002a. HPLC-UV and LC-MS-MS characterization of silymarin in milk thistle seeds and corresponding products (manuscript submitted to Journal of Chromatography A).


Figure 1: Structures of silychristin (SCN), silydianin (SDN), silybinin (SBN), taxifolin (TXF) and isosilybinin (ISBN).
Extraction of 95% Silymarin Compounds from Milk Thistle Seeds

Figure 2: Typical chromatogram of milk thistle seed extract. Retentions times of taxifolin, silychristin, silydianin, silybinin A and silybinin B were 10.059, 18.476, 21.264, 24.313 and 25.330 minutes, respectively. It should be noted that this particular seed lot contained minuscule amounts of silydianin.

Figure 3: Silybinin B concentration in mg of compound per 100 ml as a function of time at different temperatures. Results show all the batches for all temperatures. The maximum time reported is 240 min; experimentally, infinite times corresponded to 300 min for 100°C experiments, and 1020 min for 50°C, 70°C and 85°C experiments.
Figure 4: Concentration in mg of compound per gram of seed as a function of time at different temperatures. Results based on the first batch of each temperature. The maximum time reported is 240 min; experimentally, there were infinite times of 300 min for 100°C, and 1205 min for 50°C, 70°C and 85°C.

Figure 5: Compound ratio as a function of sampling points for the 85°C and 100°C experiments. Top (A) taxifolin to silybinin B ratio. Middle (B) silychristin to silybinin B ratio. Bottom (C) Silybinin A to silybinin B ratio.

<table>
<thead>
<tr>
<th>Water Temperature (°C)</th>
<th>Diffusion Coefficient (m²/s*10⁻¹⁰)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Taxifolin</td>
</tr>
<tr>
<td>50</td>
<td>0.2 ± 0.1</td>
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<tr>
<td>70</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>85</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>100</td>
<td>3.0 ± 1.0</td>
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Table 2: Diffusion coefficients (D) calculated for silymarin present in milk thistle seed

<table>
<thead>
<tr>
<th>Water Temperature (°C)</th>
<th>Dielectric Constant</th>
<th>Ratio of Compound to Silybinin B</th>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Taxifolin/SB</td>
<td>Silychristin/SB</td>
<td>Silybinin A/SB</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>70</td>
<td>0.281</td>
<td>0.910</td>
<td>0.615</td>
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<tr>
<td>70</td>
<td>64</td>
<td>0.297</td>
<td>0.935</td>
<td>0.639</td>
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<tr>
<td>85</td>
<td>54</td>
<td>0.330</td>
<td>1.118</td>
<td>0.630</td>
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<tr>
<td>100</td>
<td>56</td>
<td>0.176</td>
<td>0.772</td>
<td>0.551</td>
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</tr>
</tbody>
</table>

Table 3: Calculated ratio compound/Silybinin B as a function of temperature. These ratios were calculated at the last sampling point.
Faculty Comments:

Dr. Danielle Julie Daniels, who worked in the laboratory with Mr. Alvarez-Barreto, made the following remarks about his work:

I have not taught Mr. Barreto, hence I do not know him from a classroom perspective. However, I have worked closely with Mr. Barreto in a laboratory setting. Honesty, intelligence, rigor and hard work characterize his laboratory performance. Although this student is beginning his research career, he displays scientific maturity and understanding well beyond what could be expected from his peer reference group. As Mr. Barreto grasped the mechanics of the experiments, he became independent in terms of laboratory autonomy, generating reproducible and high quality data. This proved to be an invaluable asset for Dr. Clausen and myself. These same data sets served as a platform on which a NRI/USDA proposal was prepared and submitted.

Once the data were generated, Mr. Barreto actively participated in its analysis and spent a large part of his Christmas holiday generating a first draft of this paper. As is the due course of scientific writing, this paper bounced back and forth between Mr. Barreto and his mentors. Mr. Barreto will undoubtedly work in a research setting, as he will continue his work as a chemical engineering graduate student at the University of Oklahoma. My only regret is that we will lose a great asset.

Mr. Alvarez-Barreto's other mentor, Dr. Edgar Clausen, had this to say about his efforts:

Mr. Barreto transferred from the Universidad de Carabobo in Venezuela in the Spring, 2001 semester, largely because his mother entered the Ph.D. program at the U. of A. in food science. I became acquainted with Jose during this semester as his instructor in two undergraduate chemical engineering classes, and he subsequently decided to do an undergraduate research project for academic credit under my direction in the summer of 2001. Like many research projects, we had just "skimmed the surface" of the project by the end of the summer, but funds were not available to continue the work. Mr. Barreto volunteered to continue this now better-defined project on hot, liquid water extraction of flavanolignans from milk thistle throughout the 2001-2002 academic year without pay under the direction of Dr. Julie Carrier and me. This work built upon previous analytical work with milk thistle by Dr. Carrier, and the pioneering work of Hawthorne et al. in using hot, liquid water as an alternative solvent for the solubilization of both polar and non-polar compounds.

Mr. Barreto has done a good job on this project, including setting up the experiments and obtaining the experimental data, analyzing the data and preparing the manuscript. The analytical support was provided by Dr. Carrier. Dr. Carrier and I both believe that this is important work that may open the door for the use of water as an important extraction solvent. As a result of these efforts, Mr. Barreto earned a $500 undergraduate research award from the College of Agriculture, and his work will be subsequently published in *Discovery*, a publication of undergraduate research in agriculture. In addition, Mr. Barreto will present his work in April at the Mid-America Regional AIChE Student Paper Contest at the University of Iowa. His research effort was the foundation for a USDA NRI research proposal and will be a significant part of an additional refereed research publication. The research efforts are currently being expanded to examine high temperatures for hot, liquid water extraction, as well as the application of hot water extraction to other natural products. I was so impressed by Mr. Barreto’s dedication in the classroom and laboratory that I arranged for him to get a small scholarship from the Department of Chemical Engineering to help ease the financial burden of out-of-state tuition. Dr. Carrier and I were recently awarded a small research contract on nutraceuticals extraction from natural products, and Mr. Barreto was the natural choice for doing the laboratory work, this time with compensation. Mr. Barreto will be attending graduate school in chemical engineering in the fall at the University of Oklahoma. We will be losing a valuable research colleague who has a bright future.
ACTIN DYNAMICS REGULATE MYOSIN ASSEMBLY IN MUSCLE CELLS

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Faculty Mentor: Michael Ferrari
Department of Biological Sciences

Abstract:

During muscle cell (i.e. myocyte) development, organization of actin and myosin into the contractile unit (i.e. sarcomere) is required for proper muscle contraction. By disrupting the processes of muscle cell development, I am able to see which steps play important roles in proper maturation of myocytes. Elucidation of the key pathways in muscle development could lead to a better understanding of human cardiac hypertrophies and muscle myopathies. Since actin filament formation precedes myosin organization, I am using actin assembly inhibitors to determine if actin filaments are a necessary prerequisite for myosin organization. It is hypothesized that disruption of actin will disrupt myosin organization. In these experiments Jasplakinolide (Jasp) is applied to cultured embryonic myocytes during sarcomere assembly. Jasp binds, stabilizes, and induces polymerization of actin filaments (i.e. F-actin) making it a useful compound for determining if actin dynamics or precise thin filament length are necessary for myosin incorporation into the sarcomere. My results indicate that normal actin organization is required for correct sarcomere development. Myosin organization was reduced by more than 90% in all treatment regimes (50, 100, and 500 nanomolar Jasp). Sarcomeres failed to form and myosin appeared diffuse throughout the myocytes. My results indicate that intact, precisely regulated thin filaments are a prerequisite for normal myosin assembly. It remains to be determined if this requirement stems from actin-actin interactions, actin-myosin cross-bridge formation, or some other intermolecular interactions. Hopefully a better understanding of how sarcomeres form will provide insight into diseases involving the improper assembly of muscle such as myopathies or hypertrophies.

Introduction:

Striated muscle is a highly specialized tissue with one major function: contraction. Contraction of cardiac muscle causes the blood to pump throughout the circulatory system; contraction of skeletal muscle allows the body to move. Disruptions in the development or regulation of striated muscle can result is serious conditions such as cardiac hypertrophies or skeletal myopathies. So, a better understanding of how muscle cells develop could lead to treatment or prevention of such conditions. In skeletal muscle actin and myosin are organized into structures called sarcomeres, which contract by sliding of filaments not the compression or stretching of them. Actin filaments extend perpendicularly from a protein region called the Z line, while myosin thick filaments extend from the M line. A sarcomere is defined as the portion of the muscle that runs Z line-M line-Z line. Hundreds of sarcomeres are organized into strands called myofibrils, which run parallel to the long axis of the cell. All the aspects of this extensive organization is not completely understood, but new discoveries are always occurring.

The organization of actin and myosin into the contractile unit (i.e. sarcomere) during development is a very complicated process requiring numerous actin and myosin associated proteins along with various kinases and phosphatases. For example, actin must associate with Z line proteins like alpha-actinin and CapZ for proper orientation and polymerization of the actin thin filament. Interestingly both actin and myosin can form filaments independently in vitro, but does this hold true in vivo? In other words, are interactions between actin and myosin necessary for proper assembly inside the muscle cell (i.e. myocyte)? Interactions between myosin A bands and actin I bands appear unnecessary for the organization of actin thin filaments in both vertebrates (Ferrari in prep) and invertebrates. But, are I bands needed for A band formation?

During the formation of myofibrils, F-actin is organized into I bands prior to the incorporation of myosin thick filaments into A bands. Therefore, it is possible that intact I bands are required for proper A band formation. Mature F-actin may be needed to support actin-myosin cross-bridge interactions, which appear necessary to fine tune sarcomere organization. Titin, a massive elastic filament that extends from Z line to M line whose role in muscle development and contraction is not fully understood, may play a role as well. Thin filaments could be
involved in actin-titin binding and subsequent titin dependent myosin organization. Therefore, to determine if proper actin organization is necessary for myosin A band formation, actin inhibitors are applied to embryonic myocytes during sarcomere formation. In this study, I examined the effects of Jasplakinolide (Jasp), which disrupts normal actin dynamics by stabilizing F-actin and inducing polymerization.

Methods:

Cultured myocytes were prepared from stage 15 embryos of Xenopus laevis as previously described. Cells were treated at 6 hr in culture (approximately stage 19) with various concentrations of Jasp (50, 100, and 500 nM). This treatment time was selected because it is before the start of rapid organization of actin and myosin into sarcomeres. Some cultures were treated at 24 hr in culture (approximately stage 32) with 50nM Jasp. By 24 hr, sarcomere assembly is minimal and present only at the end of the cells. At 48 hr cultures containing more than 100 myocytes were fixed with 37% formaldehyde in (mM) 80 NaCl, 10 EGTA, 10 MgCl2, 10 Pipes, pH 6.5 and permeabilized with 37% formaldehyde/1% Triton. Tris-buffered Saline (TBS) (in mM 80 Tris HCl, 10MgCl2, 10NaH2PO4, pH 6.7) was used to rinse the cells before blocking with 1% milk in TBS. After blocking, cells were rinsed and left in TBS until stained for immunocytochemistry.

Myosin was labeled with MF20 supernatant (Developmental Studies Hybridoma Bank, DSHB), which recognizes sarcomeric myosin. Visualization of the antibody was achieved with Alexa-Fluor 488 goat anti-mouse IgG (Molecular Probes, MP). Tropomyosin, an actin-associated protein, was labeled with CH1 (DSHB) and a fluorescent secondary antibody, Alexa-Fluor goat anti-mouse 568 IgG(MP), as an indirect assessment of F-actin organization. In some experiments, actin was labeled with JLA20 (DSHB) and Alexa-Fluor 488 goat anti-mouse IgM (MP). All antibodies were left on for at least 2 hr at room temperature or overnight at 4°C. TBS washes were used between each antibody treatment to remove unbound antibodies.

An Olympus Microscope using a xenon light source with appropriate filters was used to visualize cells. Digital images were acquired with a Quantix cooled CCD camera and MetaMorph/MetaFluor imaging software (Universal Imaging Corp.). Image analysis was also done with MetaMorph/MetaFluor.

Data for sarcomere organization were recorded in both a qualitative and quantitative manner. In blind sessions, qualitative analysis was done by rating myocytes on a scale of 0-5: 0 corresponding to complete disorganization and 5 to perfect assembly. For quantitative analysis, first the area (in microns) of the myocyte from just outside the perinuclear region to the cell tip was determined. Then the total number of actin I bands and myosin A bands in this area were recorded to yield a density value. All values of both a qualitative and quantitative manner are presented as a percent of the control value. For example if control cells have an average Qualitative Tropomyosin rating of 5 and treated cells average only 1, then treated cells have an Qualitative rating that is 20% of controls. Statistical significance was determined using students unpaired two-tailed t-tests for values < .05. In figures, * indicates .05 > t-value < .001, and ** indicates t-values < .001.

Calcium imaging was preformed as previously described. Myocyte cultures were treated at 2 hr in culture with 100 nM Jasp for at least 2 hours prior to imaging. After incubation in Jasplakinolide, Fluo-4 calcium indicator (MP) was added for 1 hr. The indicator was rinsed out with standard saline (mM 117 NaCl, 0.7 KCl, 1.3 MgCl2, 2 CaCl2, 4.6 Tris, pH 7.8) and 100 nM Jasp was reapplied. Myocytes were then imaged for 30 minutes.

Results:

Our results suggest that actin filament dynamics are necessary for proper A band formation. Jasplakinolide disrupts actin dynamics but has no known effect on myosin formation or incorporation into A bands. Yet, 6-hr treated cultures show almost complete disruption of myosin based on both qualitative and quantitative analysis. (All data are presented as a percentage of the control cell’s value. See Methods paragraph 4). For cells treated with 50 nM Jasp (n = 31) the Qualitative rating (QR) for tropomyosin (TR) was 8.77% ± 2.3 while the QR for myosin (MY) was 7.61% ± 2.2. 1 band density (IBD) was very low at only 3.35% ± 1.7 along with A band density (ABD), which was 2.53% ± 1.1. When 500 nM Jasp (n = 50) was used, there was even more disruption of both actin and myosin. The QR TR was only 3.86% ± 1.6, and the QR MY was 1.24% ± 0.9. Values for band density showed the same pattern: IBD 2.00% ± 1.0 and ABD 2.23% ± 2. For all concentrations of Jasp used, values of I band and A band densities as well as qualitative ratings for both tropomyosin and myosin were significantly less than controls (Figure 1 & 2). Based on our data, actin organization is needed for proper myosin A band development.

Cultures treated at 24 hr showed some disruption but not near the levels seen in 6-hr cultures. Treated cultures (n = 51) had a QR TR that was 57.28% ± 5.6, QR MY 78.57% ± 5.0, IBD 72.16% ± 10.9, and ABD 79.36% ± 4.7 (Figure 3 & 4). These results are not surprising because mature actin filaments undergo constant remodeling, and new sarcomeres are still being added to the ends of cells. Jasp would certainly disrupt these processes. However, F-actin was less organized than myosin A bands. The Wilcoxon sign-rank test showed that in treated cells the QR TR was significantly lower than QR MY as well as that IBD was significantly less than ABD. Both nonparametric tests had significance levels less than .05. This experiment shows that after the bulk of sarcomere formation has occurred, Jasp has significantly less affect on myocyte organization.
To insure results were not because Jasp prevents basic cellular function, controls were performed to test for proper cell function. Calcium imaging served to show that myocytes maintain normal calcium dynamics in the presence of Jasplakinolide. This is important for two reasons. First, healthy myocytes will generate spontaneous calcium transients; secondly, if Jasp alters normal calcium transients then myocyte organization could be disrupted because transients are necessary for sarcomere assembly (calcium transients are spontaneous increases in the levels of cytoplasmic calcium, mediated by calcium release from the sarcoplasmic reticulum). Dishes treated with 100 nM Jasp had an incidence (% cells active/ 0.5 hr) of 37.4% ± 7 while control cells had an incidence of 34.3% ± 4.9%. Also the frequency of calcium transients in myocytes was slightly lower but close to control cells (Control 6 ± .4%; Treated 4.2 ± .8). Treated cells were able to maintain normal calcium transients based on data for incidence and frequency in the presence of 100 nM Jasplakinolide (Table 1). Therefore, the disruption of actin and myosin was not because of a loss of cell viability but rather because of the action of Jasp on actin organization.

Discussion:

Direct visualization of actin filaments with Phalloidin, a traditional actin label, was not possible at the concentrations used in this experiment because Jasplakinolide and Phalloidin compete for the same binding site on F-actin. Tropomyosin, which twists around the actin filament, was used as an indirect assessment of actin organization. Tropomyosin antibody staining and Phalloidin labeling was used in the presence of low Jasp concentrations to show colocalization of the two stains. Also some cultures were treated with concentrations of Jasp used in experiments and stained with tropomyosin and actin antibodies to show colocalization of tropomyosin and actin in the presence of high levels of Jasplakinolide.

The data show that when actin is disrupted myosin is disrupted as well, but the exact pathway by which this disruption occurs is unclear. The increased stabilization caused by Jasplakinolide could lock F-actin into an unnatural orientation. In this scenario, normal cross-bridge interactions may not occur, preventing consolidation of myosin thick filaments into A bands. In addition, Jasplakinolide-induced polymerization might generate longer than normal actin filaments. These long filaments may cause steric hindrance of myosin thick filament incorporation into the A-band region. Alternatively thin filaments may be required for titin stability. Experiments have shown that actin does interact with titin, and titin is known to have myosin binding domains. In addition, some titin binding domains are thought to act as a template for myosin organization. Therefore, disruption of titin via disruption of actin would prevent normal myosin organization.

Experiments with 24 hr treatment show that after the bulk of sarcomere formation has occurred, treatment with 50 nM Jasp does not severely alter myocyte organization. The interesting result is that actin was significantly more disorganized than myosin. This result may suggest that once thick filaments are consolidated (perhaps by titin binding or M-Line binding). A band organization can be maintained in the absence of proper actin band organization. At 24 hr titin is also organized and could support myosin; therefore, this idea supports the argument that actin-titin interactions are important for proper myosin and subsequent sarcomere assembly.

Further experiments must be done to examine through which pathway actin disruption leads to myosin disruption. To determine titin’s possible role in this pathway, experiments will be done in which cells are treated with Jasplakinolide and Phalloidin and stained for tropomyosin, myosin, and titin. Secondly, myocytes will be stained for M-line and Z-line proteins to see where actin and myosin congregate when Jasp is applied. This may help us understand if steric hindrance by actin prevents the proper formation of myosin A bands. Finally, fluorescently labeled actin monomers can be used to track actin dynamics in the presence of Jasp, which may allow us to better understand how the inhibitor affects sarcomeric organization.

References:

Figure 1. Jasplakinolide Disrupts Formation of Both A and I Bands. A. Troponyosin 50nM Jas. B. Troponyosin 100nM Jas. C. Troponyosin 500nM Jas. D. Myosin 50nM Jas. E. Myosin 100nM Jas. F. Myosin 500nM Jas.
Figure 2. Graph comparing the qualitative and quantitative data from 6hr experiments.

Figure 3. Treatment with Jasplakinolide at 24hr Results in Less Disruption. A. Tropomyosin in control cell. B. Tropomyosin in 24hr treated cell. C. Myosin in control cell. D. Myosin in 24hr treated cells. (For 6hr cells see Figure 2.)
Figure 4. Graph showing the difference in effect of jasplakinolide when applied at 24 hr as opposed to 6 hr.

Treated Cells have Normal Calcium Dynamics

<table>
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<tr>
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<th>Control(^6)</th>
<th>Treated (100 nM)</th>
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<tr>
<td>Incidence</td>
<td>34.3 ± 4.9</td>
<td>37.4 ± 7</td>
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<tr>
<td>Frequency</td>
<td>6 ± .4</td>
<td>4.2 ± .8</td>
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Table 1. Calcium imaging was done on cells treated with 100 nM Jasp to show that jasplakinolide treated cells can still maintain normal calcium dynamics. Incidence (% cells active/0.5 hr), Frequency (# transients/hr).
Faculty Comments:

Mr. Cook's faculty mentor, Michael Ferrari, wrote a very enthusiastic letter supporting the publication of Mr. Cook's article. In it he says the following:

Mr. Cook impressed me tremendously in the year he worked in my laboratory as a research assistant. Although the basic biomedical research in my lab did not relate to his career goal of becoming a practicing veterinarian, he nevertheless undertook his project with intelligence, diligence, and perseverance. Most astonishing, due to his excellent work ethic, he generated enough data to present a poster at both a regional (Midwest Developmental Biology meeting held at UM Columbia) and national (American Society for Cell Biology meeting held in Washington, DC) scientific meeting last year. This is a rare achievement for any undergraduate, especially within a year! Mr. Cook had no previous lab experience, yet in this short time he learned several advanced techniques, generated excellent data, and became familiar enough with the field to present the work with confidence and composure at the aforementioned two meetings.

My lab studies how the internal cables of muscle cells, which generate contractile force, are constructed. John made a major contribution by showing that assembly of one component of these cables, called myosin, is entirely dependent on the normal assembly dynamics of another major component, called actin. He used a specific disruptive compound of actin assembly, called jasplakinolide, to show that myosin cannot assemble properly if actin assembly is compromised. Besides presenting this work at meetings, it is currently being written up for publication in a high profile journal. John was able to achieve so much because of his high intelligence and excellent work ethic. He spent many a late night, and even weekends, in the laboratory working on his experiments. This is a level of dedication rare for graduate students, let alone undergraduates!

I imagine the letter could stop here. However, Mr. Cook's performance was even more noteworthy in an important regard. While I did "hand" the jasplakinolide project to Mr. Cook, he rapidly became familiar with the techniques and literature. This resulted in a rather amazing thing: he came to me not once, but many times with questions about trying new methodologies and suggesting alternative ways to visualize the actin and myosin. In other words, he was very creative in thinking about ways to approach the project, and he made it his own rather than simply performing a given set of directives.

I will not dwell on Mr. Cook's academic record, which is excellent. I will say that, given his performance in the lab, he has the right personality to truly capitalize on his intelligence, self-discipline, and work ethic in his chosen career. From my observations, Mr. Cook has both the intellectual aptitude and emotional attitude necessary for success. Mr. Cook was very well-liked by others in the lab, and was even a bit of a "go-to" person, even though he was just an undergraduate! He has a good sense of humor, is well-balanced, and a pleasure to have around. To be honest, I am disappointed I could not lure him here to Kansas City for graduate school; I would love to have retained him. However, he is committed to his career path. The strongest recommendation I can give Mr. Cook is that, were it not for my wife (who is a veterinarian), I would certainly have had him treat my animals after his graduation (as it stands, I'll have my wife try to hire him as an associate after he graduates!).

Mr. Cook is a well-rounded student, interested in a range of subjects. Classics professor Daniel Levine had this to say about Mr. Cook:

Mr. Cook was my student in three classes. He is a solid worker who never tries to take the easy way out. He always comes to class prepared and always learns what he is supposed to learn. I have recommended him for several scholarships with great enthusiasm. I believe that this young man is one of the finest students we have seen at the University of Arkansas in years.
CHARACTERIZATION OF WOUND-INDUCIBLE GENES ENCODING ENZYMES FOR TERPENOID BIOSYNTHESIS IN MEDICAGO TRUNCATULA

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Faculty Mentor:
Kenneth L. Korth
Department of Plant Pathology

Abstract:

In addition to having numerous applications as food flavorings and pharmaceuticals, terpenoids are an important class of defensive compounds that can accumulate in plants after pathogen infection or injury by insects. Sequences of DNA encoding putative terpene synthases and an oxidosqualene synthase, isolated from insect-damaged Medicago truncatula leaves, were selected from an expressed sequence tag (EST) database. The cDNA clones were used as radiolabeled probes to analyze gene expression in leaves treated with factors known to trigger a defense response in plants. Transcript levels for all of the genes examined increased in response to artificial wounding, insect herbivory, and methyl jasmonate (meJA) treatments, whereas salicylic acid (SA) and glucose oxidase (GOX) had no measurable effects on transcript levels. Furthermore, the genome of M. truncatula was analyzed via DNA blots for an estimation of the number of copies of enzyme isoforms and indicate that each of the enzymes examined is encoded by a single-copy gene or a small gene family. The results show that M. truncatula can serve as a valuable source for novel terpene synthase clones and potentially for strong wound-inducible regulatory elements.

Introduction:

Plants have unique responses to specific physical or chemical stimuli and this can be manifested by induction of genes putatively involved in a defense system. An example of a plant response to herbivory is demonstrated when lepidopteran larvae feed on a plant and volatile compounds are produced and systemically released from leaves. These volatile compounds can attract parasitoid wasps that are natural enemies of the herbivorous insect. In some cases wasps lay eggs in the lepidopteran larvae, and when they hatch, the wasp larvae devour the caterpillar from within (Turings et al., 1995). Thus the plant is protected indirectly, through an induced mechanism, from further damage by the herbivore (Kessler and Baldwin, 2001).

One of the most abundant and common classes of induced volatile plant compounds released in response to insect herbivory is the terpenoids. For the induced production of volatile terpenoids, genes are possibly induced to express the proteins needed for volatile biosynthesis. The chemical pathways that lead to terpenoids begin with a five-carbon building block known as isopentyl diphosphate (IPP). Two independent biosynthetic pathways can produce IPP, the mevalonate (MVA) pathway localized in the cytosol and the 2C-methyl erythritol 4-phosphate (MEP) pathway found in plastids. The five-carbon IPP units bond together in a head-to-head, head-to-tail, or head-to-middle fashion to form an acyclic prenyl phosphate. Specificity of the terpenoid produced is determined by the activity of the terpene synthase enzymes that convert the acyclic prenyl phosphate. Mono- and di-terpenes (C_{15} and C_{20}, compounds, respectively) are thought to originate via the MEP pathway, whereas sesqui-, tri- (C_{15} and C_{30}, respectively), and poly-terpenes are produced predominantly by the MVA pathway.

Terpenoids have a wide range of activities, and their applications range from flavorings to perfumes to pharmaceuticals. Individual sesquiterpenes have been shown to act as antimicrobial phytoalexins and as insect antifeedants. One form of triterpenes, known as saponins, have antifeedant and antifungal properties that aid in plant defense (Haralampidis et al., 2001).

A plant may respond differently to mechanical damage than to actual insect damage. These differential responses may be due to insect-derived oral factors that are perceived by the plant. A protein component in the saliva of some insects, glucose oxidase (GOX), may trigger a response by the plant (Felton and Eichenseer, 1999). Plant defense responses may also be triggered by the perception of volatile compounds such as methyl jasmonate (meJA). Methyl jasmonate is produced constitutively in plants, but often increases in abundance as plants undergo stress. Plants release meJA, which serves as a signal to surrounding plants that an herbivore is feeding nearby. However, salicylic acid (SA), a key modulator of systemic acquired resistance (SAR) to pathogens, may interfere with the meJA-centered defense pathway (Felton et al., 1999).

https://scholarworks.uark.edu/inquiry/vol3/iss1/1
For insect herbivory experiments, *Spodoptera exigua*, the beet armyworm, was employed to feed on plants. This caterpillar can be a serious pest of many crops such as alfalfa, bean, broccoli, corn, cotton, soybean, and tomato to list a few. Gene isolation and expression studies used *Medicago truncatula*, an excellent model plant for genetic analyses. This self-fertilizing legume possesses a small diploid genome and is easy to transform. It also has a relatively short generation time, allowing for more rapid genetic studies. In addition, a large scale genomics project is underway, including the sequencing of expressed mRNAs. To date, over 140,000 expressed sequence tags (ESTs) are available in a public database (http://www.tigr.org/tdb/mmgj/); this includes nearly 10,000 ESTs that were derived from *S. exigua*-injured *M. truncatula*.

To understand plant defense responses to insects, it is important to characterize the regulation of genes that encode the enzymes that produce terpenes and saponins. Based on sequence similarities with known genes from other plant species, we selected three putative terpene synthase cDNA clones and one putative β-amyrin synthase (responsible for saponin biosynthesis) clone from the EST database. Accumulation of mRNA for each gene was measured in response to several types of wounding and treatment with meJA, SA, and GOX.

**Materials and Methods:**

**Plant and insect maintenance:**

*M. truncatula*, line A17, was grown under standard conditions in a growth chamber at 24°C with a 16:8 hour light:dark regime. Fertilizer was administered at two-week intervals. All treatments were carried out in a greenhouse and were started at 0900 hours. Eggs of *S. exigua* were obtained from the USDA Gast Rearing Lab (Starkville, Miss.). Larvae were maintained on an artificial diet under conditions at approximately 22°C.

**DNA probes:**

DNA probes were derived from a cDNA library of *M. truncatula* leaves that had been subjected to *S. exigua* herbivory. The clones were identified in a search of the *M. truncatula* EST database based on sequence similarity with characterized terpene and beta-amyrin synthases from other plant species. Four clones were chosen for analysis. The terpene synthase clones and their Genbank accession numbers, were A4 (accession no. BF639687); A7 (accession no. BF640252); and A10 (accession no. BE321953). The putative β-amyrin synthase clone was designated B3 (accession no. BF642680).

**Insect Treatment:**

Insects were placed on plants and allowed to feed for 24 hours before samples were taken. Leaves that had been damaged by the insect were "local," and undamaged leaves on the same trifoliate as an insect-damaged leaf were "systemic." Artificial wounding was conducted by cutting leaves with scissors, and only the locally damaged leaves for this type of treatment were collected for sampling after 6 hours. Control samples came from leaves of undamaged plants.

**Chemical treatments:**

For all meJA treatments, intact plants were placed in 18-L glass chambers. Cotton swabs with volumes of 0.5 μL, 1.0 μL, and 2.0 μL meJA (calculated volatile concentration 0.125 μM, 0.25 μM, and 0.5 μM, respectively) were inserted into the soil next to each plant in separate chambers, and the open-end bottom of each chamber was covered with a layer of foil and cheesecloth. A control plant was placed in a glass chamber with no added meJA. The plant was removed from the chamber after 1 hour, and leaf samples were taken after 6 hours. A second experiment was conducted using the same technique but over an 18-hour time period.

Glucose oxidase was applied to leaves after wounding with a tracing wheel. As the wheel was rolled across the leaves, it punctured small holes in the plant, and 20 μL of 1.3 mg/mL GOX solution was pipetted onto the leaves. This level of GOX approximates the concentration measured in *Spodoptera* labial gland extracts (H. Eichenseer, personal communication). Local and systemic tissues were collected at 1 and 6 hours after the treatment. For testing the effects of SA, solutions of SA in water were sprayed on plants at 2 mM, 4 mM, and 8 mM. For control treatments, the solvent with no SA was used. Samples were taken at 1 and 6 hours after treatment.

**Nucleic acid analysis:**

Leaves were collected, immediately chilled in liquid nitrogen, and stored at -70°C until analysis. Total RNA was extracted using TriReagent (MRC, Inc. Cincinnati, Ohio) and separated on 1% agarose formaldehyde gels. The RNA was transferred to nylon membranes and hybridized with radiolabeled probes (Church and Gilbert, 1984). Insert DNA from individual cDNA clones was amplified via polymerase chain reaction and radiolabeled with 32P in random-primer reactions (Sambrook et al., 1989).

*M. truncatula* genomic DNA was isolated according to Junghans and Metzlaff (1990). DNA was digested in individual reactions with BamHI, EcoRI, or HindIII overnight at 37°C. Cleaved genomic DNA was separated on a 1.0% agarose gel, denatured, and transferred to a nylon membrane (Sambrook et al., 1989). Hybridizations were carried out as for RNA blots.

**Results and Discussion:**

**EST clone selection:**

A search of the *M. truncatula* EST database revealed the presence of at least three putative terpene synthase clones and one β-amyrin synthase clone that were derived from insect-damaged leaves. Functional assignment of the clones was based on the presence of highly conserved sequence domains for each
type of enzyme. Terpene synthase clones were designated A4, A7, and A10. The A4 sequence is predicted to encode a plastid transit signal at its amino terminus, indicating that it probably encodes a mono- or di-terpene synthase. The A7 and A10 clones bear highest sequence similarity to known sesquiterpene synthase clones, whereas the B3 clone is highly similar to a characterized oxidosqualene synthase, namely B-amyrin synthase. Consistent with their putative enzymatic functions, none of the other genes were observed on RNA blots. This suggests there might be multiple forms of similar transcripts, derived from independent genes, that are cross-hybridizing on the membranes. For the A10 transcripts, the two bands were always observed at similar levels, suggesting that they are derived from independent genes, these genes must be coordinately regulated. Transcripts for each gene were also induced in systemic tissues of insect-damaged plants, although not to the same degree as insect damage. This was not the case for A10, in which the highest transcript levels were observed after artificial damage. For clones A7 and A10, two bands were consistently observed on RNA blots. This suggests that transcripts accumulated to high levels following wounding.

**Wounding induces transcript accumulation:**

Measurement of RNA accumulation demonstrates that genes encoding terpene synthases and B-amyrin synthase were induced by artificial- and insect-wounding. For most of the genes examined, the highest levels of transcripts are observed in leaves injured by insect herbivory (Fig. 1). For each gene, low levels of RNA were present in undamaged leaves. Artificial damage also caused an increase in transcript accumulation, but generally not to the same degree as insect damage. This was not the case for A10, in which the highest transcript levels were observed after artificial damage. For clones A7 and A10, two bands were consistently observed on RNA blots. This suggests there might be multiple forms of similar transcripts, derived from independent genes, that are cross-hybridizing on the membranes. For the A10 transcripts, the two bands were always observed at similar levels, suggesting that they are derived from independent genes, these genes must be coordinately regulated. Transcripts for each gene were also induced in systemic tissues of insect-damaged plants, although not to the same degree as in damaged leaves. The A7 transcript was consistently the most strongly induced of all the genes examined. Insect herbivory is known to often elicit a greater plant response than artificial damage, probably due to the differing types of wounding or the presence of elicitor compounds associated with the insect (Korth and Dixon, 1999; Walker-Simmons, et al., 1984). The enzyme products of the genes examined here are predicted to be involved in the biosynthesis of defense compounds, so it is not surprising that transcripts accumulated to high levels following wounding. Probing for the constitutively present ribosomal RNA indicated that equivalent amounts of total RNA were present in each gel lane.

**Gene induction by methyl jasmonate:**

Treatment with meJA also led to transcript accumulation for each of the genes examined. Transcript levels were low in untreated samples, but RNA accumulation increased dramatically when plants were exposed to the lowest concentration of volatile meJA applied, 0.125 mM (Fig. 2). Levels of A4 transcripts increased with increasing levels of meJA, whereas transcripts for the other genes were somewhat lower with increasing levels of meJA. Temporal expression of transcript accumulation was tested for the terpene synthase clones after exposure to volatile 0.25 μM meJA (Fig. 3). The A4 transcripts were present to some degree even in untreated control samples in this experiment, but the RNA blot seemed to indicate that transcripts accumulated to higher levels between 2-6 hours after initial exposure to meJA. For A7 and A10, transcript levels clearly increased with time and returned to normal levels by 18 hours after the initial exposure. As in wound experiments, the A7 transcripts were the most abundant, and the A7 transcripts also were induced earlier than the other genes tested.

**Glucose oxidase and salicylic acid treatments:**

Glucose oxidase is the most abundant protein found in labial gland saliva of lepidopteran insect larvae (Eichenseer and Felton, 1999). This enzyme has been shown to affect plant responses to chewing caterpillars and to wounding when it is applied to a wound site (J. Bede and G. Felton, personal communications). Addition of GOX did not have any effect on transcript accumulation for any of the genes used in this study; levels of RNA in leaves treated with GOX did not differ significantly from those treated with water (data not shown).

In addition, treatment with SA ranging from 2-8 mM did not affect transcript accumulation (data not shown), therefore SA alone seems not to be directly involved in the regulation of these genes.

**Enzymes encoded by small gene families:**

Probing *M. truncatula* genomic DNA with the selected cDNAs revealed that this species contains low copy numbers of the genes examined. Banding patterns indicate that the terpene synthase sequences are present in one to three copies each, whereas sequences hybridizing to the B3 B-amyrin synthase clone are present in three to four copies (Fig. 4). Although the genes examined here all encoded well-conserved protein domains, it is well established that enzymes similar in primary sequence can differ greatly in terms of the specific products that they synthesize (Bohlmann et al., 1998). Therefore, independent genes that cross-hybridize on DNA blots might encode enzymes with different specificities. At the very least, our data indicate that there is a low degree of genetic redundancy for the sequences that we tested via genomic DNA blots. Knowledge of the copy number of these genes will be important if efforts are made to isolate genomic promoter sequences.

**Conclusions:**

The results from our experiments reveal that insect herbivory and chemical treatments can cause systemic gene responses. Systemic leaves of wounded plants accumulated transcripts for terpene and B-amyrin synthases, showing that gene-induction signals are being transported through the plant. In wounded leaves, the highest levels of transcript accumulation were generally observed after insect herbivory. This result is indicative of the presence of specific insect-derived elicitors or a unique type of
damage during chewing by lepidopteran larvae as compared to mechanical wounding.

Treatments with meJA, a central modulator of wound responses in most plant species, showed that this plant hormone may regulate expression of defense genes in M. truncatula. Addition of volatile meJA to intact plants caused a rapid and transient accumulation of terpene synthase- and β-amanin synthase-encoding genes.

The addition of GOX and SA, compounds known to affect expression of some plant defense genes, did not affect accumulation of any of the genes examined in this study. Although GOX can repress levels of some plant-defense gene transcripts (J. Bede and K. Korth, unpublished data), we did not see any differences in transcript levels when comparing wounded leaves with and without added GOX.

Understanding the regulation of the genes described here might aid ultimately in manipulation of plant defense responses or in the biosynthesis of valuable terpenoid compounds. The role of these genes' products in defense is suggested by the strong and rapid induction of transcripts that occurred following insect herbivory. With the basic characterization reported here, targeted studies of the function of these genes in defense can be carried out. The enzymes that these genes encode, or the promoter sequences that control their regulation, might provide valuable tools in production of plants that are more insect-resistant. This work clearly demonstrates that M. truncatula can serve as a new source of novel and valuable genes encoding enzymes involved in plant defense and terpenoid biosynthesis.

Acknowledgements:

Thanks to Dr. Jacqueline Bede for technical instruction and helpful discussions and S. Karen Gomez for technical assistance. Thanks also to Cam Romund and Dr. John Clark for helpful comments on the manuscript, and Joe Clouse, Bob Gonzales, and Richard A. Dixon (Noble Foundation) for help in providing cDNA clones. This research was supported by the Arkansas Science & Technology Authority and a C. Roy Adair Internship awarded to M. M. Cox by the Department of Plant Pathology, University of Arkansas.

References:


Figure 1. Transcript accumulation as indicated by RNA blots, in leaves following artificial damage with scissors ("wound"), or S. exigua herbivory. Leaves were collected at 6 hours after the initial damage. Membranes were hybridized with the indicated probes, and bands were visualized via autoradiography.

Figure 3. Transcript accumulation in leaves following exposure to 1.0 μl (0.25 μM) of meJA. Leaves were collected at the time indicated, after intact plants were placed in a glass chamber with 0, 0.5 ml (0.125 mM), 1.0 ml (0.25 mM), or 2.0 ml (0.5 mM) meJA for 1 hour. Membranes were hybridized with the indicated probes, and bands were visualized via autoradiography.

Figure 4. DNA blot analysis of terpene synthase and b-amyrin synthase clones. Genomic DNA from M. truncatula was digested with BamHI (B), EcoRI (E), or HindIII (H) and separated on a 1% agarose gel. Positions of DNA size markers are indicated at left. Identical membranes were hybridized with the indicated probes, and bands were visualized via autoradiography.
Faculty Comments:

Ms. Cox’s faculty mentor, Ken Korth, had the following comments about Ms. Cox’s work:

Mandy Cox started working in my lab as a part-time employee early in 2001. She was able to continue her work during the summer of 2001 as she was awarded a competitive Adair Scholarship to conduct undergraduate research in the Department of Plant Pathology. Ms. Cox received a Bachelor of Science degree in Food Science in May 2002 with a cumulative 4.0 GPA.

Ms. Cox came to my lab with little experience in laboratory work, but she quickly mastered a number of techniques in molecular biology such as PCR, bacterial plasmid preps, electrophoresis, and RNA blots. She initiated the independent research project that is described in the accompanying paper. Her work will comprise a major component of a peer-reviewed publication of which she will be a co-author. She is a careful worker and a quick learner. Most importantly, she has a strong talent for asking the right questions — a good sign that she will continue to succeed in academic (or any other) endeavors. She took a serious approach toward her coursework, as evidenced by her consistently being named to the Dean's and the Chancellor's Lists. By the time that Ms. Cox left my lab, she was among the best in the lab at performing several of the more challenging techniques that we use.

Ms. Cox showed her dedication to her research project by completing the manuscript describing her work, which unfortunately can not fully communicate the time and effort that she put forth to make it a success. She worked well with everyone in the lab, and always impressed me with her respect for others and her maturity. I have high confidence in Ms. Cox's skills, intellect and potential, and it was truly a pleasure to have her working in our lab.

A second faculty member who has worked closely with Ms. Cox, Mike Johnson, was also extremely complimentary about her work. He said:

Mandy Cox transferred from WestArk College in Fort Smith in Fall 2000 to continue her work toward the B.S. degree in Food Science. She wanted to gain some insights into plant molecular biology, and I steered her to take Dr. Ken Korth's Agricultural Biotechnology course. She then managed to get funded to do some undergraduate research in Dr. Korth’s lab to learn hands-on some of the molecular techniques discussed in the above course. The fruit of that labor, with excellent guidance from Dr. Korth, is the manuscript, "Characterization of wound-inducible genes encoding enzymes for terpenoid biosynthesis in Medicago truncatula, which Ms. Cox has authored.

My own independent observations of Ms. Cox’s prowess as an undergraduate researcher are in concert with those of Dr. Korth. Ms. Cox has worked as an hourly researcher on an important research subcontract with the National Animal Disease Center/ Iowa State University to analyze the virulence of isolates of a bacterial pathogen, Listeria monocytogenes, which came from swine or turkey sources. My able colleague, Dr. Rama Nannapaneni has been coaching her on the techniques used, and we have high confidence in the results she has collected for us on this project. It is our intent that her name be included as a co-author on the refereed paper that will be submitted soon on this cooperative research project. She is a careful worker and asks lots of questions on the front end. This to me is the hallmark of a good researcher, learning to ask good questions so as to design good meaningful experiments.

Because of her high academic achievement in formal course work and her dedicated work habits in the lab, I have invited Ms. Cox to do her M.S. degree research in my lab beginning Fall 2002. I feel fortunate to have recruited her to my lab. I expect she will continue to make the same kind of fine research progress and contributions as she has already made in Dr. Korth's lab.
THE NEED FOR PFC ABATEMENT IN SEMICONDUCTOR MANUFACTURING

Mohsen Manesh and Brian Kendrick
Department of Industrial Engineering

Faculty Mentor: Scott J. Mason
Assistant Professor of Industrial Engineering

Abstract:

Perfluorocompounds (PFCs) are highly stable chemical compounds used in two integral steps of semiconductor manufacturing: chemical vapor deposition (CVD) chambers and etch chambers. Unfortunately, PFCs are also greenhouse gases linked to global warming. This, combined with their long atmospheric lifetimes gives them global warming potentials much higher than CO₂, the principal greenhouse gas. In a series of voluntary agreements with the United States and other national governments, the worldwide semiconductor industry has set a goal of reducing PFC emissions to 90% of their 1995 levels. To reach this goal, researchers have explored four main methods of reduction: substitution of PFCs, recovery and recycling of PFCs, tool optimization, and exhaust abatement. While the first three methods have successfully reduced emissions in the CVD chambers, they have proven too costly for or inapplicable to etch chambers. Therefore, it has become apparent that further reductions must be achieved through the abatement of etch chamber exhaust.

Herein, we compare three commercially available abatement systems representative of the three techniques currently used to abate PFCs. All three systems are categorized as either downstream systems, which receive diluted exhaust from multiple etch chambers, or point-of-use (POU) systems, which receive concentrated exhaust from a single etch chamber. Though both downstream and POU configurations are equally effective in destroying PFCs, they differ in cost depending on the number of etch chambers in use and the dilution rate per chamber. Given these numbers, our Microsoft Excel-based cost model computes the total cost of each of the three commercial systems, allowing the user to determine which system is most economical for a specific factory setting.

Introduction:

Perfluorocompounds (PFCs) are a group of highly stable chemical compounds used in two integral steps of semiconductor manufacturing: chemical vapor deposition (CVD) chambers and plasma etch chambers. Unfortunately, PFCs also are greenhouse gases linked to global warming. Emissions of greenhouse gases are commonly reported in comparison to CO₂, the principal greenhouse gas, which accounts for 81.4% of greenhouse gas emissions. Although annual emissions are relatively small, PFCs have a much higher global warming potential (GWP) than CO₂ (see Figure 1). For example, SF₆ has a GWP of 23,900. This means that a given volume of SF₆ will absorb 23,900 times as much heat from the sun as that same volume of CO₂ over a period of 100 years. Moreover, since SF₆ and other PFCs have such long lifetimes, once in the atmosphere, they will practically "live" there forever.

Motivated by such international conventions as the 1992 Rio Summit and the 1998 Kyoto Convention, the World Semiconductor Council in April 1999 set an industry-wide goal to reduce year 2010 PFC emissions to 90% of 1995 emissions. Given that the annual growth rate of the industry is approximately 17%, this reduction is like a 90% reduction on a per-chamber basis. Twenty-two U.S.-based semiconductor manufacturers reiterated their commitment to this goal by signing the Memorandum of Understanding with the Environmental Protection Agency in February 1998. Similar industry-government agreements have been signed in Taiwan, Japan, Korea, and Europe. Further motivation to reduce emissions was provided by Dupont, the major supplier of PFCs, which threatened to curtail the sale of C₃F₆, the most widely used PFC, to semiconductor manufacturers if emission controls were not addressed.

Since the signing of the Memorandum of Understanding and Dupont's threat, the semiconductor manufacturing industry has actively sought methods to achieve this level of reduction. Thus far, four main methods have been examined: substitution of PFCs, recovery/recycling of PFCs, tool optimization, and exhaust abatement. While the first three methods have effectively reduced emissions in the CVD chambers, they have proven too costly for or inapplicable to the etching process, which accounts for 10%-30% of the semiconductor industry's PFC emissions. Tool optimization and substitution of PFCs with alternate chemistries have been unsuccessful due to the anisotropy, polymerization, and the precision necessary in etch applications and the recovery and recycling of PFCs has been shown to be economically infeasible. Therefore, to achieve further reductions, it has become necessary to explore methods of effective PFC abatement.
abatement of etch chamber exhaust, a task not easily accomplished given PFCs’ stable chemical structure.

POU versus Downstream Abatement

Given that abatement is requisite, this study will analyze the costs associated with the three principal methods of PFC abatement for etch chambers: thermal abatement, catalytic destruction, and plasma abatement. Abatement systems can be applied in two basic configurations: point-of-use systems and downstream systems. Point-of-use (POU) systems are placed in the foreline directly after the etch chamber and before the rough pump, such as in the four POU units in Figure 2. Downstream systems are located after the rough pump where the exhaust gas is diluted, usually with nitrogen. In this arrangement, downstream systems can receive exhaust from multiple etch chambers (see Figure 3). POU abatement systems treat a concentrated PFC stream rather than a nitrogen-diluted stream thus requiring less power. In contrast, the downstream units take in much higher volumes of gas, and therefore, require much more power. However, given their configuration, one downstream unit can receive exhaust from multiple chambers, whereas the POU systems are required one per chamber. The project hypothesis is that at some number of etch chambers, the downstream systems will be more economical than multiple POU units. To put it differently, as the number of etch chambers increases, the price of multiple POU systems will increase more rapidly than the price of a downstream system. Alternately, at some level of nitrogen dilution, POU systems will be more economical than downstream systems. That is, as the nitrogen dilution rate increases, the price of multiple downstream units will increase more rapidly than that of multiple POU systems. Both hypotheses are illustrated in Figures 4 and 5, respectively. A cost model was used to estimate the costs of specific POU and downstream systems as well as to test the project hypotheses.

Etch Chamber Exhaust and Nitrogen Dilution:

To properly evaluate the abatement of etch chamber exhaust, the etch exhaust itself must be analyzed. For this study, etch exhaust will be considered on a per chamber basis rather than a per tool basis, to avoid the complication presented in multi-chamber tools. Etch chamber exhaust varies depending on the type of etch being performed and the recipe being used. Typical exhaust rates are far less than 1 standard liter per minute (sLm) per chamber, usually less than 300 standard cubic centimeters per minute (scm) per chamber. PFCs usually constitute less than 100 scm of this exhaust.

Like the etch exhaust, the nitrogen dilution rate necessary for downstream systems, too, varies with the type of etch being performed. According to Joe Van Gompel of BOC Edwards, a “clean process” such as oxide etching requires as little as 10 sLm of nitrogen dilution at the rough pump, whereas a “dirty process” such as nitrogen etch must be diluted with 40-50 sLm. Typical nitrogen dilution rates are around 50 sLm. Nitrogen dilution rates are so large that when using a downstream system, the volume of exhaust directly from an etch chamber is, in comparison, negligible.

To analyze the costs associated with the abatement systems, the number of chambers and the maximum dilution rate per chamber must be provided by the user to the cost model.

Three Abatement Systems:

Three systems, each representative of the three main abatement methods, were chosen for this cost analysis. These units are commercially available and have been tested and proven effective in abating PFCs. The capital, utility, accessory, and installation costs were assembled from a variety of sources ranging from company sales representatives to studies performed with the equipment. The sources for each system are cited here, and the costs are summarized in Figure 6.

Litmas LB1200 and LB3000

The Litmas LB1200 and LB3000 are POU plasma abatement systems located in the foreline between the etch chamber and rough pump. These systems produce a plasma discharge which decomposes PFCs into carbon and fluorine atoms that are then combined with an additive gas (typically water vapor) to convert them into the less harmful gases HF and CO2. The destruction and reduction efficiency (DRE) of the Litmas systems has been found to be >96% for CF3 and >99% for CHF3, in common etch recipes. The costs of the two units were provided by Jerry Pearson, Vice-President of Litmas Incorporated and sources [3], [7], and [10].

BOC Edwards Thermal Processing Unit (TPU)

The TPU is a downstream thermal abatement system located after the rough pump. The unit combines a burner with a water scrubber to destroy PFCs and a variety of other gases. When PFCs are not present in the exhaust, the TPU works in “low-fire” mode, burning at 650°C. When PFCs are detected, it switches into “high-fire” mode, burning at 850°C-1000°C. The stable chemical structure of PFCs requires this high temperature to decompose. The TPU uses 6 gallons of water per minute, so a water recirculation module is recommended for each unit. Experimental DRE rates for PFCs are >90%. The costs associated with the TPU were provided by Joe Van Gompel, Product Specialist at BOC Edwards Phone and sources [11] and [12].

Hitachi Super Catalytic Destruction System (SCDS) CD-60, CD-120 and CD-200

The SCDS is a downstream abatement system located after the rough pump. It exploits chemical reactions enhanced by a catalyst, converting PFCs into CO2 and HF. The catalysts require replacement every 24 months, and the old catalyst can be recycled as a steel additive. Its built-in water recirculation module uses only 1 gallon per minute. Experimental PFC DRE rates for this system are >99%. 
Optimal combination downstrean exhaust. As the total downstream exhaust exceeds 200 sLm given by the user. The number of inputs required is determined by the total downstream exhaust, which system are neccessary to handle the exhaust given by the user's inputs. Additional annual operational costs were gathered from source [14].

Inputs into the Cost Model:

The cost model was developed in Microsoft Excel. It requires four inputs: the number of etch chambers in use in the factory, the maximum exhaust from a chamber before the rough pump (in scm), the maximum nitrogen dilution necessary at the rough pump (in sLm) per chamber, and an interest rate for all time value of money calculations.

The maximum exhaust flow from the etch chamber only affects usage of the Litmas LB1200. The cost model provides two options, ">100" scm or "<100" scm. Should the user select the maximum exhaust flow to be ">100" scm, the Litmas LB1200 will be eliminated as a possibility since it can handle a maximum of only 100 scm. In the case that the user selects "<100", both the Litmas LB1200 and LB3000 will be considered, though the latter will never be optimal as it is more expensive. If the user does not know what the maximum exhaust flow will be, it is recommended that it be left at ">100." This ensures that the LB1200 is not used when it is inapplicable.

The model asks for the maximum nitrogen dilution per chamber to avoid the complication of different dilution rates for different etch recipes. Of course, this input only affects the downstream systems. It is limited to a minimum of 1 sLm and a maximum 60 sLm. If the user is unsure what nitrogen dilution rate is necessary, it is recommended that it be set at 50 sLm. Fifty sLm is the typical rate, and a higher than necessary rate will ensure proper abatement while a lower than necessary rate will not.

Outputs of the Cost Model:

The cost model first determines how many units of each system are necessary to handle the exhaust given by the user's inputs. The POU units are required one per chamber. Therefore, the number of POU units necessary is equal to the number of chambers given by the user. The number of downstream units required is determined by the total downstream exhaust, which is defined as:

\[ \text{Total Downstream Exhaust} = \text{Number of Chambers in Use} \times \text{Dilution Rate Per Chamber} \]

The TPU can handle at maximum 200 sLm of total downstream exhaust. As the total downstream exhaust exceeds 200 sLm, 400 sLm, and 600 sLm, a second, third, and fourth TPU are required. For the Hitachi SCDS, the model calculates the optimal combination of CD60, CD120, and CD200 units to handle the total downstream exhaust. A sample output is shown in Figure 7.

The initial and annual costs of each system are defined as

\[ \text{Initial Cost} = (\text{Cost of each Unit} + \text{Cost of any Required Additional Accessories}) \times \text{Number of Units Required} \]

\[ \text{Annual Cost} = \text{Annual Operational Costs per Unit} \times \text{Number of Units Required} \]

The annual operational cost includes utility cost, maintenance cost, and any other annually recurring costs. The model includes tables similar to Figure 6, which provide the model with the unit cost, accessory cost, annual utility cost, and maximum exhaust rate for each system. Every output uses the numbers in these tables for its computations. These numbers can be altered should system specifications change, to update prices, or to substitute the specifications of another system. For example, the Hitachi system prices were estimated and can be changed if the actual prices were known. Should the other systems' prices decrease or increase, these too can be reflected in the model.

Having computed the initial and annual costs, the model proceeds to calculate the cost of ownership, using the user-given interest rate, for a period of one to six years. Using this cost allows the user to see which system will be most economical for his planning horizon and how much that system will be in present-value dollars. It assumes that the initial cost is paid up front (i.e., at time 0) and that the annual operational cost is paid at the end of each year. For each abatement system, the present-value cost of ownership is computed for the whole system as well as per chamber. These computations are presented in a table (Figure 8) and corresponding graph (Figure 9).

The model also addresses the two project hypotheses by generating a table and graph of initial cost as a function of the number of etch chambers (Figure 10) and initial cost as a function of the dilution rate (Figure 11). In Figure 10, the user-given nitrogen dilution rate is held constant while the number of chambers is varied to see at what number of chambers the downstream systems are more economical. Conversely, in Figure 11 the user-given number of etch chambers is held constant while the dilution rate is varied to see at what dilution rate the POU units are most economical. Note resemblance of Figure 10 to Figure 4 and Figure 11 to Figure 5.

Finally, the model computes the average capacity of the two downstream units (Figure 12). Capacity for both systems is defined as:

\[ \text{Capacity} = \frac{\text{Total Downstream Exhaust} \times \text{Maximum Exhaust Capacity of the System}}{\text{Maximum Exhaust Capacity of the Chamber}} \]

https://scholarworks.uark.edu/inquiry/vol3/iss1/1
Since dilution rates do not affect POU systems, their capacities were not included. The capacity computations allow the user to see which of the two downstream systems would be better utilized and if there is room for increased usage.

**Project and Research Conclusions:**

In the course of researching and developing the equations used by the cost model, some particular aspects of abatement systems became apparent. The number of POU systems required is a function of the number of etch chambers. The number of downstream units required is a function of the product of the number of etch chambers and the nitrogen dilution rate, more specifically the total downstream exhaust. That is to say, the price of an abatement system can be determined using only the number of etch chambers and the maximum nitrogen dilution rate necessary per chamber. This function could be graphically represented in a three-dimensional graph. Such a graph is simulated in the sample outputs Figure 10 and Figure 11.

The utility of this research is in illuminating and simplifying the obscure, complicated field of PFC exhaust gas abatement. Based on two simple factors, a factory manager, who may know relatively little about abatement, could use the model to determine what type of abatement is least expensive and as an estimate of its expense. With the model's per-chamber approach, complications arising from considering multi-chamber tools are avoided. Similarly, by using the maximum dilution rate, the effect that changing the etch recipe can have on the dilution rate is ignored. Simplifying these complexities, the model allows the user to determine which abatement system is most economical. Providing economically feasible solutions encourages industry to make ecologically friendly decisions, which benefits everyone. Though the cost model does not solve the problem of PFC emissions and global warming, it can help factory managers and the semiconductor industry as a whole take a step in that direction.

Figure 1: Atmospheric Lifetimes and Global Warming Potentials of PFCs with respect to CO₂

<table>
<thead>
<tr>
<th>Gas:</th>
<th>Atmospheric Half-life (Years)</th>
<th>GWP (over 100 years):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon Dioxide - CO₂</td>
<td>50-200</td>
<td>1</td>
</tr>
<tr>
<td>Halocarbon 14 - CF₄</td>
<td>50,000</td>
<td>6,500</td>
</tr>
<tr>
<td>Halocarbon 116 - C₂F₆</td>
<td>10,000</td>
<td>9,200</td>
</tr>
<tr>
<td>Halocarbon 218 - C₃F₆</td>
<td>2,600</td>
<td>7,000</td>
</tr>
<tr>
<td>Octafluoroclobutane - C₄F₈</td>
<td>3,200</td>
<td>8,700</td>
</tr>
<tr>
<td>Octafluoroclopentene - C₅F₈</td>
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<td>90</td>
</tr>
<tr>
<td>Halocarbon 23 - CHF₃</td>
<td>264</td>
<td>11,700</td>
</tr>
<tr>
<td>Nitrogen trifluoride - NF₃</td>
<td>740</td>
<td>11,700</td>
</tr>
<tr>
<td>Sulfur hexafluoride - SF₆</td>
<td>3,200</td>
<td>23,900</td>
</tr>
</tbody>
</table>
Figure 2: Basic Configuration of POU system

Figure 3: Basic Configuration of a Downstream system
Figure 4: Hypothetical Graph 1

Figure 5: Hypothetical Graph 2

Figure 6: Summary of Costs

<table>
<thead>
<tr>
<th></th>
<th>POU of Downstream?</th>
<th>Maximum Exhaust Flow per Chamber (cc/min)</th>
<th>Maximum Total Downstream Extract (l/min)</th>
<th>Unit Price</th>
<th>Required Accessory Price</th>
<th>Annual Operational Costs</th>
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</thead>
<tbody>
<tr>
<td>Litmas LB1200</td>
<td>POU</td>
<td>100</td>
<td>N/A</td>
<td>$25,000</td>
<td>$5,800</td>
<td>$300</td>
</tr>
<tr>
<td>Litmas LB3000</td>
<td>POU</td>
<td>300</td>
<td>N/A</td>
<td>$32,000</td>
<td>$5,800</td>
<td>$350</td>
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<tr>
<td>BOC Edwards TPU</td>
<td>Downstream</td>
<td>N/A</td>
<td>200</td>
<td>$120,000</td>
<td>$10,000</td>
<td>$6,500</td>
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<tr>
<td>Hitachi SCDS CD50</td>
<td>Downstream</td>
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<td>60</td>
<td>$50,000</td>
<td>N/A</td>
<td>$6,000</td>
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<tr>
<td>Hitachi SCDS CD120</td>
<td>Downstream</td>
<td>N/A</td>
<td>120</td>
<td>$90,000</td>
<td>N/A</td>
<td>$6,000</td>
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<tr>
<td>Hitachi SCDS CD200</td>
<td>Downstream</td>
<td>N/A</td>
<td>200</td>
<td>$130,000</td>
<td>N/A</td>
<td>$6,000</td>
</tr>
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</table>

Figure 7: Output - Units Required, Initial costs, and Annual Costs

<table>
<thead>
<tr>
<th></th>
<th>Number of Units Required</th>
<th>Initial Cost</th>
<th>Annual Costs</th>
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</thead>
<tbody>
<tr>
<td>Litmas LB1200</td>
<td>Not Applicable</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>Litmas LB3000</td>
<td>6</td>
<td>$226,800</td>
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<tr>
<td>BOC Edwards</td>
<td>2</td>
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</tr>
<tr>
<td>Hitachi CD60</td>
<td>0</td>
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<tr>
<td>Hitachi CD120</td>
<td>1</td>
<td>$90,000</td>
<td>$6,000</td>
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<tr>
<td>Hitachi CD200</td>
<td>1</td>
<td>$130,000</td>
<td>$6,000</td>
</tr>
<tr>
<td>Hitachi System</td>
<td></td>
<td>$220,000</td>
<td>$12,000</td>
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</table>
Figure 8: Output - Present Value Cost of Ownership Table

<table>
<thead>
<tr>
<th></th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Year 4</th>
<th>Year 5</th>
<th>Year 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litmas LB1200</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Litmas LB3000</td>
<td>$228,744.44</td>
<td>$230,544.86</td>
<td>$232,211.90</td>
<td>$233,755.47</td>
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<td>BOC Edwards</td>
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<td>$293,502.26</td>
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<td>Hitachi CD60</td>
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<td>t</td>
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<td>t</td>
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<tr>
<td>Hitachi CD200</td>
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<tr>
<td>Hitachi System</td>
<td>$231,111.11</td>
<td>$241,399.18</td>
<td>$250,925.16</td>
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<td>$275,474.56</td>
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</tbody>
</table>

Figure 9: Output - Present Value Cost of Ownership Graph

Figure 10: Output - Chamber Analysis Graph
WORKS CITED:


Faculty Comments:

The research mentor for the Manesh/Kendrick study was Scott Mason who serves the Industrial Engineering Department as Graduate Studies Chair. He made these remarks about the work of his two undergraduate researchers:

Mohsen Manesh and Brian Kendrick were the only two undergraduates registered in my graduate level "Modeling and Analysis of Semiconductor Manufacturing" course in the Fall 2001 semester. The course, which both students took as a technical elective for their Bachelor of Science in Industrial Engineering degree, requires students (either as an individual or in a team of two) to complete both a literature review on a topic of their choosing, as well as a final project. Both the literature review and final project are presented orally to the class.

First, Mr. Manech and Mr. Kendrick reviewed the open literature pertaining to the caustic emissions produced by semiconductor manufacturers and the Memorandum of Understanding signed by the Semiconductor Industry Association and the Environmental Protection Agency in 1996 to reduce PFC emissions worldwide. This literature review was completed professionally and accurately, surpassing most of the graduate students' own literature reviews in my course.

Taking their learnings on PFC emissions in the semiconductor industry to heart, they developed an "Etch Chamber PFC Exhaust Abatement Cost Model" in Microsoft Excel for their class project. This cost model performed a capacitated, present value analysis of seven different abatement solutions available to the semiconductor industry today in terms of total number of etch chambers, exhaust flow per chamber, and nitrogen dilution rate. These two students took the initiative to contact leading abatement system vendors to conduct their research, again showing a motivation level rarely matched by their classmates.

As graduate studies chair, I review the applications of all incoming graduate students to our program. I feel Mr. Manech and Mr. Kendrick have already demonstrated a level of excellence that surpasses many of the graduate applicants that I have reviewed.

Mr. Kendrick and Mr. Manesh's undergraduate faculty advisor, Terry R. Collins, is familiar with the research of the two students. He also knows them well because of the contributions they have made to the Industrial Engineering Department. He had this to say about them:

Mr. Manech and Mr. Kendrick have submitted a research article to the Undergraduate Research Awards Selection Committee for consideration toward publication in the University of Arkansas Journal of Undergraduate Research. Their research contribution in the development of this article is scholarly for it includes genuine research methodologies and applicability in the area of environmental abatement for perfluorocarbons (PFC's).

I have known Mr. Manech and Mr. Kendrick since they joined our Industrial Engineering undergraduate program three years ago. I currently serve as their undergraduate faculty advisor. Both are exemplary students, which is evidenced by their exceptional overall GPA (Brian Kendrick 3.64/4.0, Mohsen Manech 3.91/4.0), and their Chancellor's Scholar status. They are also very active in university, department and student chapter functions and activities. Mr. Manech unselfishly volunteers his spare time to tutor underclassmen in our prestigious Students Helping Undergraduate Students (SHUR) program. Other tutors in the SHUR program are compensated for their time, but Mr. Manech felt that it is more of a privilege than a job to work with first year industrial engineering students. He is also a freshman orientation leader for the University of Arkansas. Mr. Kendrick devoted countless hours in assisting with the coordination of the Ergonomics Symposium last year. This symposium was a fund-raising activity for the IE student chapter, which cleared an amazing $25,000 for the chapter. As you can see, these two students are overachievers in all endeavors.
SYNTHESIS AND CHEMISTRY OF NAPHTHALENE ANNULATED TRIENYL IRON COMPLEXES: POTENTIAL ANTICANCER DNA ALKYLATION REAGENTS

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Mentor: Dr. Neil T. Allison
Department of Chemistry and Biochemistry

Abstract:
Iron complex chemistry that opens a new door to the medicinal and pharmaceutical worlds is the aim of this research. Specifically, ortho-quinone methide moieties are intermediates in several antitumor drugs and have been identified as bioreductive alkylators of DNA. In our research, a class of iron compounds has been targeted to resemble these quinone methides. It is hoped that these new compounds could be modified to provide a window of opportunity toward the discovery of a selective mode of drug delivery. We have focused our efforts on generating a reactive transition metal complexed 5-membered ring analog of o-quinone methide based on our earlier reports of CpFe(CO)2(butadienyl) complexes. In this vein, we have elaborated this chemistry by preparing and reacting a lithionaphthalene allene with CpFe(CO)2, which gave the desired naphthalene annulated sigma complex. This complex thermally rearranged to the desired naphthalene annulated 5-membered ring quinone methide analog. Upon photolysis, this complex successfully mimicked its antecedent and alkylated alcohols. Thus, we here report our initial study of the preparation and chemistry of a transition metal complexed 5-membered ring quinone methide analog. Its reactions with alcohols have accomplished the first step toward the ultimate goal of selectively alkylating DNA.

Introduction:
Cancer has become a prevalent disease facing many people of the world today. Naturally, its ubiquitous and devastating character has led many attempts to synthesize a suitable drug for combat. Fortunately, scientists have determined the benevolent nature of quinone methides and their alkylation of nucleic acids as an effective mechanism for anticancer antibiotics. Ortho-quinone methide moieties are believed to be intermediates of several antitumor drugs.

Quinone methides are a class of compounds in which one of the oxygen atoms of a quinone is replaced by a methylene (or substituted methylene) group. The reactivity of quinone methides is mainly electrophilic in nature, which can also be directly correlated to their toxicological properties. If the alkylation process is achieved under mild, ideally biological, conditions, it could be used in a number of biomolecular applications. In fact, these reactive intermediates have been used as DNA alkylating agents and crosslinkers. Compounds that react through quinone methide and related intermediates moieties couple to the amino groups of guanine (N7) and adenine (N3) bases primarily to modify nitrogen nucleophiles; they also couple to the oxygen functional groups, most notably the phosphate and ribose oxygens as well as (O6) of cytosine and (O8) of guanine. The specificity of DNA alkylation depends greatly on the reaction pathway and the ability of the reactive intermediate to associate with particular nucleotide sequences or helical conformations.

As to the nature of quinone methides, they, especially the simple ones, are unstable and are often not isolable in a pure form. Instead, they rapidly polymerize upon concentration of their dilute solutions. So far, no “simple” quinone methide has been isolated, except for cases in which the quinone methide moiety is part of a fused aromatic system with little contribution from the quinone methide form. The observed stability of such a complex could only be attributed to the formation of a strong metal-olefin bond that remains stable even at the expense of the loss of aromaticity of the quinone methide. Therefore, the key to synthesizing these intramolecular metal-quinone methide complexes is to retain the reactive nature of the quinone methide complexes so that it can subsequently interact and modify reagents, such as DNA.

The marriage of organometallic chemistry and drug design has only lightly been explored. Recently, the Allison group has targeted a class of organometallic compounds that resembles quinone methides and may possess potential DNA alkylation abilities. Specifically, we chose a research pathway (shown in Figure 1) that would focus on the preparation and chemistry of a selected (h1-C5H5)Fe(CO)2(h1-pentatrienyl) complex that should mimic new medicines.
In efforts to synthesize these compounds, previous studies of the Allison group found that in general, butadienyl iron complexes converted to hydroxyferrocenones in very high yields as outlined in Scheme 1. The synthesis of the starting butadienyl complexes 2 was carried out with ease; yet, in the past, efforts to prepare compounds needed for our project, i.e. the analogous pentatrienyl sigma complex, had not been successful. (See Scheme 2.) The reason for this failure was thought to be due to an intermediate 4 in the mechanism that gives complex 5. This mechanism is shown in Scheme 3, in which pentatrienyllithium attacks the carbonyl to form 4, followed by concomitant alkyl migration and iodide displacement to give 5.

We suspected that intermediate 4 was responsible for previous stumbling blocks in preparation of the pentatrienyl complex. Instead of the pathway shown in Scheme 3, it was thought that the intermediate underwent cyclization to give an undesired complex 6 and ultimately decomposed as shown in Scheme 4.

We carried out model theoretical calculations for an organic compound that corresponded to 4 and found that the conversion of 4 to 6 was favorable by ~20kcal/mol. This estimate related the ease of the undesired cyclization to occur, followed by decomposition. In light of this problem, we sought a pentatrienyl ligand that contained a key annulated aromatic moiety.

Compounds that are classified as aromatic are characterized to be cyclic, having conjugated double bonds with delocalized electrons. Aromatic compounds exhibit unusual stability. This gain in stability is due to resonance energy and is the result of the delocalized and overlapping orbitals of the p electrons. With the annulated aromatic naphthalene moiety of the pentatrienyl ligand in 4¢AR, we hoped that the complex would be blocked from the undesired cyclization, i.e. 6¢AR, and facilitate migration to form the preferred iron complex 1. (See Scheme 5.) Such would be true if the energy required to break the aromaticity of the ring was too much to allow cyclization to occur. Our synthesized pentatrienyl complex would then possess DNA alkylation potential.

Methods:

The experimental design consisted of beginning with the applicable 1-bromo-2-methylnaphthalene (7) and performing a series of reactions to synthesize the desired Σ3-naphthalene annulated pentatrienyl quinone methide complexes. The series of reactions are shown in Scheme 6.

Step 1: Preparation of 1-Bromo-2-bromomethyl naphthalene (8). A round-bottomed flask equipped with a magnetic stir bar was fitted with an air condenser. The bromo-methyl naphthalene (7) (19mL, 122mmol), NBS (24.175g, 160.75mmol), and 500mL of CC14 were placed inside the flask and irradiated with a 250W flood lamp for 20h while stirring. The product was recrystallized in hot hexanes to give a mass product 8 of 26.095g in 71.3% yield.

Step 2: Preparation of 1-Bromo-2-hydroxymethyl naphthalene (9). A round-bottomed flask was equipped with a magnetic stir bar, and complex 8 (26.095g, 86.98mmol), CaCO3 (44.784g, 447.84mmol), 250mL of deionized water, and 250mL of P-dioxane were added. The reaction was refluxed at 90°C for 16h while stirring. The solution was then allowed to cool. The organic product was collected by being dissolved in 600mL of CH2Cl2 and 800mL of 1M HCl. After separation, the organic phase was washed three times with 100mL portions of saturated NaHCO3 and dried over MgSO4. The product was recrystallized in hot hexanes to give a mass product 9 of 9.08g in 44% yield.

Step 3: Preparation of 1-Bromo-2-carbaldehyde naphthalene (10). Complex 9 (6.5g, 27.3mmol) was dissolved in 180mL of CH2Cl2. In a round-bottomed flask, PCC (11.75g, 54.4mmol), the naphthalene solution, and a stir bar were placed. The reaction flask was fitted with an air condenser and allowed to stir for 1.5h. Ether (40mL) was added to the reaction mixture, and the black gun was washed with two 10mL portions of ether. This organic phase was passed through a column of a 1-in. layer of celite wet with ether. The product was purified over a column of silica gel in 10% ether/pentane solution. The mass product 10 was 5.907g in 92% yield.

Step 4: Preparation of Acetic acid 1-(2-bromonaphthalen-1-yl)hept-2-ynyl ester (11). In a large oven-dried Schlenk tube, a magnetic stir bar was added, and N2 atmosphere was established. Dry ether (75mL) and 1-hexyne (1mL, 8.8mmol) were added. The reaction flask was submerged in an ice bath. N-BuLi (1.6M, 1.6M, 5.5mL, 8.8mmol) was added dropwise over a period of 5min. The solution was allowed to stir 10min. In a small oven-dried Schlenk tube of N2 atmosphere, complex 10 (1.872g, 8mmol) was quickly added, and the flask was degassed three times. The aldehyde was dissolved in 85mL of dry ether. The aldehyde solution was added to the reaction mixture over a period of 10min, and it was allowed to stir for 1.5h. After warming to room temperature, to the reaction flask was added 100mL of ether and 75mL of DI H2O. After separation, the organic was washed twice with 75mL portions of DI H2O and dried over MgSO4. The product was purified by passing it over silica gel in ether. The mass product 11 was 2.562g in 89% yield.

Step 5: Preparation of 2-Bromo-1-(3-methylhepta-1,2-dienyl)naphthalene (12). In a large oven-dried Schlenk tube, a magnetic stir bar was added and N2 atmosphere was established. Quickly, LiBr (2.345g, 27mmol) and CuI (5.142g, 27mmol) were added to the flask. The flask was degassed three times. The reagents were dissolved in 90mL of dry ether and allowed to stir 30min in an ice bath. MeMgBr (3M, 9mL, 27mmol) was added dropwise over a period of 5min. The reaction was stirred 30min. In a small oven-dried Schlenk tube of N2 atmosphere, complex 11 (3.2g, 8.9mmol) was added and dissolved in 25mL of dry ether. This solution was added dropwise to the reaction mixture over a period of 5min, the ice bath was removed, and the solution

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was allowed to stir for 24 h. To extract the compound, 40 mL of DI H₂O and 50 mL of ether was added to the Schlenk tube. The solution was filtered over a Buchner funnel. After the addition of 50 mL of ether and 30 mL of saturated NH₄Cl, the filtrate was separated. The organic phase was washed three times with 30 mL portions of DI H₂O and dried over MgSO₄. The product was purified by passing it through silica gel in 2% ether/pentane solution. The mass product 12 was 1.828 g in 65% yield.

**Step 6: Preparation of Σ²-Pentatrienyl Sigma Complex (13).** To a medium oven-dried Schlenk tube, a magnetic stir bar was added and N₂ atmosphere was established. Dry THF (20 mL) and complex 12 (1.008 g, 3.52 mmol) were added to the Schlenk tube. The tube was placed in an acetone/dry ice bath at -88°C. It was allowed to stir 10 min. Over a period of 5 min, 1.6 M n-BuLi (2.2 mL, 3.52 mmol) was added. The solution was allowed to stir for 1 h. In a small oven-dried Schlenk tube of N₂ atmosphere, quickly FpI (0.623 g, 3.52 mmol) was added, and after a N₂ atmosphere had been re-established, the crystals were dissolved in 8 mL of dry THF. Over a period of 10 min, the FpI solution was added to the medium Schlenk tube. After stirring 10 min, the ice bath was removed, and stirring was continued for an additional 20 min. The reaction mixture was coated with 9.8 g of 6% water/alumina gel. It was immediately transferred to the top of a N₂ saturated 6% water/alumina column, keeping in mind that this product is unstable in the presence of air. The product was eluted at a rate of one drip per second with 2.5% ether/pentane solution. The mass product 13 was 0.814 g with a 62% yield.

**Conversion of Σ²-Pentatrienyl Sigma Complex to 5-Membered Ring Quinone Methide Complexes (14).** Complex 13 was thermodynamically converted to 14 by placing it in a dry flask in dark atmosphere over a period of two weeks. This quinone methide complex was purified by passing it through a N₂ saturated 6% water/alumina column with 2.5% ether/pentane solution. Two bands were present with remnants of complex 13 eluting first off the column followed by the complex 14.

**Alkylation Reactions.** Four small aliquots of the 5-membered ring quinone methide complexes were dissolved in 1.5 mL of CDCl₃ and placed in a NMR tube. 10 mL (10 equivalents) of diethylamine, 5 mL (4 equivalents) of ethanol, 20 mL (20 equivalents) of methanol, and 10 mL (10 equivalents) of methanol were placed in the tubes, respectively. The first three tubes were placed in an ice bath of 0°C, and the last tube was placed in a dry ice/acetone bath of -78°C. All reactions were photolyzed at 75 W. These reactions were monitored through Nuclear Magnetic Resonance. See Figures 4, 5, 6, and 7 for results.

**Results and Discussion:**

Preparation of the desired h¹-naphthalene annulated pentatrienyl quinone methide analog was successfully accomplished as shown in Scheme 6. Reaction of 1-bromo-2-carbadehyde naphthalene (10) with hexynyllithium followed by acylation gave 11 in an 89% yield. Reaction of 11 with CuI/MeMgBr yielded the allene 12 in 65% yield. Reaction of the naphthalene annulated vinyl allene bromide 12 with n-BuLi followed by (C₅H₅)₂Fe(CO)₂ gave sigma complex 13 in 62% yield. This h¹-sigma complex is identified through ¹H-NMR by its characteristic peaks at δ 6.76 and δ 5.08. The absorption at δ 6.76 has an integration of 1, resulting from the single proton on the allene, and the peak at δ 5.08 has an integration of 5, resulting from the 5 protons on the cyclopentadienyl group. [See Figure 2.]

At room temperature, this compound was cleanly converted to the diastereomeric stable quinone methide complexes 14 with a half-life of two weeks. The compound is identified through ¹H-NMR by characteristic peaks at δ 4.12 and δ 3.52, 3.58. The former allene peak at δ 6.76 had disappeared, and two new peaks had appeared at ca. δ 3.5 with an integration of 1; this represents the single proton on the h¹-complex. Also, the peak at δ 5.08 had shifted to δ 4.12, representing the 5 protons on the cyclopentadienyl group. [See Figure 3.]

Initial alkylation reactions were performed to mimic the quinone methide analog reactions in the presence of cancerous DNA. The test reagents chosen (diethylamine, ethanol, and methanol) all represented the characteristics of amine or alcohol groups contained on DNA. In the process of alkylation, the quinone methide analog would attach itself to DNA; this reactivity is a key factor in the nature of an anticancer drug. The results of the four reactions (Figures 4, 5, 6, and 7) reveal that there were definite changes in the course of the reactions as compared to the ¹H-NMR spectrum of the quinone analog starting complex (Figure 3). In all four reactions, a set of twin novel peaks are formed at δ 6.45, 6.55. However, more tests must be done on the products of the alkylation reactions to accurately determine what is exactly happening, if DNA alkylation is indeed occurring, and what is being formed.

One can note that when comparing the methanol reactions at 0 and -78°C (Figures 6 and 7) the ideal condition for photolysis is in a dry ice/propanol solution of -78°C. Here, the reaction was much cleaner and would better facilitate identification of the newly formed products. This alkylation reaction with methanol at -78°C was recently submitted for analysis via Electrospray Mass Spectroscopy. However, there has not been sufficient time to thoroughly analyze the results and begin the process of identifying the products of the alkylation reaction.

Indefinitely, this research project has provided a strong foundation in the chemistry of transition metal pentatrienyl complexed 5-membered ring quinone methide analog. It has proven successful in the synthesis of the desired complex. Thus, we have made a contributable step toward the potential of an anticancerous drug. With a promising future, we can continue in further research to study alkylation reactions with this newly synthesized product.
References:

Figure 1

Scheme 1

Scheme 2

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Scheme 6

\[ \text{Br} \quad \text{CH}_3 \quad \text{NBS} \quad \text{Br} \quad \text{Br} \quad \text{Br} \quad \text{CaO}_3 \quad \text{H}_2\text{O} \quad \text{PCC} \]

\[
\begin{align*}
\text{Br} & \quad \text{H}_2\text{O} \\
\text{Br} & \quad \text{Ac}_2\text{O} \\
\text{Br} & \quad \text{CH}_3\text{MgBr, CuI} \\
\text{Br} & \quad \text{n-BuLi} \quad \text{OCH}_3\text{Fe} \\
\end{align*}
\]

10
11
12
13

\[
\begin{align*}
\text{D} & \quad \text{Fe} \quad \text{CO} \\
\text{Fe} & \quad \text{CO} \\
\end{align*}
\]

14a
14b

Figure 2: Sigma Allen Complex 13

https://scholarworks.uark.edu/inquiry/vol3/iss1/1
Figure 3: 5-Membered Ring Quinone Methide Complexes 14

Figure 4: Diethylamine Alkylation Reaction
Figure 5: Ethanol Alkylation Reaction

Figure 6: Methanol Alkylation Reaction at 0°C
Faculty Comments:

Neil Allison, Ms. Means' faculty mentor, is lavish in his praise of her work. He says:

My contact with Ms. Means has been extensive. First she attended my organic chemistry lab course (Organic 1, CHEM 3702) that I taught in fall 1999. In the spring 2000, she was enrolled in my organic chemistry lecture course (Organic 11, CHEM 3713). At this time she decided to carry out honors research work in my group. Ms. Means is currently working in my research lab on a chemistry problem that focuses on model studies of potential anticancer and anti-tumor drugs. She has chosen to carry out these studies as her senior honor's project in her four-year honors scholar program. This course of study is the most academically rigorous program on our campus. I would say that she is truly a multi-task person that solves all problems with gusto. Even with all of her obligations, I have found that Ms. Means is not a "bookworm" type. She is completely honest and reliable. She has an amiable personality and is well liked by our faculty and her peers. Probably most impressive on the research front is that she also is a leader in my laboratory. When she is present, all of the students are excited about their research! This rare quality is not found in many people, and to have a person that is excited about research with an effervescent personality really tends to ignite the whole lab. The research that Ms. Means is currently accomplishing is comparable to what I would expect from a good third-year graduate student. She grasps theoretical chemical concepts instantly. In fact, after she read a grant proposal that I was writing in the area of her research, she was able to offer suggestions! I would never have expected this from an undergraduate student and only would expect a third or fourth year graduate student to be mature enough for this task. Her laboratory skills are the best I've seen from any undergraduate student (and most graduate students) in my laboratory. She has accomplished a completed project, one that I am currently writing up as a communication for publication in an American Chemical Society journal. To be published as a communication means that it must be "urgent, fast breaking research". In this paper we report that she has accomplished a new multi-step synthesis of a previously unknown compound. We anticipate that this compound will be an important...
model for a new class of drugs based on an organometallic system (something previously unknown). All factors considered, of our current chemistry students I would rank Ms. Means at the top in research. Before coming to this university I studied at the University of California, Berkeley, the University of Cologne, and the University of Florida, Gainesville. I would rank Ms. Means, at her stage in her career, among the best undergraduate students that I’ve seen at these universities. Over the past 22 years, I’ve probably written 160 or so letters of recommendation for students, half of whom I would consider as outstanding students. Ms. Means is certainly in the very top handful (3 to 5) of these students.

I must also mention, although not related to research, that Ms. Means also takes on other, perhaps more mature, responsibilities. Through her compelling need to help people she is carrying out missionary work in a very impoverished area of Haiti for two months this summer and during spring break. Traci is active in helping people. I just found out that she purchases food for an elderly woman who is housebound and takes it to her apartment. In the past Ms. Means has also helped visually impaired students. This includes note taking, reading, etc.

Ms. Means’ faculty advisor, Dale Johnson, is also very complimentary; his remarks follow ...

I have been Traci Means’ academic advisor for the past four years so I am familiar with her great academic strengths, and I have followed her research through conversation with Ms. Means and her mentor Professor Allison. Ms. Means is a special student; she is quick to grasp concepts and can use them in new situations with ease. She has never chosen the easy academic path and is completing the four-year honors degree leading to a BS in Biophysical Chemistry, perhaps the most demanding undergraduate degree option offered by the Department of Chemistry and Biochemistry. In her own words, “I chose this rigorous major because I believe it is interesting, challenging, and will best prepare me for my next academic goal, entering medical school.”

Professor Neil Allison has raved about her work ethic and the ease with which she learns laboratory skills. Despite a busy academic schedule, she is the most productive member of his research group, surpassing the output of some of our graduate students. I have read a draft of a publication that is in progress based on her undergraduate thesis. It will be an important contribution to both organometallic and biosynthetic chemistry and will serve as the foundation for a new research direction for Allison’s research group. This is the sort of contribution you hope will come from the efforts of an excellent graduate student. To see this productivity from an undergraduate is extremely impressive.

A third chemistry professor, Wally Cordes, is equally enthusiastic about Ms. Means’ research. He says ...

It is easy to say positive things about Traci Means. The expectations of the chemistry department are high; even so, the research independence developed by Ms. Means is extraordinary, and her over-all academic record is equally outstanding.

Professor Allison has had a large number of excellent undergraduate students work in his lab during his tenure here, and for him to say that Ms. Means is about the best he has ever had is a significant statement. The research she has done is not a trivial project: the extremely air-sensitive materials she has worked with require the highest level of experimental technique. And the chemical reactions she has developed and perfected are all very sophisticated organic reactions. In reading the abstract of her work I can see how Dr. Allison compares it to the work of a third-year graduate student. Organic synthesis is not an easy kind of research for an undergraduate student to pursue; it requires more patience and perseverance than many other kinds of chemical research. There are many minor details that must be handled to have decent yields of final products. In most cases of an undergraduate student doing this kind of research one expects the student to make daily visits to the research director asking for advice on how to overcome “the problem of the day”. That’s why it also impresses me when Dr. Allison said Ms. Means would go to the lab and attack these problems on her own, so that by the next time he saw her she would tell him how she had solved the problems and attained the desired results.

The research will be submitted for publication in one of the highest prestige research journals of the American Chemical Society, and I’m confident it will be published. In addition to the organic chemistry developed, it has important pertinence to the “hot” area of research for selective drug delivery.
DEVELOPMENT OF A YEAST BIOSENSOR STRAIN FOR THE IDENTIFICATION OF GENOTOXIC COMPOUNDS

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Abstract:
Advances in combinatorial chemistry have provided the pharmaceutical industry with innumerable lead compounds that could potentially serve as therapeutic agents. One of the challenges in the further development of such compounds is to rapidly, yet inexpensively, distinguish those that have undesirable effects such as genotoxicity. Thus, a simple biological assay that would permit the identification of potential DNA mutagens, and be adaptable to high-throughput technologies would be cost-effective in screening such lead compounds. The current methods use the Ames and SOS tests involving prokaryotic organisms, while systems that utilize mammalian cell culture and/or animal testing are time-consuming and expensive. Our research has focused on developing the yeast, Saccharomyces cerevisiae, as a convenient and inexpensive eukaryotic biosensor for the identification of genotoxic compounds. The yeast biosensor uses two distinct bioluminescent reporters within the same cell. The first reporter is the Renilla (sea pansy) luciferase gene, which is expressed at a continuous rate to allow standardization. The second reporter is the firefly luciferase gene fused to gene promoters that are induced when cells are exposed to DNA mutagens. By monitoring changes in the ratio of firefly to Renilla luciferase activity upon exposure to potential mutagens, one can rapidly assess genotoxicity. We have demonstrated the sensitivity, reliability and convenience of the dual luciferase assay itself, and are continuing to optimize the sensitivity of the biosensor system.

Introduction:
Since the identification of DNA as the molecule of inheritance, the quest for uncovering its maintenance and reproductive mechanisms has been ongoing. Explanations and proposed schemes for these mechanisms have since sparked questions as to how these processes are manipulated in response to environmental stimuli. Much of the work in these areas has been done with rapidly growing prokaryotic organisms such as Escherichia coli. These organisms contain several fundamental differences from eukaryotes, such as DNA packaging and repair mechanisms, that restrict inferences into the human population (1). To overcome this problem, the yeast Saccharomyces cerevisiae has become an important model organism for eukaryotic research (2). Because it possesses the typical features of eukaryotic cellular architecture and metabolism, and its DNA repair mechanisms are strikingly similar to humans, it presents an ideal model system for investigation (1).

One question that has arisen from this research is how the genetic response to DNA mutagenic agents can be monitored. For example, in pursuit of new therapeutic agents, the pharmaceutical industry seeks a fast, cheap, and reliable means of quickly identifying compounds that may be genotoxic (3). The current standard uses the well-established Ames and the SOS tests, which involve prokaryotic organisms (1). Alternative methods that utilize mammalian cell culture and/or animal testing are both time consuming and expensive (1). The work described here demonstrates that a simple eukaryote, the Baker's yeast Saccharomyces cerevisiae, may provide a useful tool for identifying genotoxic compounds.

The RAD54 gene encodes one of the proteins in S. cerevisiae involved in DNA damage repair (4). Important to how the studies described here, the transcription of RAD54 is tightly regulated (5), being induced in yeast cells only when the cells are exposed to a variety of DNA mutagens (6). This transcriptional induction results from the binding of transcription factors to specific DNA sequence elements in the RAD54 promoter region in response to DNA damage (5). In the studies described herein, we take advantage of the transcriptional induction of the RAD54 promoter in response to DNA mutagens to develop a biological sensor (biosensor) for genotoxins.

This work is an expansion of previous research in which a bioluminescent gene reporter system was developed for S. cerevisiae to monitor changes in gene expression in response to environmental stimuli (7). In this system, the luminescent reporter is firefly luciferase that, when exposed to the substrate luciferin, emits light and the rate of light emission is a direct indicator of the amount of firefly luciferase (8). When the firefly luciferase...
is coupled to a regulated gene promoter such as that of RAD54, the relative changes in luminescence can be directly correlated to changes in transcriptional activity (9). In many different organisms, firefly luciferase has served as one of the best nontoxic and most sensitive methods to measure changes in gene expression (10).

The yeast system developed by McNabb et al. (7) is a dual-luciferase assay in which two different bioluminescent reporters are contained within the same yeast cell (Figure 1). The second reporter is the Renilla (sea pansy) luciferase, which uses a different substrate for bioluminescence and can be assayed sequentially with the firefly luciferase (9) (Figure 2). The Renilla luciferase is fused to a promoter (SPT15) that is expressed at a constant rate under all environmental conditions. This reporter serves as an internal control, allowing multiple samples to be compared directly providing enhanced accuracy to the assay. By monitoring the ratio of firefly:Renilla luciferase activity, one can rapidly and accurately determine the induction of a test reporter when exposed to changes in environmental conditions (i.e. exposure to DNA mutagens). In this paper, we describe our initial studies directed toward the development of a yeast biosensor strain to monitor genotoxicity.

Materials and Methods:

Yeast strains and media.

For generation of the yeast biosensor, the Saccharomyces cerevisiae strain DMY229 (Maia ura3-52, his3Δ200, leu2Δ1, lys2Δ202, can1Δ::SPT15-Rluc) was used. Rich (YPD) medium and synthetic complete (SC) medium lacking appropriate auxotrophic selections were prepared as previously described (11).

Plasmid construction.

The promoter region of the RAD54 gene was obtained by polymerase chain reaction (PCR) using genomic DNA isolated from S. cerevisiae (12). The PCR reactions contained 100 ng of yeast genomic DNA as template, 100 pmols of the primers oDM0351 (5'-GGCCGGAATCCATGATAGAGCCACG, CATATAC-3') and oDM0352 (5'-GGCCGGAATTCA GTTATAAGGAATATATGACC-3'), 2.5 mM MgCl₂, 0.2 mM of each nucleotide and 1X PCR buffer (Promega) and 2 units of Taq DNA polymerase (Promega). The PCR product was purified, subjected to restriction enzyme digestion with BamHI and EcoRI and ligated into the BamHI and EcoRI sites in the polylinker region upstream of the firefly luciferase gene in the plasmid pDM553 (7). The ligation was introduced into E. coli DH5[alpha][F-][phi]80lacZAM15Δ(lacZY-argF)U169, endA1, recA1, hsdR17(rl-mk+), deoR, thi1, supE44, [lambda]-, gyrA96, relA1] by transformation using standard procedures as previously described (13). To verify the correct plasmid construction, the plasmid DNA was isolated from bacteria and subjected to restriction digestion. The RAD54-firefly luciferase (RAD54-Fluc) fusion plasmid was designated pDM573.

Construction of the yeast biosensor strain

For construction of the yeast biosensor strain, DMY229 was grown overnight in YPD and subsequently inoculated into fresh YPD and allowed to grow for four hours at 30°C. The plasmid pDM573 was linearized by restriction enzyme digestion with NcoI within the URA3 gene of the plasmid. The linearized plasmid was introduced into DMY229 by the lithium acetate transformation (14) and the cells were plated on SC medium lacking uracil (SC-Ura). Linearizing the plasmid within the URA3 gene targets integration of the plasmid to the mutated ura3-52 locus by homologous recombination (15) resulting in cells that can grow on medium lacking uracil (SC-Ura). Three independently isolated Ura⁺ colonies were tested for firefly luciferase activity in response to mutagenic treatment.

Luciferase assays.

Assays for firefly and Renilla luciferase activities were measured using the dual-luciferase kit according to the manufacturers instructions (Promega). Briefly, 10 to 20 µl of yeast cells were taken directly from a growing culture, diluted with 100 µl of passive lysis buffer (9), and 10 µl of the lysate was immediately transferred to the lumimeter. Firefly and Renilla luciferase activities were determined by the sequential addition of 100µl of each substrate as described (9). Bioluminescence was quantified using a Turner Designs TD-20/20 single tube luminometer with an integration time of 10 seconds for each substrate. Each individual dual assay required approximately 30-45 seconds to complete.

Results:

Identification of the biosensor strain.

The integration of the linearized RAD54-Fluc plasmid (pDM573) into the ura3-52 locus of the yeast genome by homologous DNA recombination could occur by recombinational events that result in Ura⁺ colonies lacking a functional RAD54-Fluc gene. To verify that the individual Ura⁺ colonies contained a functional RAD54-Fluc, three individual clones were screened for transcriptional induction in response to exposure to the DNA mutagen ethyl methanesulfonate (EMS), a DNA alkylating agent. Previous studies have demonstrated that the RAD54 promoter is induced in response to EMS treatment of cells (1); therefore, growth of the putative biosensor strains in the presence of EMS should result in the transcriptional induction of firefly luciferase activity. The three strains were grown for 19 hours in YPD medium in the absence or presence of 0.1% (vol/vol) EMS. The cells were subsequently assayed for firefly and Renilla luciferase activities (Table 1). Strain 10 and strain 12 clearly demonstrated Fluc activity that was induced approximately two- to three-fold in response to EMS exposure at this concentration, while strain 11 had negligible activity. The rest of the studies were conducted using strain 10 since it demonstrated the highest overall luciferase activity suggesting that it might provide the greatest sensitivity and accuracy.
Growth conditions of the biosensor strain during exposure to DNA mutagens.

To determine whether differences in the growth medium for the yeast biosensor strain altered the sensitivity of the bioluminescence assay or altered the mutagenic capacity of the EMS being tested, strain 10 was grown under two different conditions: rich medium (YPD) or synthetic complete medium lacking uracil (SC-Ura). Thus, strain 10 was inoculated into YPD or SC-Ura in the absence or presence of 0.1% (vol/vol) EMS for 20 hours at 30°C. Cells were subsequently assayed for firefly and Renilla luciferase activities (Table 2). One, the basis of these data, the growth conditions, do not appear to alter the transcriptional induction of the RAD54 promoter in response to mutagen exposure. Moreover, the level of the response is essentially identical under both conditions. Thus, our subsequent studies were performed using YPD medium since yeast cells grow more rapidly in rich medium.

Dosage sensitivity of the biosensor strain.

To examine the dosage sensitivity of the luciferase assay, studies were performed using various concentrations of both EMS and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Strain 10 was inoculated to YPD medium in the presence of various concentrations of the mutagens. After growth in the presence of EMS (22 hours) or MNNG (5 hours), yeast cells were assayed for firefly and Renilla luciferase activity. As shown in Figure 3, growth in the presence of EMS demonstrated luciferase activity that was proportional to the amount of mutagen in the medium. A reproducibly detectable transcriptional response was observed at 0.01% EMS, and increased proportionally with the EMS concentration. At concentrations of EMS higher than 0.25%, the strain failed to grow due to genotoxicity. The mutagenesis with MNNG showed a threshold of 5 μg/ml before a detectable change in firefly luciferase activity was observed, after which, the response increased in proportion to the amount of mutagen. Between 50 and 100 μg/ml of MNNG the transcriptional response appeared to be approaching saturation, suggesting that the maximum inducibility of the RAD54 promoter had been achieved. Thus, for the two mutagens tested, the yeast biosensor strain responded in a manner that was predictable based on previously published data (1). At present, the sensitivity of the biosensor system is not yet sufficient to be used for the detection of trace levels of mutagen in a given setting. Further improvements in the biosensor strain (as described in the discussion) may allow us to use S. cerevisiae as a simple eukaryotic alternative for the identification of genotoxic chemicals.

Discussion:

In an era of combinatorial drug development and high-throughput drug screening, pharmaceutical companies can readily identify numerous drug candidates acting as effectors of given therapeutic targets. One of the problems associated with the identification of numerous agents is ruling out the toxic compounds that will not prove fruitful as curative agents. For the sake of cost efficiency, drug companies must rapidly and inexpensively discard these toxic candidates, such as DNA mutagens, to focus resources on the most promising compounds. Thus, an assay that can rapidly identify potential mutagens and be adaptable to a high-throughput screening platform is a valuable tool for the pharmaceutical industry.

The long-term goal of this research is to generate a system whereby pharmaceutical companies can conveniently screen potential drug candidates for mutagenic activity. Moreover, such a system could also be exploited for the detection of environmental mutagens or genotoxins sometimes found in food products (i.e. aflatoxins). For example, water samples from pools or lakes suspected to be contaminated with a mutagenic agent could be assayed with the biosensor strain. The inducible expression of RAD54-Fluc would provide a rapid indication of mutagen contamination and further studies could then be initiated and public health measures employed more rapidly. The simplicity and speed of the assay makes it adaptable for use in the field rather than having to wait for laboratory results, which may require several days, thereby allowing preventive health measures to be instituted rapidly.

While the experiments described in this paper show our initial stages in the development of the yeast biosensor strain, further improvements to the system may add to both the accuracy and sensitivity of the system. For example, the permeability of yeast cells to different chemicals will clearly impact whether or not the biosensor strain responds to a given genotoxin. We chose MNNG and EMS for our initial studies because these chemicals are known to enter yeast cells and to alter RAD54 expression; however, when screening unknown chemicals for mutagenic activity, cell permeability is an important issue to consider. Fortunately a number of mutant yeast strains exist that have alterations in the cell wall that cause increased permeability. Such strains may prove useful in the continued development of the biosensor system. Alternatively, a genetic screen for mutants with increased sensitivity to a variety of genotoxic agents may allow us to identify strains with increased permeability, thereby broadening the number of chemicals that can enter the yeast cells. Such permeability mutants may also increase the lower level of detection for those genotoxins known to activate the reporter. An additional improvement in the system would be to use other promoters, such as RNR2, which are upregulated in response to DNA damage (6). The speed and convenience of the luciferase assay makes it plausible to use multiple independent biosensor strains to screen for genotoxins, thus enhancing the range of potential compounds that can be identified.

The studies described here provide the first application of the yeast dual-luciferase assay to the development of a yeast biosensor useful for identify genotoxic chemicals. However, it should be emphasized that the yeast dual-luciferase system is not restricted to just screening for genotoxins. One could conceivably
screen for any small molecules that alter a cellular process that could ultimately be assayed by changes in transcription. For example, high-throughput screens for small molecules that disrupt the interaction between two proteins known to be involved in causing disease could be identified using the dual-luciferase assay coupled with the yeast two-hybrid system (15). We have already constructed a two-hybrid yeast reporter strain based on dual-luciferase technology and have shown that protein-protein interactions can be accurately monitored via the induction of firefly luciferase activity (7). Thus, the number of potential applications for such a simple eukaryotic biosensor system are endless and should prove applicable to the pharmaceutical and biotechnology industry in the future.

References:

Table 1: Identification of the biosensor strain.

<table>
<thead>
<tr>
<th>Strain name</th>
<th>Fluc/Rluc (0% EMS)*</th>
<th>Fluc/Rluc (0.1% EMS)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>strain 10</td>
<td>29 +/- 0</td>
<td>69 +/- 0</td>
</tr>
<tr>
<td>strain 11</td>
<td>none detected</td>
<td>none detected</td>
</tr>
<tr>
<td>strain 12</td>
<td>8 +/- 0.8</td>
<td>16 +/- 1.0</td>
</tr>
</tbody>
</table>

* values shown are the ratio of firefly to Renilla luciferase. All values were multiplied by a factor of 1000 to obtain whole numbers.
Table 2: Effect of growth medium on the genotoxicity assay.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Fluc/Rluc (0% EMS)*</th>
<th>Fluc/Rluc (0.25% EMS)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>YPD</td>
<td>29 +/- 0</td>
<td>77 +/- 7</td>
</tr>
<tr>
<td>SC-Ura</td>
<td>22 +/- 0</td>
<td>60 +/- 2</td>
</tr>
</tbody>
</table>

* values shown are the ratio of firefly to Renilla luciferase. All values were multiplied by a factor of 1000 to obtain whole numbers.

Figure 1. Schematic depiction of the dual-luciferase assay system within the nucleus of a yeast cell. The RAD54-Fluc (firefly luciferase) is integrated at the ura3-52 locus and the SPT15-Rluc (Renilla luciferase) is integrated at the can1 Δ locus (7).

Figure 2. Schematic depiction of the sequential luciferase assay technique. A) Sample of lysate is added to a tube in the luminometer; B) the firefly luciferase (Fluc) substrate (luciferin) is added and the luminescence of the sample is measured for 10 seconds; and C) the Renilla luciferase (Rluc) substrate (coelenterazine) is added along with a quenching agent (Promega) that specifically inhibits further Fluc luminescence and the luminescence arising solely from the Rluc is read for 10 seconds.

Figure 3. Dose response of the yeast biosensor strain to EMS. The yeast strain was grown in YPD medium containing increasing concentrations of EMS and assayed for firefly (Fluc) and Renilla (Rluc) luciferase activity. The data shown represent the Fluc/Rluc ratios for each sample. Three independent measurements were done at each concentration of EMS.

Figure 4. Dose response of the yeast biosensor to MNNG. The yeast strain was grown in YPD medium containing increasing concentrations of MNNG and assayed for firefly (Fluc) and Renilla (Rluc) activity. The data shown represent the Fluc/Rluc ratios for each sample. Three independent measurements were done at each MNNG concentration.
Faculty Comments:

Ms. Reed's faculty mentor, David McNabb, had glowing things to say about Ms. Reed's research. He made the following comments:

I first met Robin when she joined my research lab in the fall of 2000. Robin quickly established herself as invaluable asset to the lab. Since I had just arrived at the University of Arkansas campus and was just setting up my research laboratory, there was an enormous amount of work just to get the lab functional. Robin was in the lab every day helping me unpack, set up equipment, and get organized. Once the lab was functional, she quickly moved on to her research project in which she has made great progress.

Robin's research continued work that I initiated as a postdoctoral fellow on the development of the yeast dual-luciferase assay system as a new technology for monitoring changes in gene expression in the yeast Saccharomyces Cerevisiae. Robin performed many of the experiments that demonstrated the utility and accuracy of the assay system as well as established several of the parameters for its use. Robin also demonstrated the usefulness of the dual-luciferase reporter system in two-hybrid screening, a technique employed commonly for studying protein-protein interactions using yeast. This component of Robin's research is part of a manuscript that is currently being prepared for publication and Robin is one of the co-authors. Robin has since taken the dual luciferase assay system to the level of industrial application. She has demonstrated the utility of the yeast dual-luciferase assay as a potentially powerful screening technique for identifying genotoxic chemicals, the subject of this article. Such a screening technology would be applicable to pharmaceutical companies that are engaged in the development of new therapeutics through combinatorial chemistry. I consider myself lucky to have had Robin as my first undergraduate researcher in the laboratory, and wish that all students displayed her level of motivation for hard work and success.

In addition to her research in my laboratory, Robin has spent the past summer at the University of Arkansas Medical School doing research on tuberculosis with Dr. Kathleen Eisenach. Robin seized the opportunity to work with Dr. Eisenach to broaden her scientific knowledge and technical expertise. During her undergraduate years at the U of A, Robin has also volunteered her time at the public health clinics in Fayetteville, as well as volunteered as a member of a medical mission to Nicaragua to provide free medical care to members of a small community. Thus, her enthusiasm for learning, helping others, and her dedication to hard work is truly inspiring.

Robin's overall academic record at University of Arkansas has been consistently excellent. She has received several awards from the university including: University Scholar, the Chancellor's List, and Fulbright College Student Ambassador. Robin was also the recipient of a SILO/SURF award this year that partially funds her research efforts in my laboratory.

On a personal level, Robin is a very friendly, mature, responsible, and caring individual. I am continually amazed by her academic abilities, work ethic, social responsibility and maturity. Robin will be attending medical school this coming Fall and I have no doubt that she will continue her excellent academic performance to become a well-rounded physician capable of interacting with patients and providing them with the medical information and care they need.

Ms. Reed's faculty advisor, Mack Ivey, had very complimentary things to say about her. He wrote:

Robin is an exceptional student. I have known her for approximately 3 years. I served as her academic advisor, and I have had her in several classes. She is currently enrolled in my Cell Physiology course, having taken my Microbial Genetics class last fall. Robin never misses class or review sessions, where her questions are always pertinent and thoughtful. Her performance has been spectacular. In Microbial Genetics, she placed in a tie for the top position in the class of 62 students. She has achieved this while maintaining an extremely active calendar outside of the classroom. She stays busy with her honors thesis research under the capable guidance of Dr. David McNabb. Her extracurricular interests are many, and include service as a Student Ambassador.

Robin is a natural leader. She is highly respected by her peers, and by members of the faculty with whom she has made acquaintance. She is pleasant, outgoing, and friendly. She does not do anything half-heartedly. I am certain that her research will be productive, and that the work she presents will have been carried out carefully and with great diligence and determination.

Jeannine Durdik also had opportunities to observe Ms. Reed's work. She says:

I had Robin in Immunology lecture and lab and in Mechanisms of Pathology classes. Robin distinguished herself in all three courses. Robin Reed ranks in the upper 1-5% of the pre-medical track seniors and the graduate students at this institution. Robin is the sort of student who will do additional work—in this case added computer problems in which the task was diagnosing virtual patients in the Mechanisms of Pathology course out of sheer interest. She really enjoyed the problem solving and asked if something similar couldn't be added to the other course, Immunology. My point is that she likes to think. I assert that thinking is what being a scientist and a scholar is all about.
PROPERTIES OF MODIFIED TRYPTOPHANS IN A MEMBRANE-SPANNING CHANNEL

Erin M. Scherer
Department of Biological Sciences

Faculty Mentor: Roger E. Koepppe, II
Department of Chemistry and Biochemistry

Abstract:

An emerging concept in biology assigns the amino acid tryptophan specific roles at the membrane/water interface that help to determine the conformation and biological function of membrane-spanning proteins. Previous studies involving the antibiotic model system gramicidin A (gA) have illustrated the importance of the indole ring of tryptophan (Trp) in anchoring proteins to a bilayer membrane and promoting ionic currents. To further investigate these phenomena, derivatives of Trp that have lost hydrogen-bonding ability (1-methyl-Trp), have an altered dipole moment (7-aza-Trp), or both (1-methyl-7-aza-Trp) were chosen for incorporation into gA. Gramicidin analogues that incorporate these modified Trps were then analyzed by single channel experiments. In addition, methods were developed for the selective exchange of indole hydrogen with deuterium (a heavy isotope of hydrogen, $^2\text{H}$) using a Raney nickel catalyst. The $^2\text{H}$ labels enable determinations of the orientation of each Trp indole ring with respect to the membrane surface using solid-state deuterium NMR spectroscopy. The last method pursued involves the application of ab initio molecular modeling programs to calculate the side-chain dipole moments of Trp, 1-methyl-Trp, 7-aza-Trp, and 7-aza-1-methyl-Trp. Therefore, this project combines both experimental and theoretical aspects of scientific research.

Results from the single-channel experiments of [7-aza-Trp] and [7-aza-1-methyl-Trp] gA analogues indicate that there is a positive correlation between channel conductance and the magnitude of the side chain dipole moments. A new methodology involving a Raney nickel catalyst was also successfully developed that allows for $\approx75\%$ of 7-aza-Trp’s sixth hydrogen to exchange with deuterium. In addition to these experimental results, the ab initio program PQS was used to generate theoretical predictions of Trp side chain dipoles that were comparable to experimentally determined dipoles, and that allowed for the calculation of 1-methyl-Trp’s side chain dipole. Beyond the immediate results, the more general implication of this project is the fundamental knowledge gained concerning the interactions of Trp with other amino acids, water, and lipids. These studies will contribute to a better understanding of folded proteins—especially those that span biological membranes.

Introduction:

With approximately 90 percent of the human genome nucleotide sequence elucidated$^{1,2}$, a continuing challenge for biochemists and molecular biologists will be to determine the mechanisms responsible for the inherent folding and tertiary structure that render encoded proteins biologically active. At present, the tertiary structures of thousands of proteins have been determined. However, the vast majority of these proteins are globular, water-soluble proteins; only a minority (less than 40) are transmembrane and/or channel proteins.

Several of these characterized transmembrane proteins contain the amino acid tryptophan (Trp). Within each protein, Trp displays a strong preference for the membrane/water interface that may be attributed to the dipole moment and hydrogen bonding ability of the indole ring of Trp. Previous studies involving the channel protein gramicidin A (gA) suggest that three of the four Trps in its amino acid sequence must form hydrogen bonds with the corresponding membrane interface in order for gA to maintain its native conformation and biological activity$^3$. In fact, if all but one of the four Trps are substituted with phenylalanine (a more hydrophobic amino acid that does not have the ability to form hydrogen bonds), another gA conformation appears that exhibits no significant ion transport activity$^4$. The passage of ions through gA has been largely attributed to the ability of Trp to attract ions into the channel by means of its indole ring’s dipole moment and to hydrogen bonding that involves the amine group in the Trp indole ring.

The structure and biological function of gramicidin A is well established, making it a useful model system for further investigations of Trp. The gA peptide has the following sequence: HCOVal—Gly—Ala—Leu—Ala—Val—Val—Val—Trp—Leu—Trp—Leu—Trp—Leu—Trp—NHCH$_2$CH$_2$OH (L-amino acids are in italics), and folds into a single-stranded helical
subunit. This subunit must then couple with another identical subunit in a head-to-head manner to display positive ion (e.g., sodium, potassium, cesium) conductance indicative of channel activity as it occurs in nature.

My research addresses both the hydrogen bonding and dipolar properties of the indole ring. To investigate these characteristics, several methods were developed. First, analogues of tryptophan with chemically modified indole rings were chosen for incorporation into gA. (Figure 1).

![Figure 1: Indole side-chains of Trp, 1-methyl-Trp, 7-aza-Trp, and 7-aza-1-methyl-Trp.](image)

The side chain of 1-methyl-Trp has lost hydrogen-bonding ability at N1; 7-aza-Trp as an altered dipole moment from Trp and introduces additional hydrogen bonding ability at N7; and 7-aza-1-methyl-Trp has a combination of the properties described for 1-methyl-Trp and 7-aza-Trp. As the dipole moments of some of these Trp derivatives were not well established experimentally, I decided to pursue a theoretical method that utilizes computer molecular modeling techniques to accurately determine each side-chain dipole. Finally, to eventually understand how each Trp derivative orients in the membrane after incorporation at position 9, 11, 13, or 15 in the gA sequence, a protocol for the selective exchange of indole hydrogen with deuterium (a heavy isotope of hydrogen, \(^2\)H) was needed in anticipation for future \(^2\)H-NMR experiments.

**Amino Acid Synthesis and Purification:**

Of the three Trp derivatives, only 1-methyl-Trp and 7-aza-Trp are available commercially. Therefore, 7-aza-1-methyl-Trp must be synthesized from 7-azaTrp. (Figure 2). Prior to synthesis, the amino acid (α-) amine of (D,L)-7-aza-Trp was "protected" with a BOC-ON reagent, which prevents it from forming bonds out of sequence during peptide synthesis. The indole nitrogen (N1) of Boc-(D,L)-7-aza-Trp was then methylated with triethylamine under anhydrous (water free) conditions at -78°C. The BOC-ON and methylation protocols were adopted from Rich et al. (1995), although several minor adjustments had to be made to the methylation procedure before I was able to consistently obtain a 50% yield of 1-methyl-7-aza-Trp with 95% purity.

![Figure 2: Schematic representation of Boc-D,L-7-azaTrp and Boc-D,L-7-aza-1-methylTrp synthesis reactions. A) D,L-7-azaTrp reacts with BOC-ON in the presence of a base (triethylamine). This results in the formation of Boc-D,L-7-azaTrp. B) The N at position 1 in Boc-D,L-7-azaTrp is deprotonated by anhydrous butyllithium, and methylated with methyl methanesulfonate, to yield Boc-D,L-7-aza-1-methylTrp.](image)
Additional concerns are introduced because 7-aza-Trp is commercially available only as a "racemic" mixture (equal parts D- and L-isomers). As illustrated previously, only the L-isomer of Trp, or modified Trp, is present in the functional peptide. If the stereochemistry is not precise, folding of the helical gA structure will be distorted so as to prohibit the passage of positive ions through the channel interior\(^6\). Thus, to obtain functional peptides, the D- and L-isomers of 1-methyl-7-aza-Trp and 7-aza-Trp were separated using a Chirobiotic T chiral column on the basis of their "handedness," (Figure 3).\(^7,8\) Chirobiotic T is a chiral packing material that consists of Teicoplanin, a glycopeptide, which has been covalently bound to silica gel. Teicoplanin effectively forms a series of cavities to "capture" both L- and D-amino acids, but with variable affinity. An alternative resolution was to separate the two peptide diastereoisomers resulting from the single substitution of one D,L-Trp derivative. This separation is possible because functional and non-functional gA analogues induce unique conformers that elute at different rates from a reversed-phase column\(^9\).

Peptide Synthesis and Purification:

Haiyan Sun and I synthesized a total of 12 singly-substituted gA peptides using standard solid-phase peptide chemistry: [1-methyl-Trp]\(^11,13,15\), gA, [7-aza-Trp]\(^9,11,13,15\), gA, and [7-aza-1-methyl-Trp]\(^9,11,13,15\), gA. Fmoc is the protecting group for all the amino acids except 7-aza-Trp and 7-aza-1-methyl-Trp, which are Boc-derivatized amino acids. Each consecutive amino acid is joined to the growing peptide by an amide bond until the sequence is complete. The peptide is subsequently "cleaved" from the resin using ethanolamine and "formylated" at the amino-terminus using para-nitrophenylformate. As the Boc-group differs from the protecting group of the other amino acids, the peptide must be taken off the synthesizer and deprotected manually using trifluoroacetic acid at these steps.

Once synthesized, each gA analogue was purified on a reversed-phase high performance liquid chromatography column. The final peptides were analyzed at Weill Medical College of Cornell University by single-channel experiments for conformational and functional changes.

Results from single-channel experiments indicate that singly-substituted [1-methyl-Trp] gA analogues form two distinct channels. One channel (A) is remarkably similar to native gA and has a shorter lifetime and higher conductance than the other channel (B), (Figure 4).

Hybrid channel experiments, in which a reference subunit of known helix sense is paired with the peptide of interest, confirmed the A channel to be right-handed. The B channel, which is present only as a minor population, represents a second channel type that is the subject of continuing investigation as to its structural conformation.

Only the right-handed channel type was present in samples of [7-aza-Trp] and [7-aza-1-methyl-Trp] gA. This suggests that there are properties of the 7-aza-group that influence gA to form characteristic right-handed channels. Results also indicate that there is a positive correlation between the dipole moment and observed channel conductance, as for the magnitude of the dipole decreases between [7-aza-Trp] and [7-aza-1-methyl-Trp] gA analogues (which have similar dipole directions), so does the channel conductance.

Dipole Moment Calculation:

Using the \textit{ab initio} molecular modeling program PQS, I was able to accurately calculate the dipole moments for the side chains of all three derivatized amino acids, as well as Trp, for comparison with experimental data. The results illustrated below are based on the converged geometries of each side-chain (Table I; Figure 5).
Table 1

<table>
<thead>
<tr>
<th></th>
<th>Trp</th>
<th>1-methyl-Trp</th>
<th>7-aza-Trp</th>
<th>7-aza-1-methyl-Trp</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental Dipole</strong></td>
<td>2.1 D⁹</td>
<td>-</td>
<td>1.47 D¹⁰</td>
<td>1.34 D⁸</td>
</tr>
<tr>
<td><strong>Calculated Dipole</strong></td>
<td>2.05 D</td>
<td>2.22 D</td>
<td>1.80 D</td>
<td>1.67 D</td>
</tr>
</tbody>
</table>

Figure 5: Orientation of the calculated dipole moment in the side chains of Trp, 1-methyl-Trp, 7-aza-Trp, and 7-aza-1-methyl-Trp.

Electron orbitals were approximated by Gaussian functions of basis set 6-31G*, and all initial geometries were generated by the semi-empirical program PM3. Density functional theory was applied to approximate the electron correlation, and self-consistent field theory was applied to calculate the orbital coefficient.

**Selective Deuteration:**

It was necessary to develop a protocol for the selective exchange of indole hydrogen with deuterium (²H) to eventually determine the orientations of each Trp derivative within a phospholipid bilayer after incorporation into the gA sequence. The selective deuteration of the indole ring of 1-methyl-Trp was achieved by employing a deuterated trifluoroacetic acid catalyst¹¹, (Table 2). This method proved unsuccessful for 7-aza-Trp, for reasons we believe may be related to the protonation of the 7-aza-group (N7). An alternative protocol from Yau and Gawrisch (1999)²² was then considered, which uses Raney nickel as a catalyst for the selective deuteration of indole and 1-methyl-indole. After making several alterations to the procedure, I found that 7-aza-Trp could be dissolved in a 1% NaOH solution before combining with deuterated Raney nickel. Upon sufficient reaction time, the Raney nickel was filtered and amino acid precipitated out of solution using citric acid. Analysis by ¹H nuclear magnetic resonance spectroscopy (NMR) confirmed an ~75% selective exchange (loss of signal) of the 6H with ²H, after three weeks and at room temperature.

Table 2.

<table>
<thead>
<tr>
<th>Proton position</th>
<th>Trp % ²H</th>
<th>1-methylTrp % ²H</th>
<th>7-azaTrp % ²H</th>
<th>7-aza-1-methyl-Trp % ²H³¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>~70</td>
<td>&gt;90</td>
<td>~0</td>
<td>&lt;10</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>&lt;10</td>
<td>&lt;5</td>
<td>~0</td>
</tr>
<tr>
<td>5</td>
<td>~20</td>
<td>~70</td>
<td>0</td>
<td>&lt;10</td>
</tr>
<tr>
<td>6</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;5</td>
<td>~75</td>
</tr>
<tr>
<td>7</td>
<td>~0</td>
<td>&lt;10</td>
<td>~0</td>
<td>~75</td>
</tr>
</tbody>
</table>

* Raney nickel

Discussion:

The results presented in this paper have provided new methods for the methylation of Boc-D,L-7-aza-Trp, the chiral separation of D,L-7-aza-Trp and D,L-7-aza-1-methyl-Trp, and the selective deuteration of 7-aza-Trp. The developments of the first two methods are of significant interest, for the future synthesis of doubly and multiply substituted and labeled peptides. In addition, it is anticipated that the last described method will transfer to other 7-aza-Trp derivatives, thus providing a method for the selective exchange of indole H with ²H for these compounds.

With sufficient quantities of deuterated 7-aza-Trp and 7-aza-1-methyl-Trp through the selective exchange protocol, it will be possible to synthesize additional singly-substituted gA analogues with ²H-7-aza-Trp and ²H-7-aza-1-methyl-Trp at positions 9, 11, 13, and 15. These peptides will then be analyzed by solid-state deuterium NMR, so that we may ultimately understand the orientations of 7-aza-Trp and 7-aza-1-methyl-Trp within the membrane.

New discoveries were made with regard to both the side-chain dipole moments of Trp, 1-methyl-Trp, 7-aza-Trp, and 7-aza-1-methyl-Trp, and the effect of the latter two amino acids on channel function. The ab initio calculations for the Trp derivative side-chain dipoles are comparable with the magnitudes of the experimental dipoles and have allowed for the accurate prediction...
of the side-chain dipole moment of 1-methyl-Trp. Key results to date also indicate that the channel conductance is positively correlated with the magnitude of the dipole moment; however, future experiments regarding the orientation of these singly substituted amino acids will be necessary to confirm the dipole orientations with respect to the ion translocation pathway.

These findings allow us to better understand those properties of Trp that are essential to the tertiary structure and biological function of gA. Overall, the implication of this project is the fundamental knowledge gained concerning the interactions of Trp with other amino acids, water, and lipids. These detailed studies will ultimately contribute to better understanding of folded proteins — especially those that span biological membranes.

Endnotes:

Faculty Comments:
Ms. Scherer's mentor, Roger Koepp, has extraordinarily positive comments about her and her work. He says ...

I write to express enthusiasm for the superb job that Erin Scherer has done in her undergraduate research concerning the development and discovery of new molecular tools for basic research in membrane biochemistry. Ms. Scherer has pursued her research with a remarkable and unique combination of creativity, independence, perseverance and versatility. I will comment on each of these qualities in turn.

Creativity — Beginning with a mere suggestion from me that she investigate the electrostatic and hydrogen-bonding properties of modified indole rings in membrane-spanning peptides, Ms. Scherer defined a broad scope for the project and infused her creative abilities into each aspect of the research. On the experimental side, she pursued new strategies for the labeling of modified rings that were intractable by the methods in current use in my laboratory (see "perseverance," below). Not content with experiments alone, Ms. Scherer on her own sought to fill a gap in the existing literature by doing ab initio calculations (for which I have no expertise) to determine the dipolar properties of her modified indole rings; she obtained the help of Dr. Peter Pulay to perform these calculations. Furthermore, she, on her own, wrote to a leading pharmaceutical company to request a gift — for research purposes — of a particular chemical isomer of one of her molecules that has been reported but is not commercially available. She has gumption and drive, combined with innovative curiosity, that are rare among graduate students or indeed established scientists at any level.

Independence — As noted above, Ms. Scherer expanded the scope of her project, in terms of theoretical as well as experimental aspects, far beyond my original suggestions. She designed and performed her own experiments and calculations in a highly original fashion.

Perseverance — Some of Ms. Scherer’s early experiments involving organic synthesis and catalyzed hydrogen/deuterium isotope exchange reactions did not go well. In particular, the standard trifluoroacetic acid-catalyzed isotope exchange on indole rings, that others in my laboratory were using, did not work for the “7-aza” modified rings that Ms. Scherer needed to use. Undaunted, she pursued a new method, based on catalysis by Raney nickel. Although precedents for this method had been reported for indole itself, the approach to 7-aza-indole was unknown and Ms. Scherer continued to endure failure after failure with difficult experimental procedures. Fortunately, her persistence was rewarded. By suitably modifying the existing procedures, she developed a new method for introducing deuterium into 7-aza-indole rings. The method will have general applicability in our laboratory and others for the preparation of labeled samples as probes for analysis for magnetic resonance spectroscopy in biological membranes.

Versatility — I have already mentioned the two major aspects of Ms. Scherer’s versatility, namely her willingness and flexibility to modify her experimental approaches to achieve the necessary chemical products and her interest in combining theory with experiment. I have not seen other undergraduate students assume such a broad combined perspective toward understanding an overall research question.

In summary, Erin Scherer bubbles with ideas and possesses the practical ability to carry them to fruition. Her inquiring mind makes it a pleasure to work with her.
Biology Professor Claudia Bailey taught Ms. Scherer and has followed her progress closely; she says ...

Many student research projects are offshoots of faculty endeavors. Ms. Scherer has displayed significant understanding of the research project and was awarded a SILO/SURF Undergraduate Research Fellowship. Her research has encompassed several semesters of work, and she has mastered numerous techniques. She will submit her Honors Thesis in April. Ms. Scherer’s project is considerably more sophisticated, complex and technical than most of the honors projects on campus. Ms. Scherer is engaged in a research project in Roger Koeppe’s lab where she is investigating the role of the indole ring of tryptophan, an amino acid present in the antibiotic gamacdin, in augmenting the interaction between ions, water and the plasma membrane of cells. She will employ deuterization of the amino acid solid phase peptide synthesis to modify the structure of the antibiotic. The newly synthesized molecule, once inserted into a membrane, should modify the flow of ions through the membrane. Ms. Scherer then utilizes solid state deuterium NMR to determine the localization of Trp relative to the protein and its orientation in the membrane.

Ms. Scherer’s work is generating data that contribute to the fundamental knowledge for predicting the effect of the modification of protein tertiary structure on the functional state of the protein. This work impacts protein chemistry, membrane structure and function, and drug design. Her body of work should have significance in the biochemistry and medical chemistry communities, and I would expect it to be publishable in a peer-reviewed professional journal. This is a highly sophisticated project for an undergraduate and attests to the confidence Dr. Koeppe has in Ms. Scherer’s abilities. I have seen few student research projects that compete with Ms. Scherer’s research in categories of significance, rigor, complexity, and quality.

In 2001, I recognized Ms. Scherer’s abilities when she was among the top five students in cell biology, all of whom achieved a 100% level of performance. Ms. Scherer wrote the most articulate essay answers I have read in many years. Not only was she factual, but her logical development of the topics indicated a thorough understanding of the principles involved. In class, her questions indicated good critical thinking and often helped other students to "break the silence" barrier, encouraging others to participate. Her enthusiasm for learning was always evident. In her discussions with me about her current research these same qualities continue to be apparent.

I also am impressed with Ms. Scherer’s involvement in summer research programs. She has sought opportunities to expand her experience in the research laboratory while remaining focused on her goal to apply her research skills to the medical sciences. The work in Dr. Dave Wessinger’s lab at UAMS to determine a preliminary pharmacokinetic profile for a psychoactive anti-pertussive agent available in over-the-counter cough medications was fascinating. Both the UAMS and UAF research programs have significant clinical potential that she can explain and evaluate.

Ms. Scherer remains focused upon a professional research career and undoubtedly will continue to make contributions in her field. She is a highly worthy candidate for the Outstanding Undergraduate Research Award.

Chemistry Professor Lothar Schafer taught Ms. Scherer in his honors colloquium. He writes ...

I know Erin Scherer as her teacher in CHEM 3923H, the Chemistry Department's honors colloquium, in which she is currently enrolled.

Ms. Scherer's research is involved with Gramicidin A as a model system for studying the properties of tryptophane. Tryptophane is an important amino acid because in membrane proteins it aligns between the phospholipid (water-repellant) and the aqueous phases. Tryptophane also has an important function in controlling the flow of ions through membrane channels. When it is removed from gramicidin, conductance is decreased. In the recent past Dr. Koeppe’s research in this area has found a great deal of national and international recognition. It is a first class program, and Ms. Scherer’s project is really at the cutting edge in this field, not a typical undergraduate-level project. Her research is of general significance because it can potentially lead to the development of antiviral and antibiotic drugs. Most importantly, I expect it to generate important information on how amino acid residues contribute to tertiary protein folding, one of the last true frontiers of protein chemistry.

In our discussions in CHEM 3923H, I have found Erin Scherer to be a brilliant young lady, very articulate, highly intelligent and able to think critically and independently. It is a pleasure to witness how vigorously she can discuss concepts that have caught her interest.
SIMULATION AND MODELING OF POND FORMATIONS ON THE ASTEROID 433 EROS

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Abstract:

"Ponds" are surface features that appear to be the result of the deposition of finer material on the asteroid's regolith, the layer of loose sediment that blankets the entire asteroid. Ponds were discovered by the NEAR Shoemaker spacecraft in January, 2001, on the asteroid 433 Eros. The objective of experimentation is to simulate the development of these ponds in the Andromeda planetary sciences environmental chamber.

The Andromeda Chamber offers a unique ability to simulate surface environments that occur on Eros. The chamber was used to simulate the processes that occur as Eros emits gas through its outer surface, including the process of fluidization in which the loose soil takes on the properties of a fluid. This was accomplished by placing sand with a large particle size distribution in the chamber and analyzing the processes that occur as nitrogen is forced up through the surface. The amount of gas that is emitted, the rate at which it is emitted, and the depth of the soil stimulant the gas is passed through were varied. Particle size sorting and subsequent pond formations exhibited a strong, exponential dependence on flow rate of a gas, as well as a linear dependence on the depth of the gas source.

Introduction:

Asteroids are material left over from the formation of the solar system. One theory suggests that they are the remains of a planet that was destroyed in a massive collision long ago. More likely, asteroids are material that never coalesced into a planet. Much of our understanding about asteroids comes from examining pieces of meteorites collected from the surface of the earth. Because asteroids are material from the very early solar system, they offer a glimpse into the origins of the formation of the solar system through analysis of their composition and characteristics.

The NEAR-Shoemaker (NEAR) mission was designed to be the first to observe a near earth asteroid (NEA) in-situ and answer many of the questions regarding the characteristics of an asteroid. Launched on 17 February, 1996, the NEAR-Shoemaker mission was a comprehensive surface characterization of the S-type near earth asteroid 433Eros. The NEAR mission's objectives were to determine the physical and geological properties of a near-earth asteroid, to clarify relationships among asteroids, comets, and meteorites, and to further our understanding of how and under what conditions the planets formed and evolved. The NEAR mission provided many interesting and exciting observations into the characteristics of 433 Eros. Results from the mission include a detailed mapping of the asteroid's surface and determinations made for its size, shape, rotation rate, mass, density and composite ion.

One of the remarkable discoveries from this mission occurred in late January, 2001, when observations revealed the development of smooth, distinctive deposits in the depths of craters previously formed by impact of a foreign object on the surface of the asteroid. These deposits, labeled "ponds" appear to be the result of the deposition of finer material separated from the upper portion of the Asteroids regolith, the layer of loose sediment that blankets the entire asteroid, and emulate characteristics of a fluid.

At the present time no accepted explanation exists for the processes that have caused these ponds to form. However, the development of these "ponds" does not appear to be random. Eros' Ponds are not distributed randomly over the surface of the asteroid. Although they do occur at all latitudes, 91% of the existing ponds occur within ±30° of the equator [7]. In particular there are zones within a few degrees of the equator centered west of the long ends of the asteroid, where the gravitational pull is the lowest that have distinctly more and larger pond deposits.

This lack of randomness leads to a search for the explanation of how these ponds were formed. Although there are as of yet no proven theories on the origin of development of these features, it is believed that ponds are the result of a fluidization of particles, in which the loose particles take on the properties of a fluid and subsequent size sorting. It is possible that this process occurred as volatiles were released from beneath the surface of 433 Eros.
and passed through the particles as it proceeded toward the surface of the asteroid. The present work described here seeks to simulate the processes that occurred as Eros emitted gas through its outer surface.

Experimentation:

The Andromeda facility, located at the University of Arkansas, Fayetteville, is part of the Arkansas-Oklahoma Center for Space and Planetary Sciences. Andromeda has been specifically established as a planetary/asteroidal surface simulation chamber. The chamber was donated by JPL to the Center in 2000 and was previously used for experiments in mineral-atmosphere interaction on Mars. After refurbishment, the chamber was commissioned for planetary simulation work in the summer of 2001.

Current instrumentation on Andromeda includes an atmosphere and pore gas sampling system, connected to a 16 port multiposition valve, in turn connected to a Varian CP-2000C micro-gas chromatograph system. A mass spectrometer will be added in the near future. Surface light reflectance is measured with a fiber-optic based USB-2000 Ocean Optics UV/Visible spectrometer, and plans are being drawn up for addition of an additional infrared source and IR spectrometer. Illumination is provided by a Spectral Energy solar illuminator, equipped with space AM-0 and a special “Martian surface” filter assembly. Wireless X-10 cameras are used to provide imagery of the interior during experiments and their signal is broadcast to either VCR units or directly to a computer.

Andromeda is equipped with atmosphere, temperature, and pressure control systems, allowing a broad range of environmental simulations. While it is not possible, due to safety and instrumentation limitations, to fully simulate ionizing radiation environments, facilities will be available for both 90 Sr-based beta irradiation and X-ray irradiation within the chamber.

A volume of sand measuring approximately 0.04 cubic meters of sand was placed inside the andromeda chamber to create a depth of 0.2286 meters from the surface of the sand to the bottom of the sample bucket. A tube connected to a container of N₂ gas was buried at various depths ranging between 0.0762 meters and 0.2032 meters. The flow rate of N₂ was controlled by a valve connected to the tank of N₂ gas, and ranged between 0.298 m/s and 2.97 m/s. Experiments were conducted to test the dependence of pond formations to depth of volatile source and flow rate of volatile through the bed of particles, and a wireless camera system was used to observe the fluidization process as it took place.

Results:

The first set of experiments was designed to determine the dependence of pond formation on depth of volatile source. This was accomplished by burying the tube connected to the tank of N₂ gas at multiple depths while the flow rate of gas was kept constant. The resulting pond formations on the surface of the sand were then observed and measured. The resulting data displayed a linear dependence of pond formation versus depth (Figure 9).

The second set of experiments was designed to determine the dependence of pond formation on flow rate of volatile through the bed of particles. This was accomplished by burying the volatile source at a constant depth while the flow rate of N₂ gas through the bed of particles was varied. The resulting data displayed an exponential dependence of pond formation versus flow rate at constant depths of .0762 meters and .2032 meters (Figure 10, Figure 11).

The radius of pond formations was measured after each experiment to determine the surface area that each encompassed. Due to the inability to distinguish an outer limit of size sorting of several of the pond formations, small pebbles were scattered along the surface in order that the outer limit was more easily determined.

Discussion:

Although there are as of yet no absolute theories on the mechanism for pond formation, two theories have been decidedly the best to date with the third presented here.

Photoelectric size sorting and seismic agitation from impact are two present theories describing the mechanism for pond formation.

I. Photoelectric size sorting

Mark Robinson, a geologist at Northwestern University and member of the NEAR-Shoemaker team, believes that the “ponds” geography is consistent with some of the predicted aspects of photoelectric sorting [7]. Photoelectric sorting is caused when radiation causes sediments in one area to all become equally charged. This causes the sediments to repel one and sort dependent on size, causing electrostatic levitation. Photoelectric sorting was first observed by Surveyor-7 along the western lunar horizon as a result from the forward scattering of sunlight by electrically charged dust grains. However, the transport and depositional mechanisms of this process are not well understood. Also this process does not explain why granular material should fall back into gravitational lows, nor does it explain why the surfaces of ponded deposits should be gravitationally level, to such an extent that sharp boundaries develop. Therefore, this theory cannot alone explain the mechanism of pond formation.

II. Seismic agitation from impact

A.F. Cheng et al. propose a simple model of seismic agitation from impacts to account for pond formation on Eros, based upon observations of 433 Eros from the NEAR mission.
[3]. Large ponds on Eros are found preferentially in an equatorial belt near the ends of the elongated asteroid, where seismic shaking after impacts may have greatest amplitude. A dry, granular material can exist in a stable condition when in a slope below the angle of repose, but can become mobilized by agitation to create a debris flow. The Cheng et al. model involves a continued interaction of boulders with a consolidated substrate during shaking to induce a relative motion between fine granular materials and the boulders, because the boulders can move downslope only to a limited extent compared with fines. Boulders, as defined in the model, represent a material with a size large enough to rest upon the consolidated substrate and to protrude above the shallow layer of consolidated fines. The model invokes gentle shaking that causes slope failure without tossing the boulders a large distance. It predicts a terrain where topographic highs appear to have a larger population of boulders; whereas, topographic lows appear smoother because they become filled with fine particulates and many of the boulders are covered.

III. Fluidization of Particles by Surface Degassing

The third theory on the mechanism of pond formation, presented here is pond formation upon degassing of the asteroid that occurs when gas is released through the surface. The flow of gas through these depositions as they move toward the surface may cause a fluidizing mixture of the sediments, therefore, they contribute to the processes that govern the formation of these “ponds”.

Particle sorting can arise after fluidizing mixture of granular materials. The degree of separation depends on material properties of the granules such as size, density, shape and particle resilience [1]. The greater the differences in the particles the more efficient the separation becomes. For fluidized systems in which a gas is passed upward through a bed of particles, the separation of granules depends upon the relative gas drag experienced by each particle. When the gas reaches a minimum fluidization velocity, the bed attains fluid-like characteristics, and the particulate solids are free to move according to their physical properties. In general, larger and heavier particles tend to settle out of a gas stream before smaller and lighter particles. This type of segregation is an important aspect of many processes in engineering and geophysics.

IV. Absolute Mechanism of Pond Formation

Each of the present theories does not absolutely explain the mechanism of pond formation. Each neglects certain facts of the characteristics and distributions of ponded deposits on 433 Eros.

Photoelectric size sorting as a mechanism for pond formations leaves many questions unanswered. The particle size fraction that can be levitated on an asteroid is not certain, as it depends on poorly known characteristics such as particle shapes, electrical resistivity, and photoelectron yields [7]. Also small particles may simply be ejected with a velocity greater than the escape velocity of 433 Eros. Since the dominating charging currents are photoelectric and solar wind, which have shallow penetration depth, single charging episodes can levitate at most only a monolayer of grains [5]. Once levitated, these grains must not fall back randomly over the surface but must return to the surface in gravitational lows, guided by electrostatic forces. This sequence must happen repeatedly to build up meter-thick deposits in ponds. Electrostatic levitation can not alone explain why grains should fall back into gravitational lows, nor can it explain why the surfaces of these deposits should be gravitationally level, to such an extent that sharp boundaries develop.

Seismic agitation from impacts neglects the destruction of pond formations while predicting that impacts create fluidization of sediments causing ponded deposits. While it has been proven experimentally that seismic shaking will induce a particle size sorting in a bed of sediments, a certain frequency is required to do so. Impacts near formed ponds will cause the sediments to redistribute in a chaotic manner. This neglects the distribution of ponded deposits on 433 Eros, as they are distributed in a manner very near to each other. However, the ponded deposits are not close enough, as one impact would form all in the distributed area. Thus, Seismic agitation cannot absolutely explain the existence of ponded deposits and their distribution on Eros.

Fluidization of particles by surface degassing also leaves distribution characteristics of ponded deposits on Eros unexplained. Ninety-one percent of the existing ponds occur within ± 30° of the equator [7]. This would mean that a large vein of volatiles would have had to lie beneath the surface of 433 Eros along this area. Also, the fact that the ponded deposits on Eros would have had to have been formed in the early stages of the asteroids creation leaves many speculations as to the characteristics of Eros in its early stages of development.

It seems as if there may have been a combination of photoelectric size sorting, seismic agitation, and fluidization by degassing as the mechanism for pond formation. Each theory alone cannot account for all of the characteristics of pond formations on 433 Eros. However, when combined, they are able to describe all observed characteristics.

Conclusion:

Experimentation showed that it was possible to induce particle size sorting by fluidization of a bed of particles in a degassing process. The size of the resulting ponds depended heavily upon depth of a volatile source and the flow rate at which a gas was passed through the bed.

Depth of source as related to resulting pond formation appears to be a linear regression. The data predict that as the depth of a volatile source is increased, the resulting pond will decrease in size. There also appears to be a range of depth for which a pond will be formed. At too shallow a depth, or too great, no pond will appear. Instead, there will either be a chaotic
mixing in the case of shallow depth or no pond formed at all in the case of too great of a depth.

Formation of ponded deposits seems to depend more heavily on the velocity at which a gas is released through a bed of particles. The data suggest that flow rate governs the area covered by the pond at any depth. There is a somewhat linear progression in flow rate versus area of the resulting ponded deposit. There is however a minimum velocity required to begin any fluidization process in a bed of particles, as observed during experimentation. Experimentation also predicts a range in which fluidization will cease to take place in a ratio with the depth of the source.

The data gathered from these experiments suggest that it was possible that ponded deposits on Eros 433 were the result of a fluidization process caused by degassing of a volatile through a bed of particles. However, this does not seem to be an absolute explanation as to the existence of ponded deposits on Eros 433, nor do any other theories presented to date. It seems as if there may have been a combination of seismic agitation and fluidization by degassing as the mechanism for pond formation as a result of the data gathered and observations of the surface of 433 Eros. A greater amount of study into the distribution and characteristics of ponded deposits may provide a more complete explanation as to the mechanism of their creation.

Acknowledgements:

I thank Dr. Derek Sears for his funding and encouragement of this project, as well as Drs. Paul Benoit and Mikhail Kareev for their tireless efforts in aiding with the experimentation and instrumentation. Without them none of this work would have been possible.

References:


Figure 1. A view of 433 Eros from the NEAR-Shoemaker spacecraft. (http://near.jhuapl.edu/index.html)

Figure 2. Various Ponds located on the surface of the asteroid 433 Eros. (http://near.jhuapl.edu/index.html)
Figure 3. Pond distribution on the surface of Eros 433. (http://near.jhuapl.edu/index.html)

Figure 4. The Andromeda Laboratory.

Figure 5. The science rack of the Andromeda environmental chamber.

Figure 6. Sample bucket for the Andromeda environmental chamber.

Figure 7. Experimental pond formation.

Figure 8. Experimental pond formation.
### Table 1. Pond Radius at Constant Depth.

<table>
<thead>
<tr>
<th>Flow Rate (Meters/Second)</th>
<th>Radius of Pond (Meters)</th>
<th>Constant Depth (Meters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.97</td>
<td>0.0476</td>
<td>0.2032</td>
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<tr>
<td>1.49</td>
<td>0.0381</td>
<td>0.2032</td>
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<tr>
<td>1.19</td>
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<td>0.2032</td>
</tr>
<tr>
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<td>0.0176</td>
<td>0.2032</td>
</tr>
<tr>
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<td>0.0191</td>
<td>0.0762</td>
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<tr>
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<td>0.1016</td>
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</tr>
<tr>
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<tr>
<td>1.49</td>
<td>0.1524</td>
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</table>

### Table 2. Pond Radius at Constant Flow Rate.

<table>
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<tr>
<th>Depth of Source (Meters)</th>
<th>Radius of &quot;Pond&quot; (Meters)</th>
<th>Constant Flow Rate (Meters/Second)</th>
</tr>
</thead>
<tbody>
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<tr>
<td>0.203</td>
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<td>0.298</td>
</tr>
</tbody>
</table>

Figure 9. Depth of Source vs. Radius of Pond at a Constant Flow Rate (.0762 meters).

Figure 10. Flow Rate vs. Radius of Pond at Constant Depth (.0762 meters).

Figure 11. Flow Rate vs. Radius of Pond at Constant Depth (.0762 meters).
Faculty Comments:

Mr. Thompson's faculty are very enthusiastic about the quality and value of his work. Professor Benoit made the following remarks:

Mr. Thompson is an excellent, bright young student. I have worked with him for almost a full year during his summer work as a NSF REU summer researcher and continuing through the Fall and Spring semesters as a researcher. During that time, he has worked on several major projects, verging on the scale of graduate work, while carrying a full load of classes in physics. On a personal level, Mr. Thompson is affable and gets along with just about everyone. He bubbles with enthusiasm over his work. I think you will find him an excellent representative of our advanced undergraduates.

Professor Kracher, who worked with Mr. Thompson at the Center for space and Planetary Sciences, had the following to say:

This letter is in support of Justin Thompson who is submitting an article for Inquiry. Mr. Thompson is working in the Arkansas-Oklahoma Center for Space and Planetary Sciences. I am research assistant professor in the same institution.

Mr. Thompson has been working with the Andromeda chamber, a facility to simulate the conditions on other planets and asteroids. In particular he has been doing research on the formation of features that were discovered by the NEAR Shoemaker spacecraft on the asteroid Eros. These features, referred to as “ponds,” apparently consist of sharply delineated areas of fine-grained material that are found in certain craters. Mr. Thompson has used his background in mathematics to develop models that improve our understanding of the formation of these ponds. He has also collaborated on experiments in the Andromeda chamber to simulate pond formation and test the mathematical models.

Mr. Thompson has a good understanding of the principles of scientific research and is able to work independently. His grasp of mathematical modelling has enabled him to make valuable contributions to the research efforts of the Center, particularly the modelling of pond formation on Eros. He has recently attended the Lunar and Planetary Science Conference in Houston, where a poster contribution, on which he was a co-author, was presented. Mr. Thompson is planning to pursue his work in space science research.

Professor Derek Sears also worked with Mr. Thompson. He said ...

Mr. Thompson was an REU student in our group last summer, and he worked on some preliminary experiments on dating sediments by their optical luminescence. He enjoyed the work, which required considerable field work with little supervision from me. He did the work in a competent and responsible fashion and made a poster presentation at the end of the summer. He impressed me sufficiently that I invited him to join the group on a permanent basis, and he is now involved with the Andromeda environmental chamber research, investigating the surface processes on asteroids. In this connection, he has just returned from the Lunar and Planetary Science Conference in Houston. Mr. Thompson is a bright and enthusiastic undergraduate student with a natural flare for research and an extremely good ability to work on a team. His enthusiasm is infectious.
Figure 2. Graph comparing the qualitative and quantitative data from 6hr experiments.

Figure 3. Treatment with Jasplakinolide at 24hr Results in Less Disruption. A. Tropomyosin in control cell. B. Tropomyosin in 24hr treated cell. C. Myosin in control cell. D. Myosin in 24hr treated cells. (For 6hr cells see Figure 2.)