An Integrated Approach to Use Genetic Resources for Resurrection Plants to Enhance Breeding-Extension Programs to Improve Drought Tolerance.

Mel Oliver
& Paxton Payton
USDA-ARS
Columbia, MO: Lubbock, TX

Robert Sharp
University of Missouri

John Cushman
University of Nevada, Reno

12 Step Proposal Plan

1) **National need ("the Problem")**
   - **(what is the big picture for your research?)**
2) **Scope of your own research**
   - **(what problems are you capable of addressing - "A man must know his limitations")**
3) **Assemble a strong collaborative team**
   - **All have same basic interest and work well together**
4) **Stakeholder Needs - National and Local**
   - **What is the "real world" focus**
5) **Focus the research to Stakeholder needs**
   - **Focus your science to the needs of the stakeholders - short and long term if possible**
6) **"Integrate" research to drive Outcomes**
   - **What are the outcomes the research drives within the integrated program**

12 Step Proposal Plan (cont)

7) **"Integrate" research within the Education/Extension Framework**
   - **Does the research focus impact and enhance the Education/Extension aspects.**

8) **Think both short and long-term outcomes**
   - **What impacts can you have? Can you deliver a sustained benefit to Stakeholders?**

9) **Plan within the limits of what is achievable**
   - **Again - you must acknowledge your limits of what is doable in the time frame**

10) **Strong management plan**
   - **Important to be able to measure progress and track achievements**

11) **Write succinct proposal**
   - **Allocate areas to each Team member - coordinate - P.I. coalesces input for clarity.**
   - **Preliminary data helps!**

12) **Funding - Now the fun (and responsibility) begins**

Area of Need

"We must learn to produce nearly three times as much food for the more populous and more prosperous world of 2050, and from the farmland we are already using, in order to save the planet's wildlands."

Norman Borlaug 2002

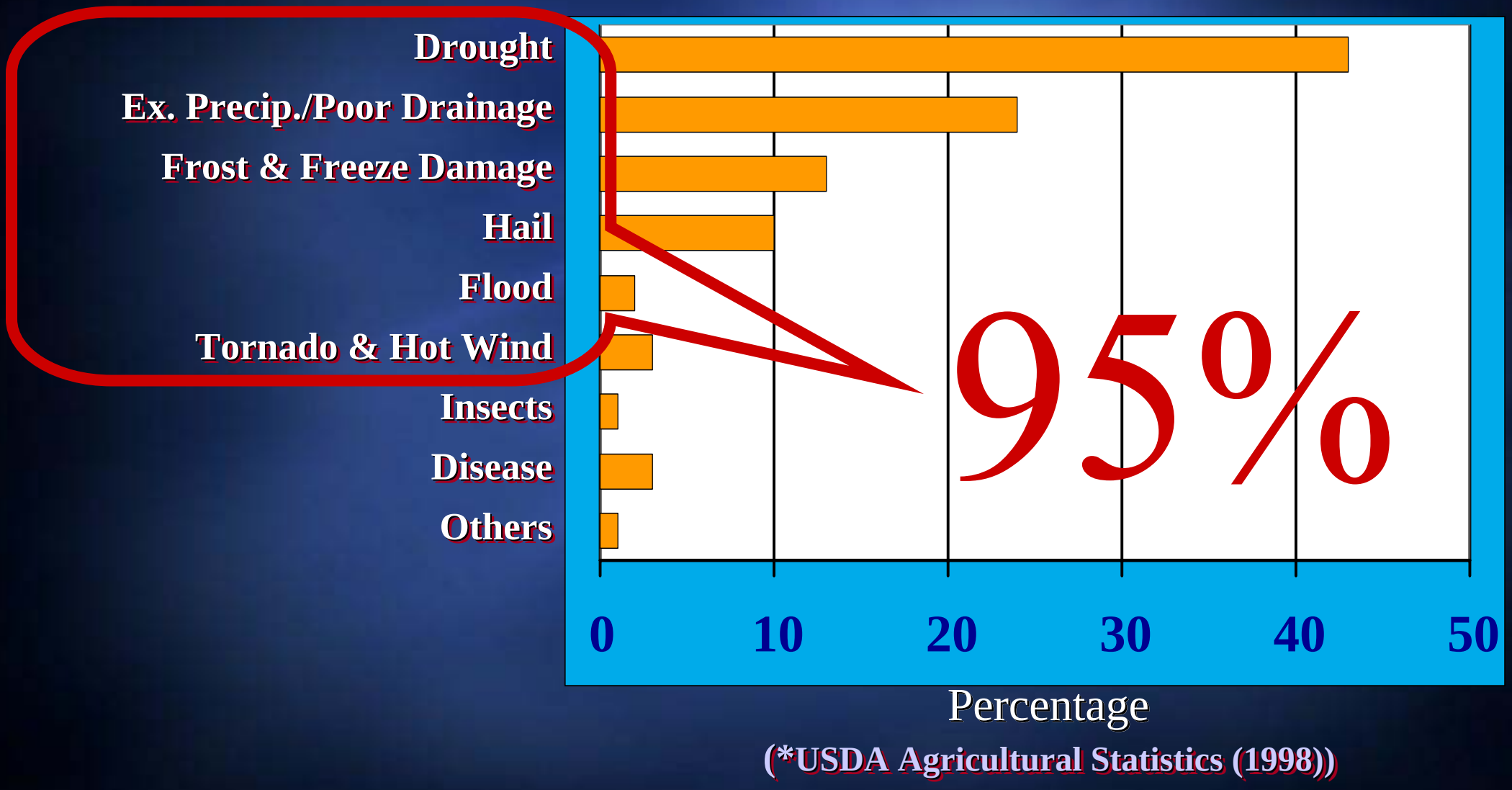
A Hungry World...

- **World population has doubled since 1950:**
  - **Pre 1950: 3 billion**
  - **1950 -> 2000: 6 billion**
  - **2000 -> 2050: 9 billion**

- **Increasing population will increase demand for food/energy:**
  - **45% increase expected by 2020**
  - **30% increase due to increased meat consumption**
  - **75% increase in food and feed demands**

- **Global consumption patterns are changing:**
  - **Increased demand for animal protein and biofuels**
  - **= increased demand for grain**

Average Percentage of Indemnities by Hazard, all crops, 1948-1996*



(*USDA Agricultural Statistics (1998))

Step 1

Drought Tolerance

Step 2

Team Interests

- **Mel Oliver** - ARS - Dehydration/Desiccation Tolerance in Plants
- **John Cushman** - UNR - Plant Stress Tolerance - Salt and Dehydration
- **Robert Sharp** - UM - Plant Stress Physiology - Drought
- **Paxton Payton** - ARS - Plant Stress Responses - Drought and Oxidative Stress

FY 2007 Priority for Integrated Activities

Plant breeding and germplasm enhancement, with particular emphasis on development of drought tolerant agricultural plants and on training scientists in plant breeding. Applicants are encouraged to utilize germplasm from the National Plant Germplasm System (NPGS).

Integrated projects

"Integrated" means to bring together the three components of the agricultural knowledge system (research, education, and extension) around a problem or issue. In FY 2007, integrated project proposals must include research, education, and extension/outreach objectives (at least two of three). In general, strong integrated projects will be stakeholder driven, issue focused, and outcome based. They will exhibit a collaborative team approach, contain strong plans for project management and project evaluation, and produce sustained education/extension initiatives.

Focus of the Proposal:Forage

- **Missouri**
  - #6 in Livestock
  - > 6 million cattle
  - > 200,000 horses
  - > 4 million acres of forages (pasture)
  - > 7 million tons of forage
- **Nevada**
  - Range Livestock production predominates
  - > 1/2 farms run cattle or sheep
  - 67% of State total farm receipts - cattle & calves and forage
  - #1 crop - forage at > 500,000 acres

Identify Your Stakeholders (UNR):

- The Nevada Farm Bureau
- The Nevada Cattleman's Association
- The Nevada Hay and Forage Growers Association
- The Nevada Department of Agriculture

Identify Your Stakeholders (UM):

- The Missouri Farm Bureau
- The Missouri Cattleman's Association
- The Missouri Forage and Grassland Council
- The Missouri Department of Agriculture

Assessing Stakeholder Needs:

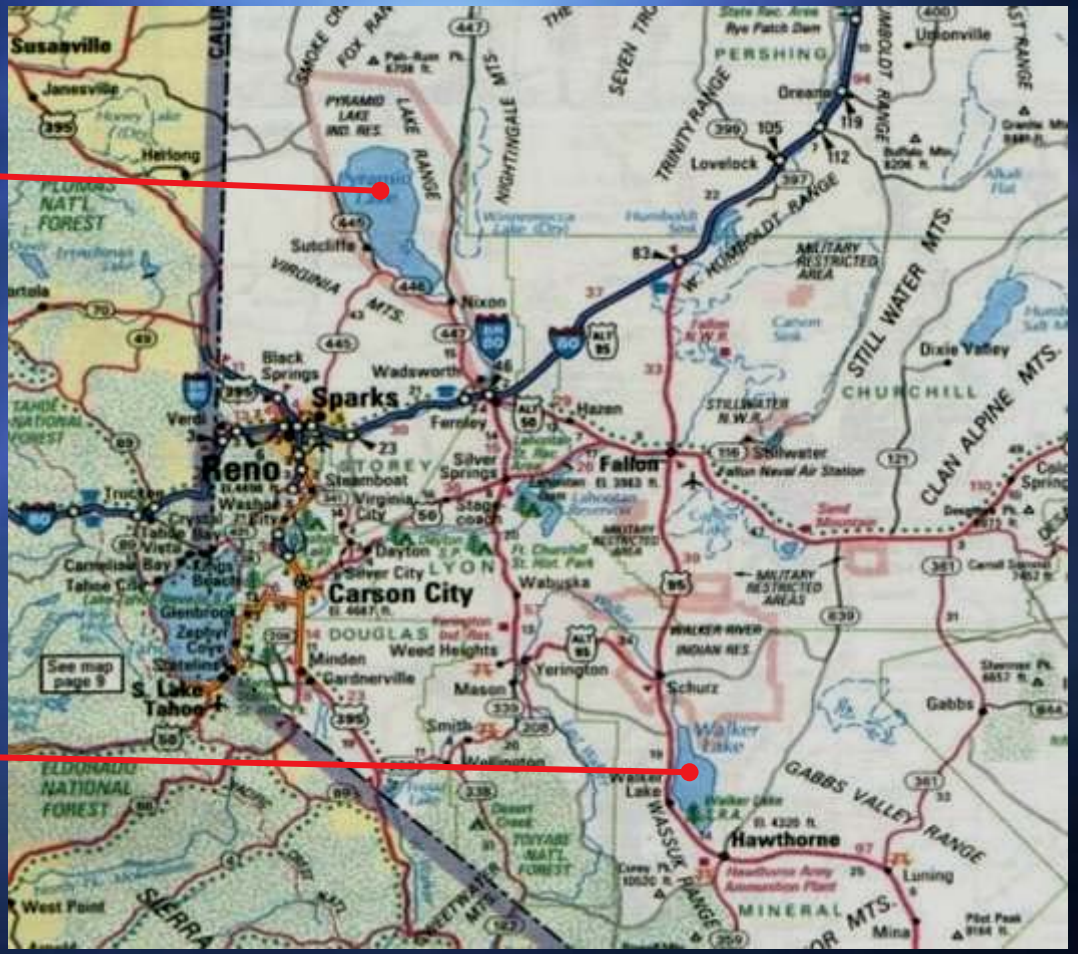
- Water was by far the most critical resource
- Issues related to water use were a top priority
- Growth has resulted in bitter conflicts between current water right holders (agricultural producers) and development interests in the cities
- Development of drought tolerance crops
- Development of alternative crops

Survey results of Dr. Loretta Singletary
University of Nevada Cooperative Extension

Nevada's Vanishing Freshwater Lakes

Pyramid Lake

Walker Lake



Step 4/5

Assessing Stakeholder Needs:

- *"A drought is like any other natural disaster in that its destructive nature adversely impacts the livelihood of Missourians"*
- *"Missouri farmers and their livestock are feeling the worst effects of the drought"*
- *"Pastures are hit the hardest with 75 percent listed as poor or very poor"*
- *"Farm families across many counties in Southeast Missouri are being hit hard by a severe drought, which is --- leading to economic hardships for livestock producers,"*

Governor Matt Blunt

Assessing Stakeholder Needs:

"I am enthusiastic about this project because the goals of the proposal meet many identified critical needs of the agricultural producers in Nevada"

- Jay Davison
Area Forage/Alternative Crops Specialist
University of Nevada Cooperative Extension

Assessing Stakeholder Needs:

"No other natural phenomenon reduces pasture growth, and thus beef production in Missouri, more than does drought"

- Robert Kallenbach
Extension/Research Assoc. Professor MU
Board Member: The Missouri Forage and Grassland Council

Assessing Stakeholder Needs:

“Increasing energy costs related to pumping water from deep aquifers are damaging the economic viability of agricultural operations in Nevada”

- Jay Davison
Area Forage/Alternative Crops Specialist
University of Nevada Cooperative Extension

Plant Breeding

"…the number of new entrants into the plant breeding industry has continued to decline ….. Many fear that if current trends are not reversed, the plant breeding industry will soon face a critical shortage of skilled breeders "

"Ensuring the future supply of well trained plant breeders will depend on ….. their* [public and private sectors] *ability to learn to collaborate more effectively "

"The basic education of plant breeders remains a quintessentially public good, … "

Morris et al., 2006. Hort Science. 41: 30-39

Scope of Plant Breeding

- **Traditional "tool-kit"**
  - **Quantitative genetics**
- **Modern "tool-kit"**
  - **Quantitative genetics**
  - **Laboratory-based tools**
    - **Genomics - both structural and functional**
    - **Transformation**
    - **Marker Assisted Selection**
    - **Physiological assessment (quantitative traits)**

Forage: Consequences of Drought

- Drop in production (biomass) compared to long term forage production averages
  - Stocking rates are set by long term forage production averages
- Greater demand on forage resource - overgrazing
- Lowers quality of forage
  - Animals range farther and eat more to compensate thus reducing body condition in mature animals and reduced gain in immature stock
- Drain on stored carbohydrates in crown and roots
  - Loss of root vigor and fewer basal buds - loss of subsequent crops regardless of moisture
- Increase in toxic compounds - e.g., nitrates and alkaloids

Goals

- To educate and hone the skills of a generation of Plant Breeders with tools from the “Modern Toolbox”
- To bring the modern fields of genomics and quantitative molecular genetics to our stakeholders
- To develop novel strategies for the improvement of drought tolerance in forage species (and crops)
- To explore alternate forage species based on resurrection grasses

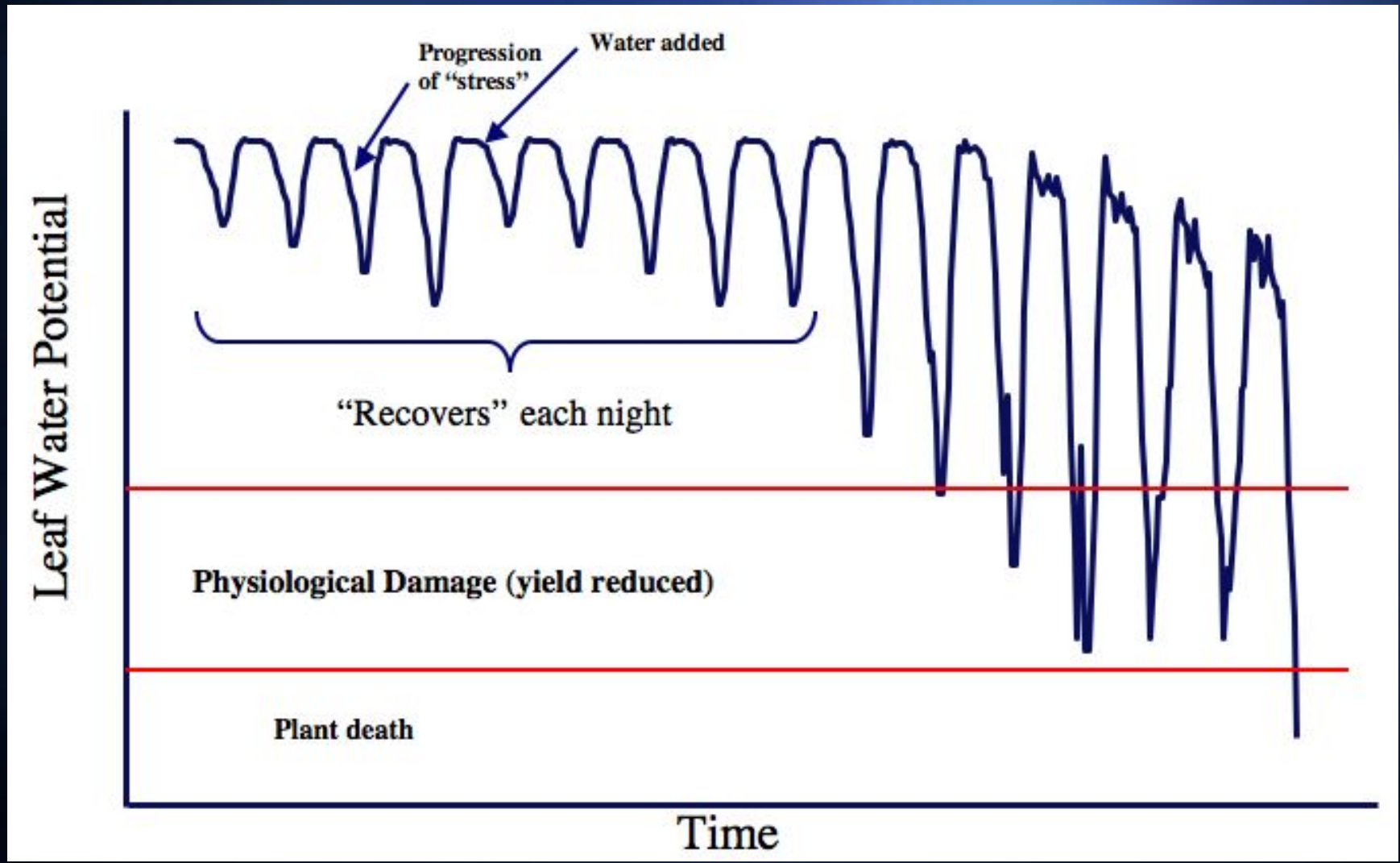
Objectives:

1. Develop/enhance courses in:

- Plant Breeding, Propagation, & Biotechnology (UNR)
- Plant Environmental Stress Physiology & Ecology (UM)
- **Integrated Research**
  - Identify novel genetic determinants for desiccation/dehydration tolerance
    - *Selaginella lepidophylla* (Club moss) (UNR)
    - *Sporobolis stapfianus* (African Inselberg grass) (UM)
  - Identify distinct water deficit stress responses by comparing closely related species pairs that differ in desiccation/dehydration tolerance

2. Develop integrated research and extension projects using *Sporobolus* as low water input forage grasses

Water Stress Cycle



Step 6

Drought and Desiccation Tolerance

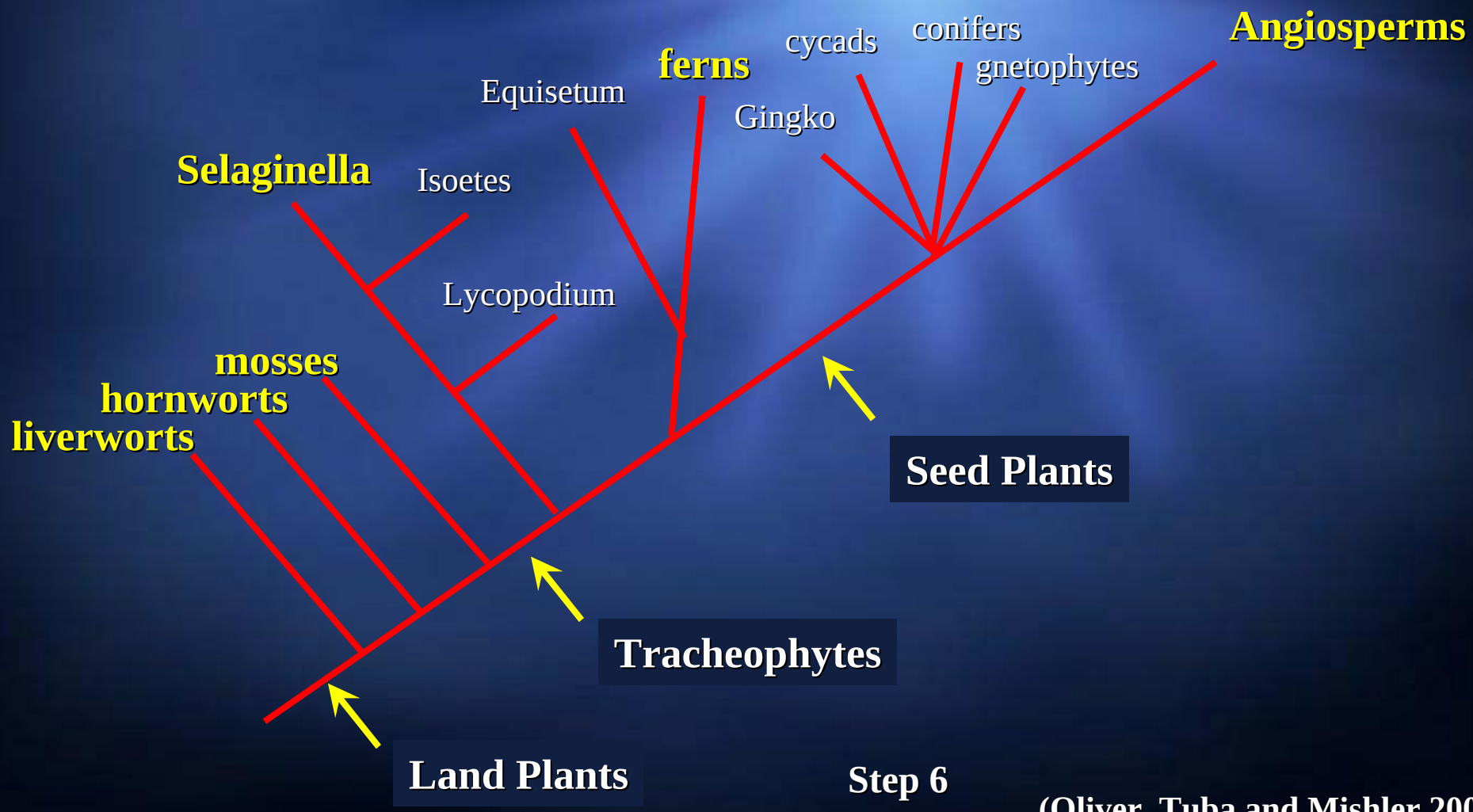
Drought tolerance = tolerance of sub-optimal water availability

Desiccation tolerance = tolerance of complete drying to equilibrium with the air

Drought tolerance mechanisms include ways of maintaining cell water content (e.g., stomatal closure), whereas desiccation tolerance consists of ways to survive the complete loss of water.

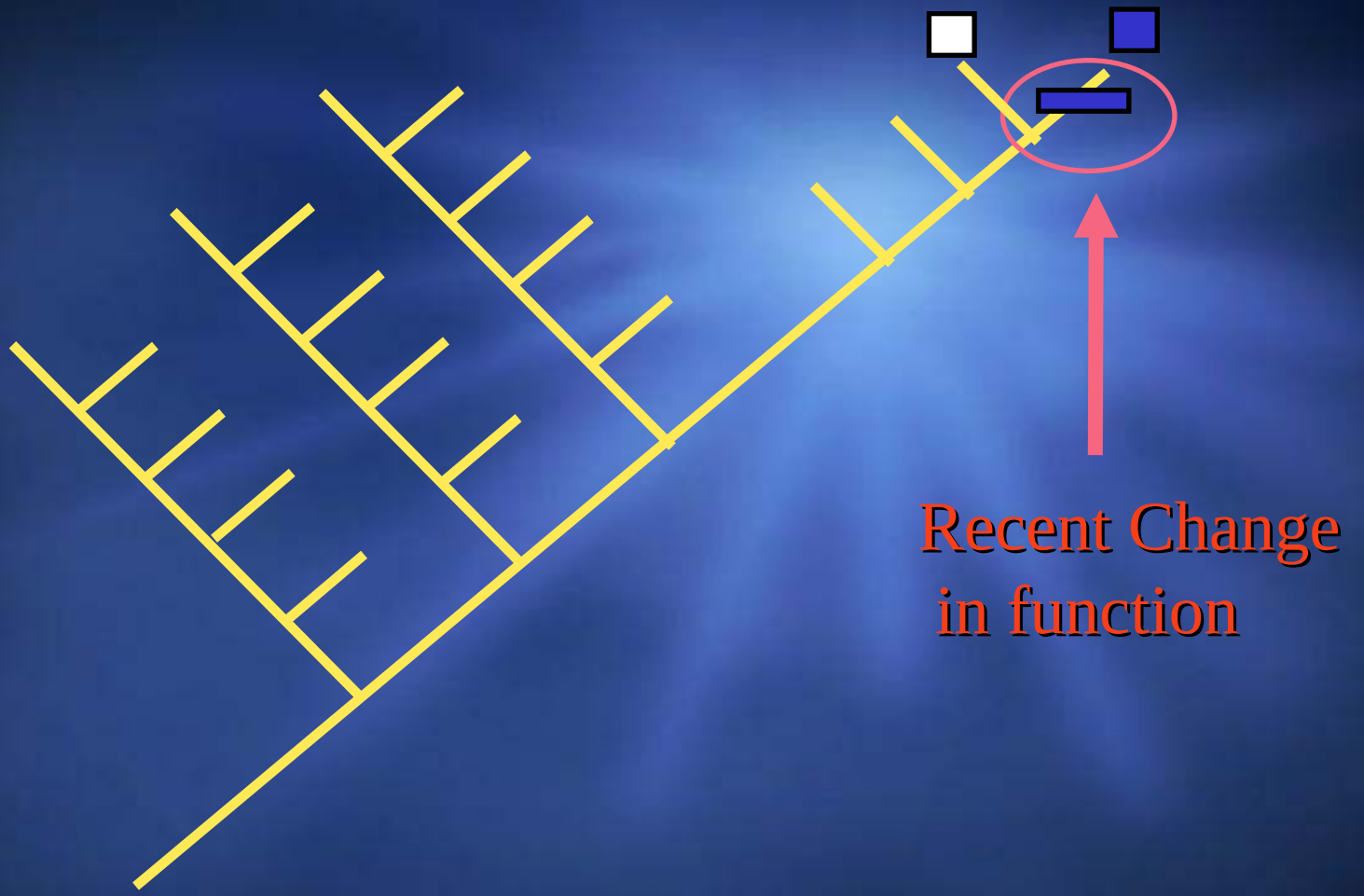
There maybe some genes common to both traits, in particular those genes that respond to the cellular dehydration but it is also likely that a large number of genes are unique to each trait.

Distribution of Desiccation Tolerance in the Plant Kingdom

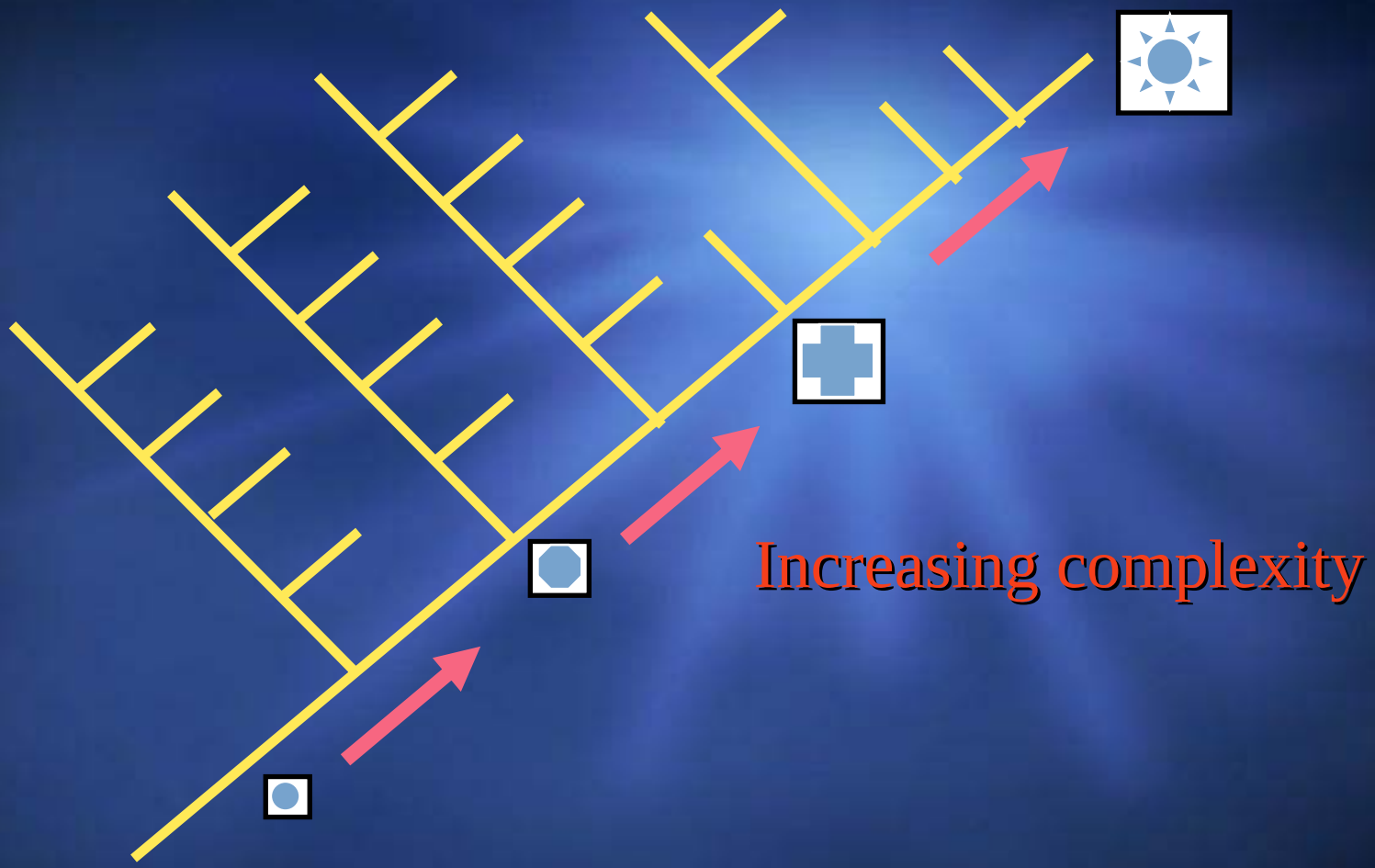


Step 6

(Oliver, Tuba and Mishler 2000



- A phylogenetically close comparison
  = low background differences



Ancestor-descendant comparison using reconstructed ancestral states

Step 6

Selaginella lepidophylla (Selaginellales, Selaginellaceae)

Selaginella lepidophylla (rehydrated)

- **Resurrection fern or Rose/Flower of Jericho:**
  - **Club or spike moss**
  - **Native to Mexico, Peru, and Southwestern U.S.**
  - **Survives complete desiccation, resurrects within hours**
  - **Useful model to elucidate mechanisms of drought tolerance, ionizing radiation, and extreme temperatures**
  - **Accumulates trehalose (90% soluble carbohydrate) for osmotic adjustment/membrane stabilization**

- **Genome sequencing of S. moellendorfii: In progress by JGI;**
  - **Non anhydrobiotic relative incapable of desiccation tolerance and resurrection.**

Selaginella lepidophylla (desiccated)

http://is.tc.cc.tx.us/~mstorey/1407.html

Sporobolus stapfianus Gand. [Poaceae]

- **Resurrection grass**
  - **Native to South Africa, Kenya, Somalia, Nigeria, and Ethiopia**
  - **Survives complete desiccation, resurrects within hours**
  - **Useful model to elucidate mechanisms of drought tolerance, ionizing radiation, and extreme temperatures**
  - **Accumulates sucrose for osmotic adjustment/membrane stabilization**
- **Large ETS collection**
- **Desiccation sensitive sister species**

Step 6

Research Approaches:

Genomics Resources

for Gene Discovery and Educational Platform

- **Conduct rapid gene discovery via EST sequencing:**
  - **454 Life Sciences (Roche)**

- **Conduct mRNA expression profiling comparing sister species**
  - ***Selaginella lepidophylla*** **(DT) -** ***Selaginella moellendorfii*** **(DS)**

  and

  - ***Sporobolus stapfianus*** **(DT) -** ***Sporobolus pyrimidalis*** **(DS)**

- **Conduct mRNA expression profiling to reveal gene networks controlling DT and recovery**

- **Transgenic assessment of gene function**

Research-Extension Objectives:

- **Develop Integrated Research-Extension Projects:**
  - **Establish breeding populations in greenhouse and field**
  - **Survey existing germplasm collections of *Sporobolus***
  - **Develop drought tolerance and productivity profiles for selected lines**

Field Plots (2007) - Reno:

Education/Outreach Objectives:

- **Establish breeding populations:**
  - ***Sporobolus stapfianus* (DT)**
  - ***Sporobolus pyrimidalis* (DS)**

- **Conduct productivity trials under different irrigation (water-deficit) regimes:**
  - **Growth performance**
  - ***Forage quality***

Educational Objectives:

- **Develop/enhance courses in:**

  - Plant Breeding & Biotechnology Lecture Course (UNR)

  - Plant Breeding & Biotechnology Lab Course (UNR)

  - Ecology of Grazingland Systems (UM)

Plant Breeding & Biotechnology Course (UNR)

- Taught at University of Nevada, Reno

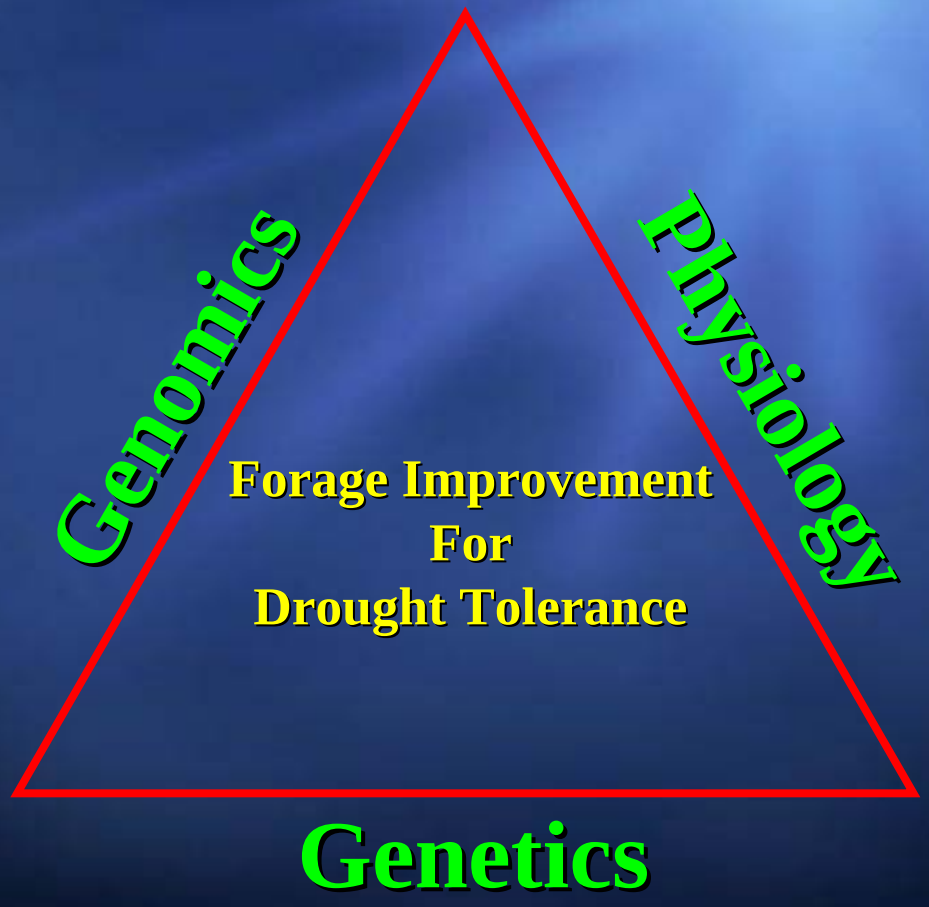
- Major Concepts Covered:
  - Origins of agriculture
  - Principles of plant breeding
  - Genetics
  - Biology of plant reproductive systems
  - Molecular genetics
  - Concepts in plant biotechnology

- Problem-based learning:
  - Modern food production systems
  - Consumption habits and human health
  - Environmental issues
  - GMOs
  - Environmental stress tolerance

Ecology of Grazingland Systems Course

- **Taught at University of Missouri - Columbia:**
- **Major Concepts Covered:**
  - **Components and functions of grazing lands; variation with ecoregion**
  - **Research needs, objectives and techniques in soil-plant-animal research, forage-livestock ecology and systems in grazing lands**
  - **Role of forages in conservation practices, nutrient management, water management, wildlife habitat, and sustainable agriculture**
  - **Industries involved with forages and livestock**
  - **Networking and team building**

Plant Breeding: Project Education Goals



Outcomes

- **Short-term**
  - **Course development - both UNR and MU**
  - **Educational and Extension Materials**
  - **Genomic resources for resurrection plants**
  - **Sporobolus breeding population**

Outcomes

- **Medium-term**
  - **Student development (measured by course enrollment and examination results)**
  - **Stakeholder awareness (measured by attendance at meetings and correspondence)**
  - **Assessment of Sporobolus as a forage for rangeland use**
  - **Novel genetic strategies for drought tolerance improvement programs.**
  - **Transgenic plant models for drought tolerance assessment programs**

Outcomes

- **Long-term**
  - **Breeders skilled in the modern aspects of plant improvement and phenotype assessment (measured by student follow-on contacts)**
  - **Markers for drought tolerance for use in ongoing Breeder education program and drought improvement programs for forage.**
  - **Alternate forage crop - as measured adoption by producers**
  - **Novel genetic targets for biotech approach to plant improvement**

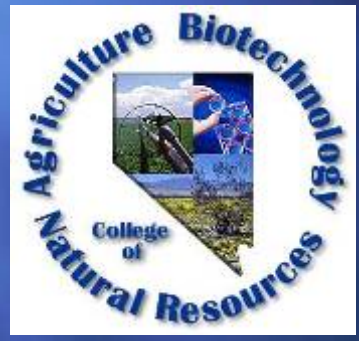
Management Plan

- **P.I: Oliver**
  - **Overall project management, *Sporobolus* cultivation and dehydration treatments, RNA isolations, curation, comparative genomics, molecular biology, bioinformatics, Arabidoposis transformation and testing. Integration with education and extension program at UMC. Establishment of a project web-site (Las Plantas Secas)**
- **Co-PI: Cushman**
  - **Selaginella species cultivation and dehydration treatments, RNA isolations, EST Database (ESTAP) molecular biology, bioinformatics, array analysis, Arabidoposis transformation and testing. Education and extension program management at UNR.**
- **Co-PI: Payton**
  - **EST Database, bioinformatics, photosynthesis, detailed physiological assessments, Arabidoposis transformation and testing.**
- **Co-PI: Sharp**
  - **Physiology, protocol assessment and management, detailed physiological assessments. Integration with Education and extension program at UMC.**

Management Plan

- Communication
  - Monthly Conference/video-conference call
  - Annual meeting of team (at common meeting -ASPB, PAG, GC)
- Dissemination of “outcomes”
  - Periodic tours of UNR field site
  - Extension publications
  - Newspaper, trade-magazines, newsletter, and blog
  - Extension Agent Training
  - Scientific journals and meeting presentations
  - Website - “Las Plantas Secas”
- Stakeholder Advisory Boards
  - Missouri and Nevada

Acknowledgments



National Research Initiative Competitive Grants Program (2007-02007)

Thank You

Steps 11 and 12