UCR/VIETNAM COLLABORATIONS

ALUMNUS: XUYEN THI NGO

HANOI UNIVERSITY OF AGRICULTURE

MENTOR/ADVISOR:SEYMOUR D. VAN GUNDY

EMERITUS PROFESSSOR & DEAN

ADVISOR: MARTHA OROZCO-CARDENAS

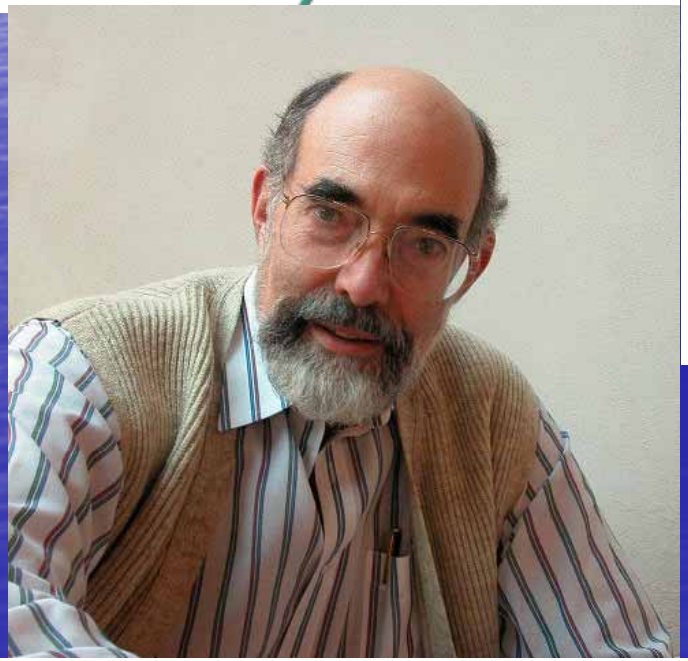
PLANT TRANSFORMATION RESEARCH CENTER

COLLEGE OF NATURAL & AGRICULTURAL SCIENCES

UNIVERSITY OF CALIFORNIA RIVERSIDE

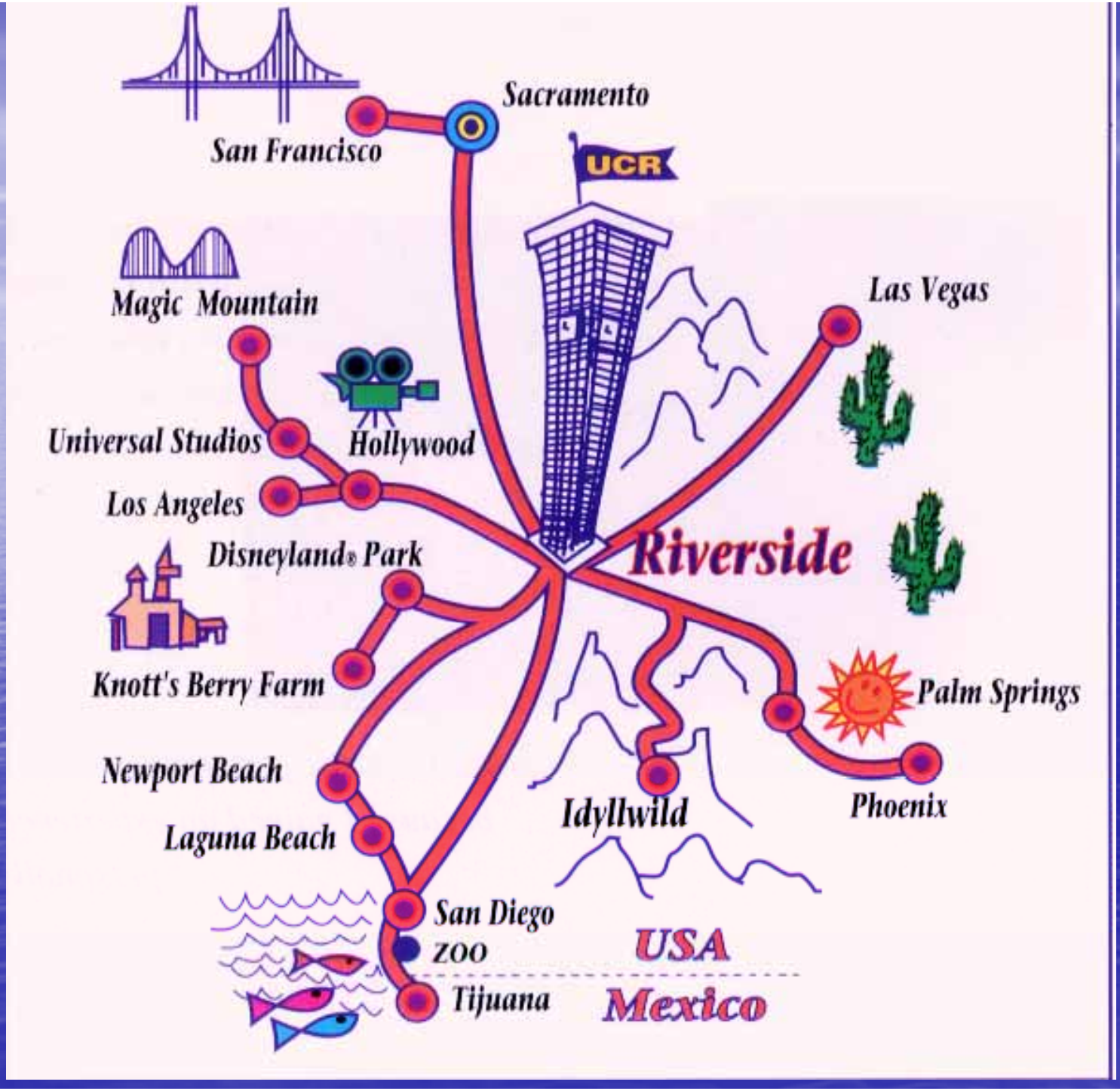
Welcome from
Timothy P. White

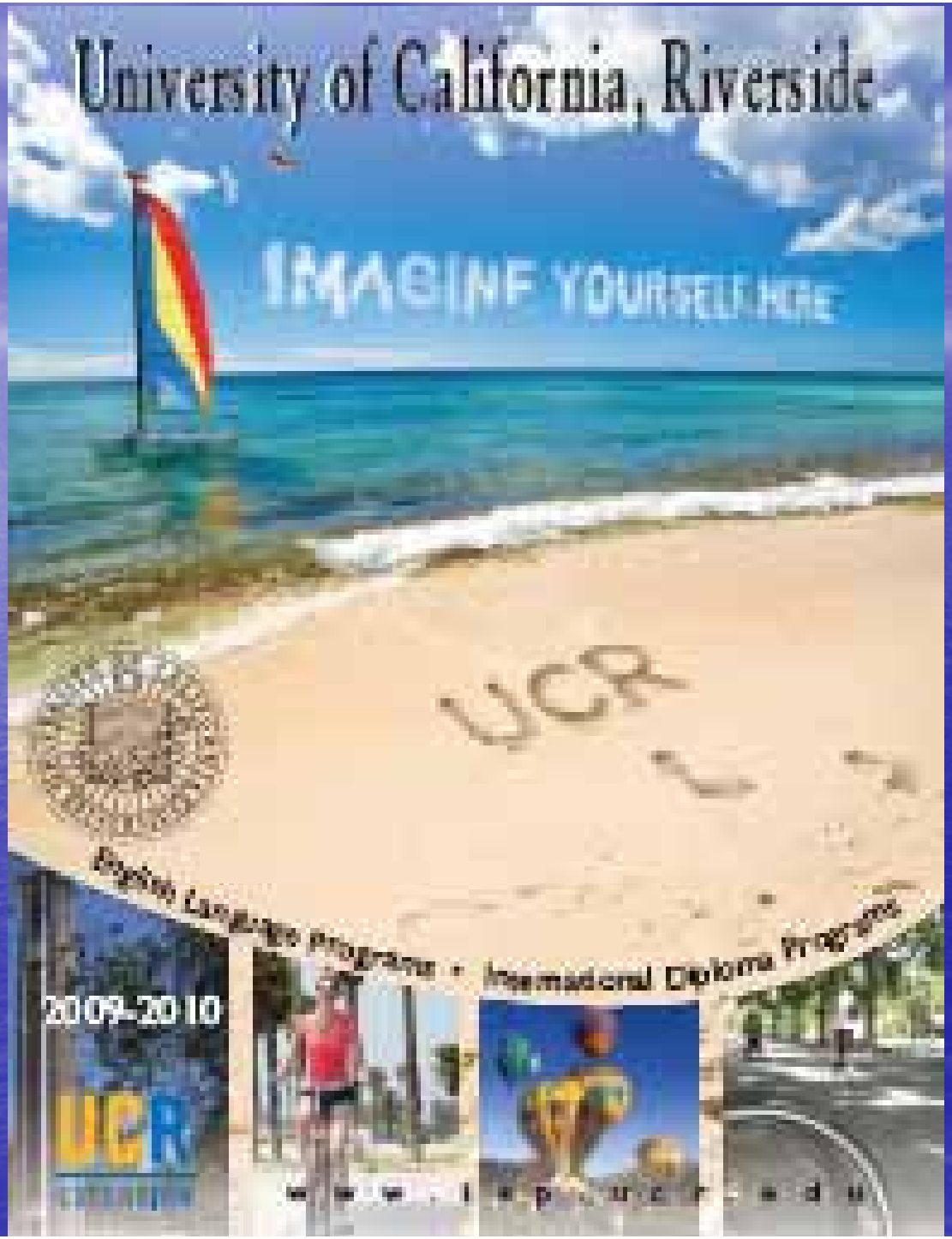
Welcome from
Sharon Duffy



Welcome from
Thomas Baldwin

N
UC Davis
UC Berkeley
UC San Francisco
UC Santa Cruz
UC Santa Barbara
UCLA
UC Riverside
UC Irvine
UC San Diego


Sacramento
San Francisco
UCR
Magic Mountain
Las Vegas
Universal Studios
Hollywood
Los Angeles
Disneyland® Park
Riverside
Knott's Berry Farm
Palm Springs
Newport Beach
Idyllwild
Phoenix
Laguna Beach
San Diego
zoo
USA
Tijuana
Mexico


University of California, Riverside
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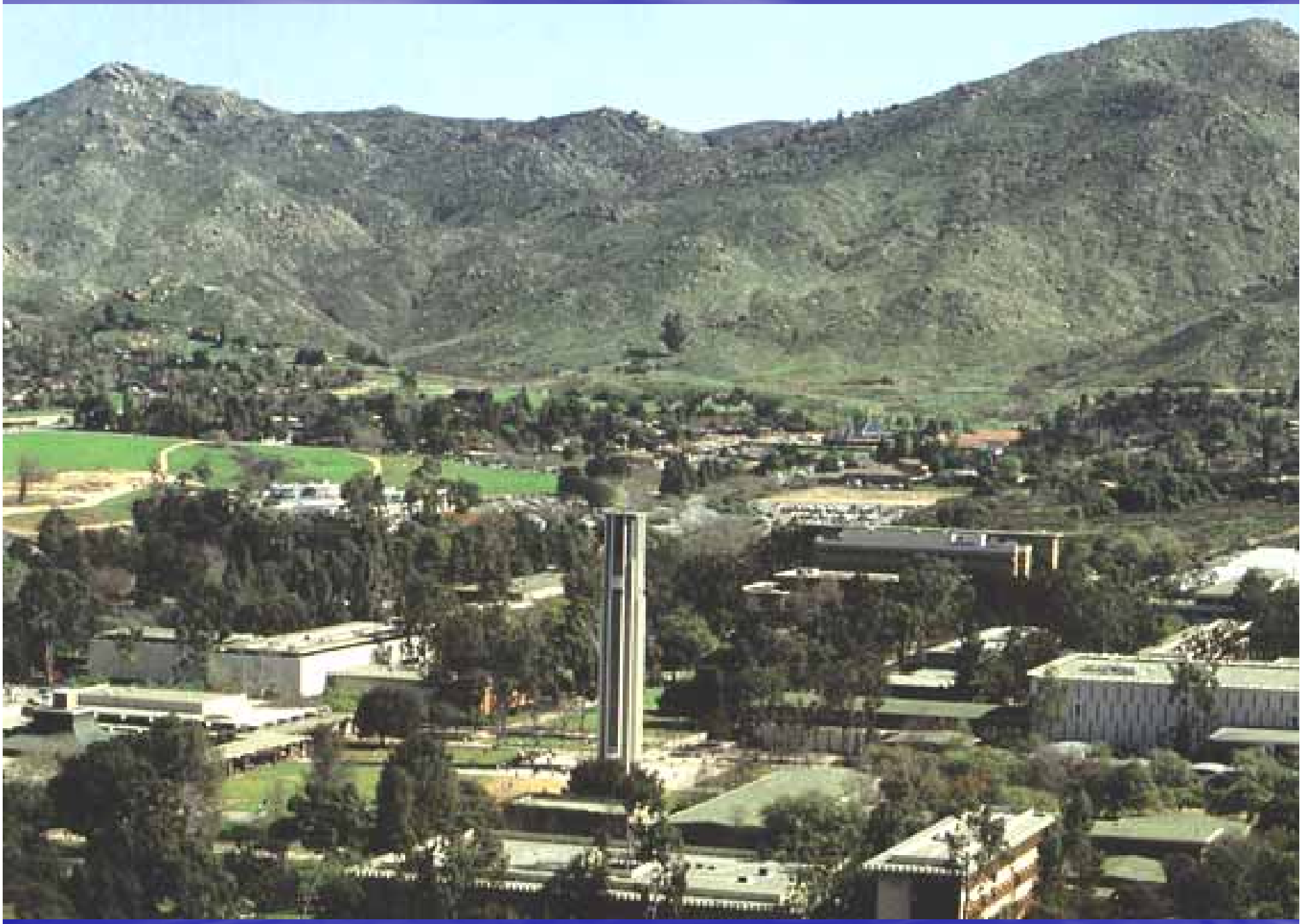
2009-2010
UCR



Science Library

UCR FACT SHEET 2007-2008

STUDENT ENROLLMENT: 18,104

ETHNICITY:

: African American ***6.7%***

: Asian/Asian American ***38.7%***

: Chicano and Latino ***23.8%***

: Native American ***0.4%***

: White/Caucasian ***20.2%***

: other ethnic ***10.2%***

U.S. News & World Report (2008) ranked UCR (1900 schools)

- **5th** in the nation in category of most diverse universities
- **9th** among the "up-and-coming" universities nationwide
- **40th** among the best national public universities
- **89th** among best national universities, public or private
- **100th** for undergraduate engineering programs

The Faculty Scholarly Productivity Index (2007)

- **1st in Nematology**
- **4th in Environmental Sciences**
- **4th in Entomology**
- **5th in Natural Resources and Conservation Biology**
- **10th in Plant Pathology**
- **10th in Botany and Plant Biology**

UCR EXTENSION CENTER

INTERNATIONAL VILLAGE

UCR-IEP Pathways

2000 MOU

UNIVERSITY OF CALIFORNIA EXTENSION AND SUMMER SESSIONS

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO — SANTA BARBARA • SANTA CRUZ

RIVERSIDE, CALIFORNIA 92521-0112

MEMORANDUM OF UNDERSTANDING ON COOPERATION

BETWEEN

UNIVERSITY OF CALIFORNIA, RIVERSIDE, USA

&

HANOI AGRICULTURAL UNIVERSITY, VIETNAM

With this Memorandum of Understanding, University of California, Riverside, USA, and Hanoi Agricultural University, Vietnam, agree to recognize one another as affiliated institutions and to co-operate where feasible and mutually acceptable in applying for the project of quality enhancement for teaching and research at Hanoi Agricultural University, funded by the World Bank and/or other sources of funding.

Ratification of this Memorandum of Understanding will not result in any material, financial or other obligation for either of the two institutions. Both universities will be at liberty to determine the framing of their joint initiatives in accordance with their respective policies.

This Memorandum is effective until April 21, 2004, but may be terminated by either university with a minimum of six months' written notice being given to the other party.

Dr. John F. Azzaretto, Vice Chancellor
Public Service and International Programs
University of California, Riverside

Professor Dr. Nguyen Viet Tung, Rector
Hanoi Agricultural University

Dr. Sheila Dwight, Director
International Education Programs
University of California, Riverside

Ngo Thiauyen
Dept. of Plant Pathology
Hanoi Agricultural University

Date: 30 August, 2000

2008 MOU

MEMORANDUM OF UNDERSTANDING ON COOPERATION

Between
UNIVERSITY OF CALIFORNIA, RIVERSIDE, USA
And
HANOI UNIVERSITY OF AGRICULTURE, VIETNAM

With this Memorandum of Understanding, University of California, Riverside, USA and Hanoi University of Agriculture, Vietnam, agree to recognize one another as affiliated institutions and to co-operate where feasible and mutually acceptable for the enhancement of teaching, research, and faculty and student exchanges.

Ratification of the Memorandum of Understanding will not result in any material, financial or other obligation for either of the two institutions. Both universities will be at liberty to determine the details of joint initiatives in accordance with their respective polices. Separate agreements will be required when resource commitments or other obligations are involved.

This Memorandum of Understanding is an extension and continuation of the MOU signed August 30, 2000, and is effective for five years, but may be terminated by either university with a minimum of six months' written notice being given to the other party.

Dr. Timothy P. White, Chancellor
University of California, Riverside

Professor Dr. Tran Duc Vien, Rector
Hanoi University of Agriculture

Dr. Thomas O. Baldwin, Dean
College of Natural & Agricultural Sciences

Dr. Nguyen The Hung, Dean
Faculty of Agronomy

Dr. Sharon A. Duffy, Acting Dean
University Extension

Date: Nov. 26, 2008

Dr. Nguyen Tat Canh, Head
International Cooperation

Date:

Vietnam Exchanges 2004-2008

- 1- VEF Researcher 2007-08
- 3-Fulbright Researchers 2000 - 2009
- 2004 = 2 students
- 2005 = 2 students
- 2006 = 2 students
- 2007 = 9 students
- 2008 = 39 students
- 2008 summer = special group of 20

Agricultural Experiment Station

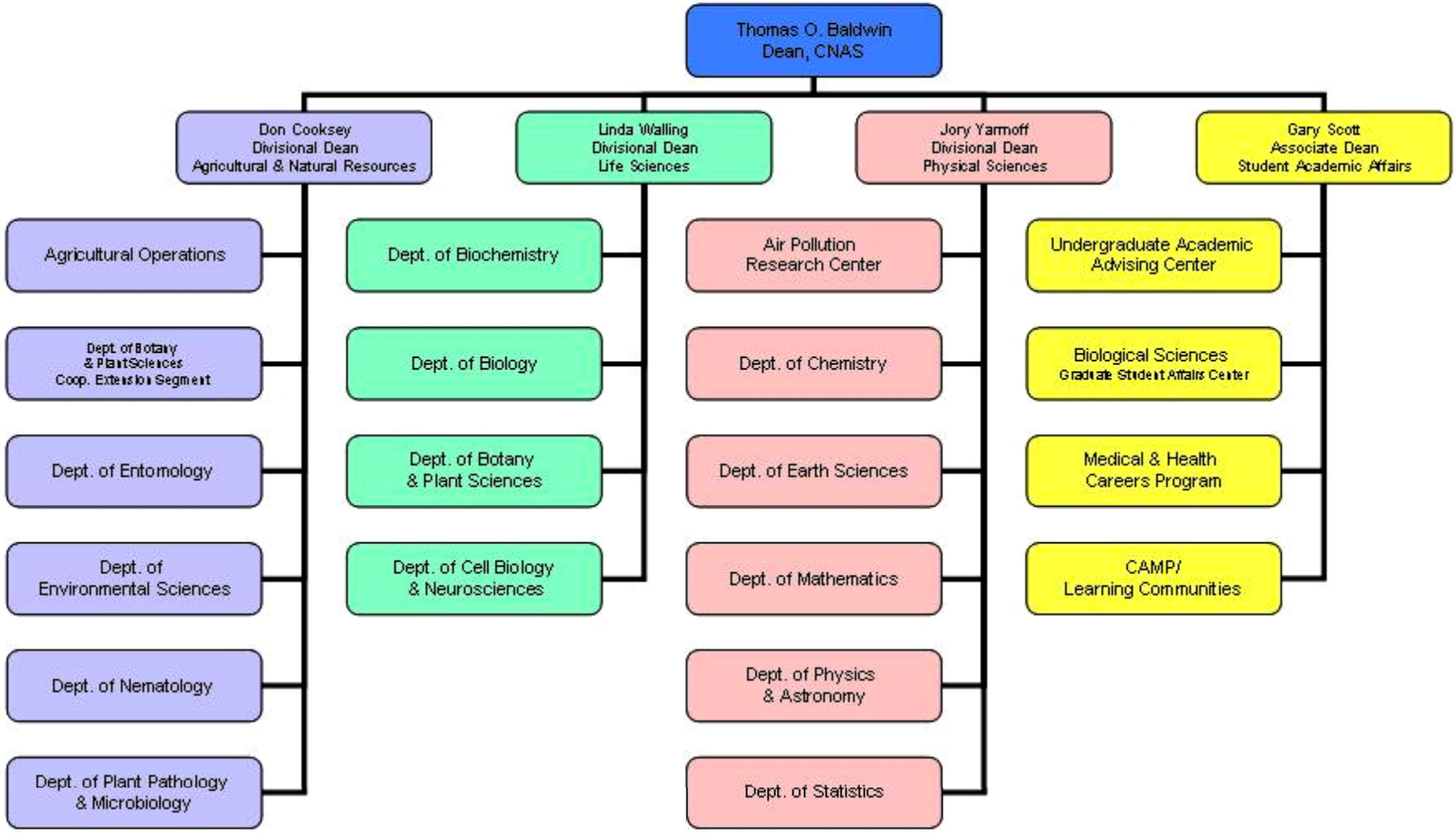
1907 – 2007

University of California, Riverside

- Citrus Experiment Station in Riverside established by University of California in 1906 to support region's prosperous citrus industry and other subtropical crops
- Established Graduate School of Tropical Agriculture
- Established as general UC campus in 1954

ANR UNIVERSITY of CALIFORNIA

College of Natural & Agricultural Sciences
Academic Dept. Organization



REV. 7/08

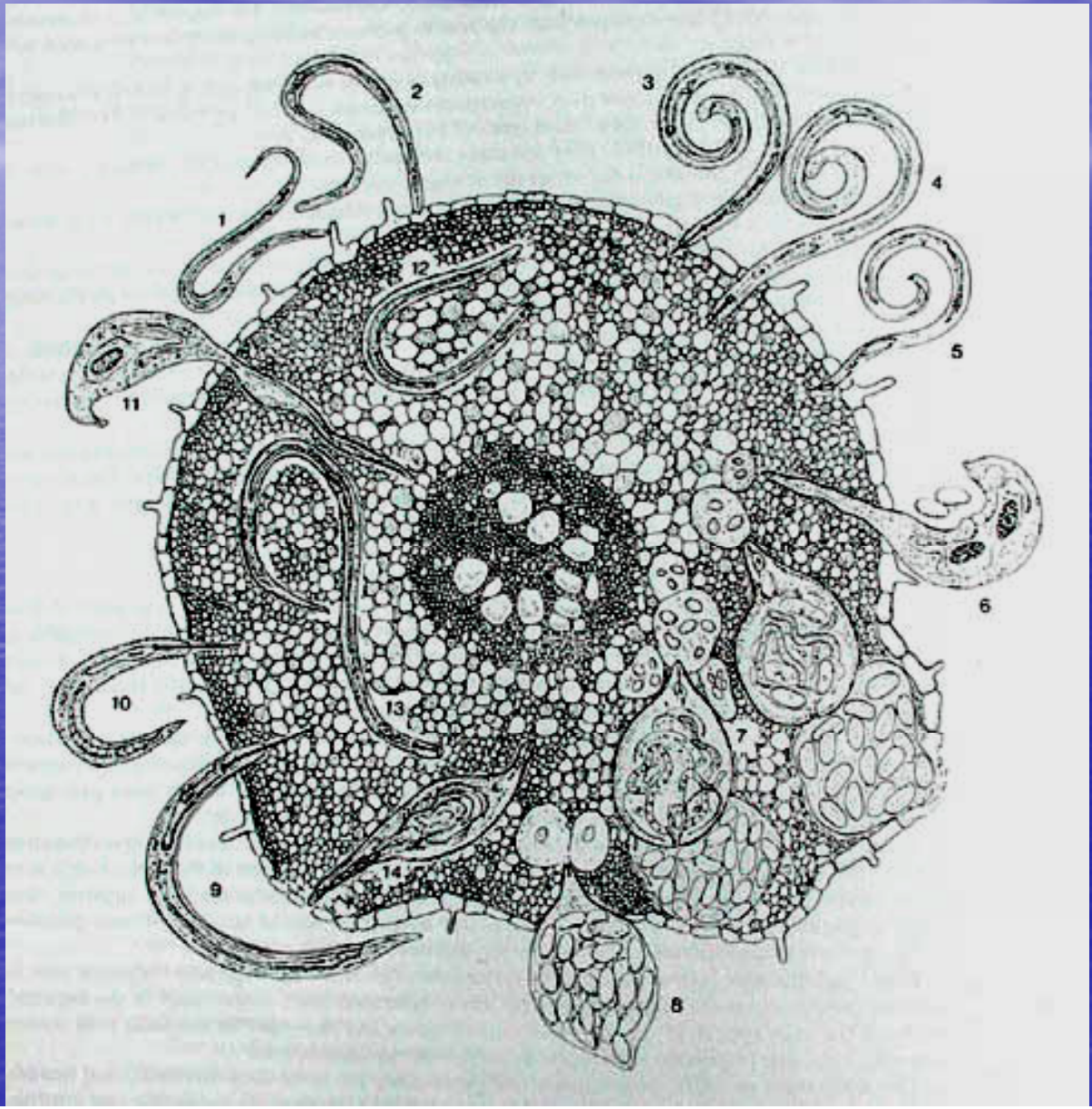
UCR DEPARTMENT OF NEMATOLOGY

Roundworms: - the most abundant, common and genetically diverse multi-cellular organisms on earth.

- A Major Component of Soil and Marine Ecosystems
- Nematodes as parasites:
  - Agriculture
  - Medicine
  - Veterinary science
  - Biological control agents
- 2002 Nobel prize in medicine *Caenorhabditis elegans* --a model in genetics and development

FAMOUS NEMATOLOGISTS UCR/UCD

PLANT PARASITIC NEMATODES

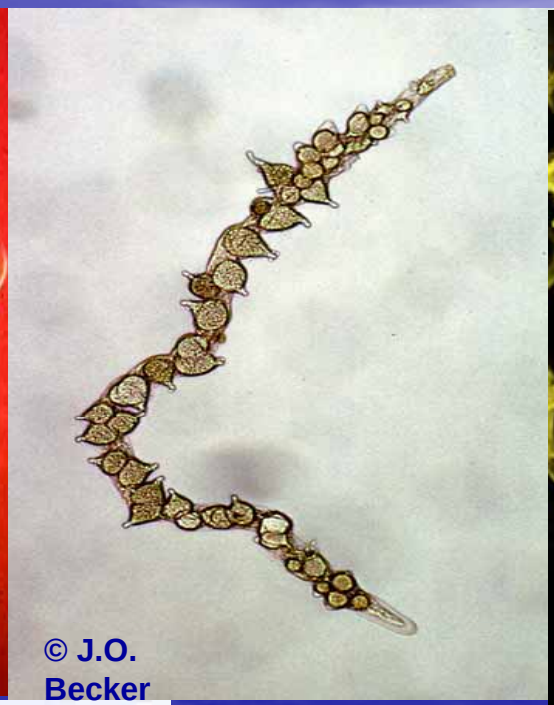


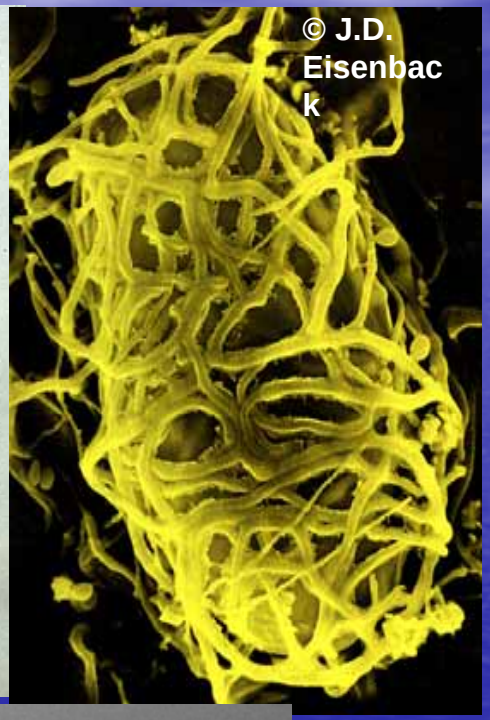
Plant Parasitic Nematodes: Symptoms

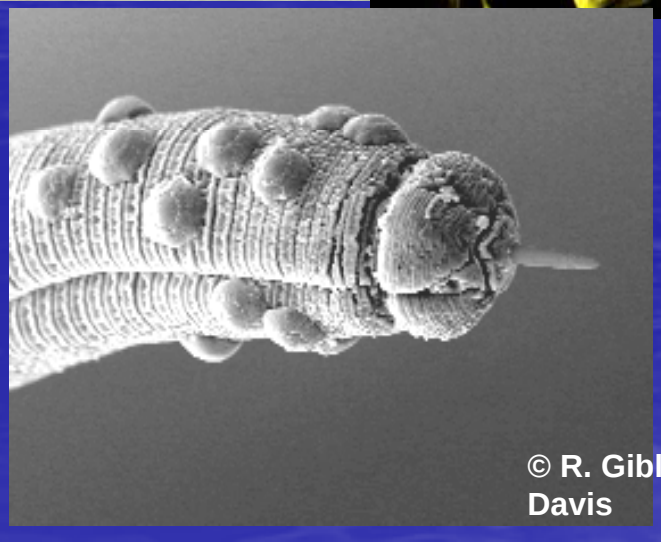
Beet cyst nematode

Heterodera schachtii

Plant Parasitic Nematodes Enemies....







COLLABORATIVE PUBLICATIONS in Nematology

- 2001-T.N. Xuyen, S.D. Van Gundy & M.C. Menuel
- 2002- Ngo, Thi Xuyen & J.O. Becker
- 2004- Ngo, Thi Xuyen & S.D. Van Gundy

Disease Resistance in Plants

Non-host resistance

Basal resistance

Race-specific resistance

Plant Endogenous small RNAs play an important role in gene regulation in plant immunity

Plant-Pathogen Recognition

↓

Signaling

Small RNAs

Small RNAs

Oxidative burst
Ion fluxes
Salicylic acid accumulation

Gene expression reprogramming

Cell death
Phytoalexin biosynthesis
Cell wall strengthening
.....

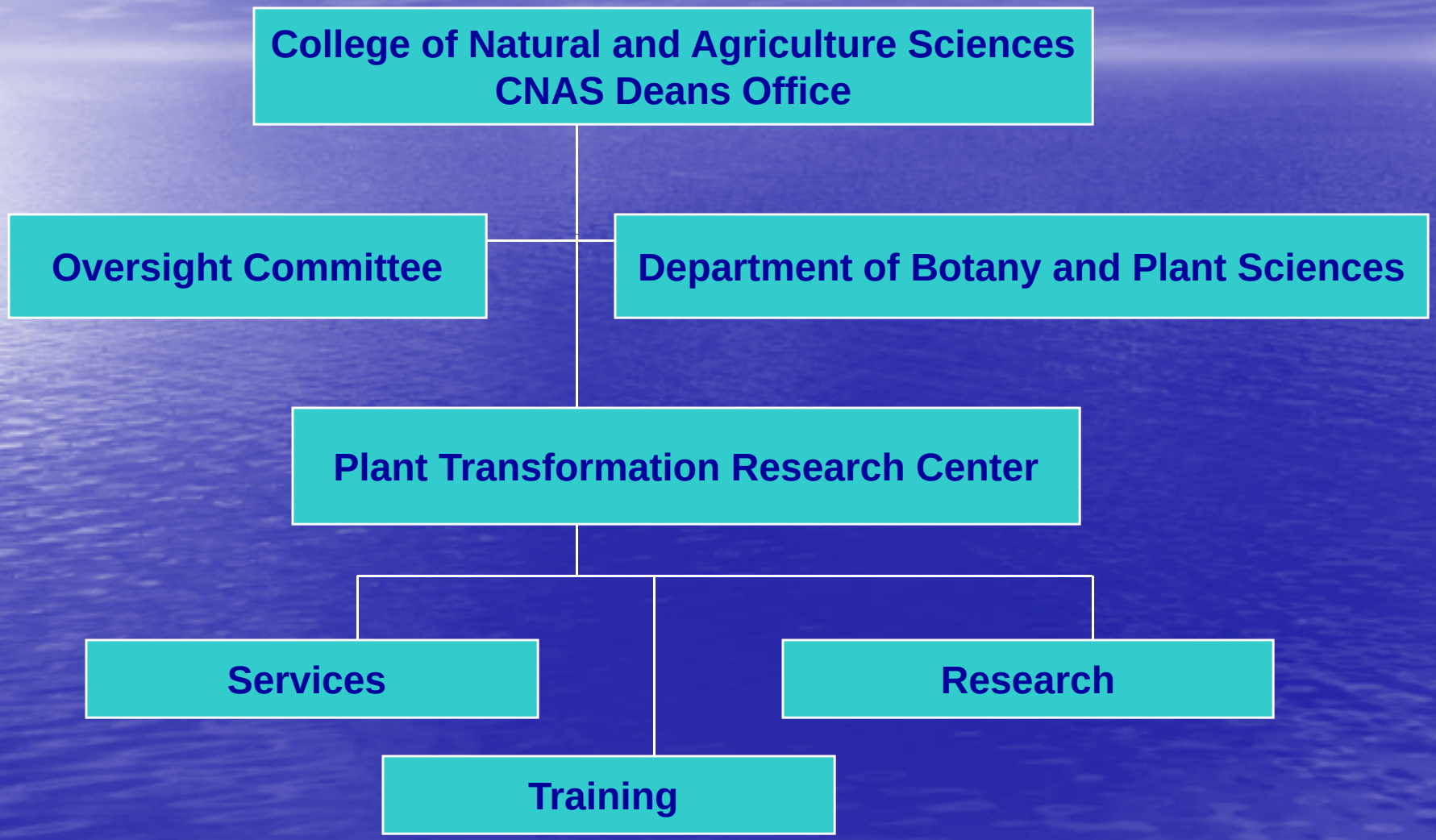
Small RNAs

Small RNAs

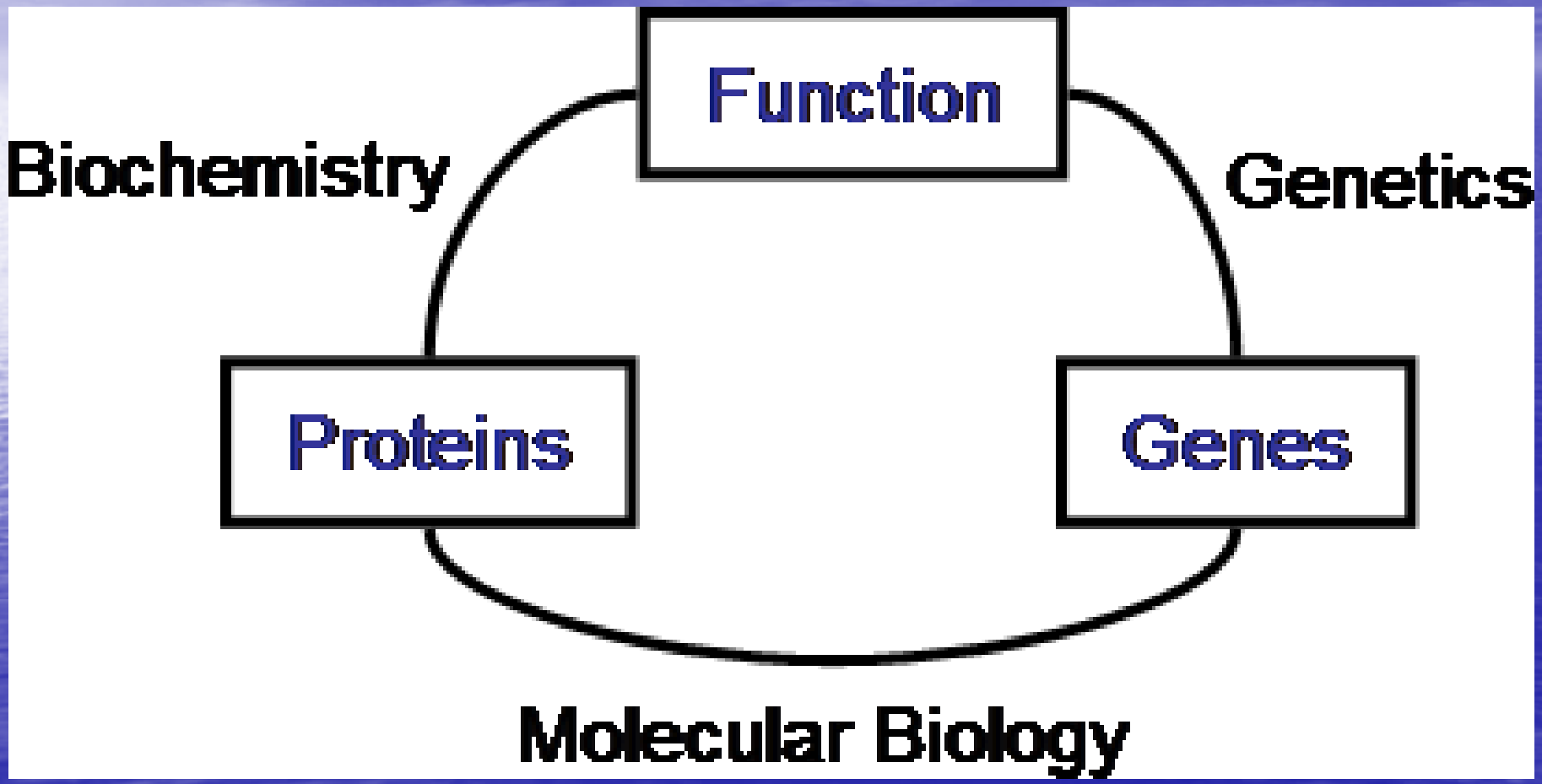
↓

Defense Responses and Pathogen Inhibition

Organizational Structure



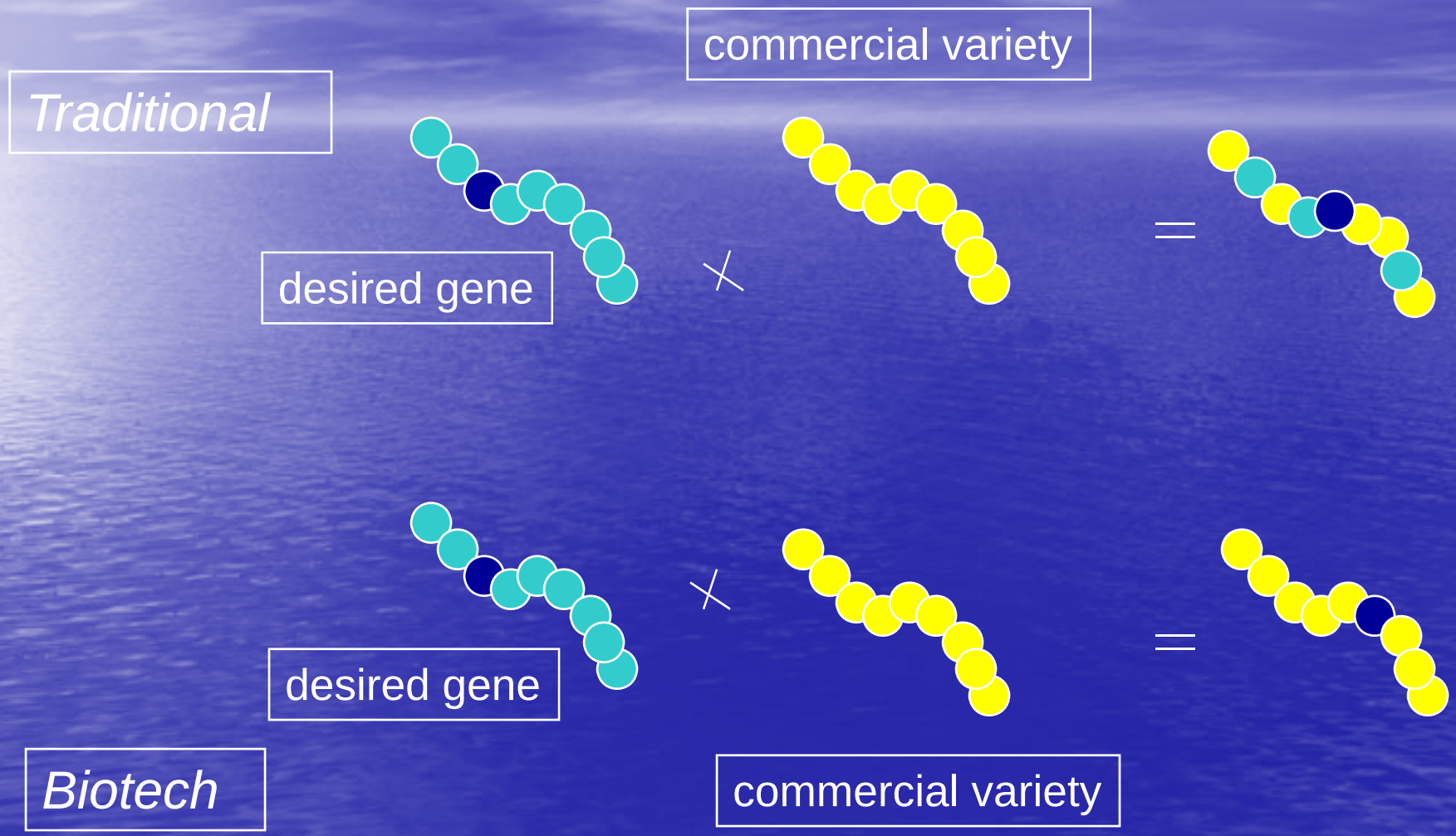
PLANT TRANSFORMATION



Transgenic Technology

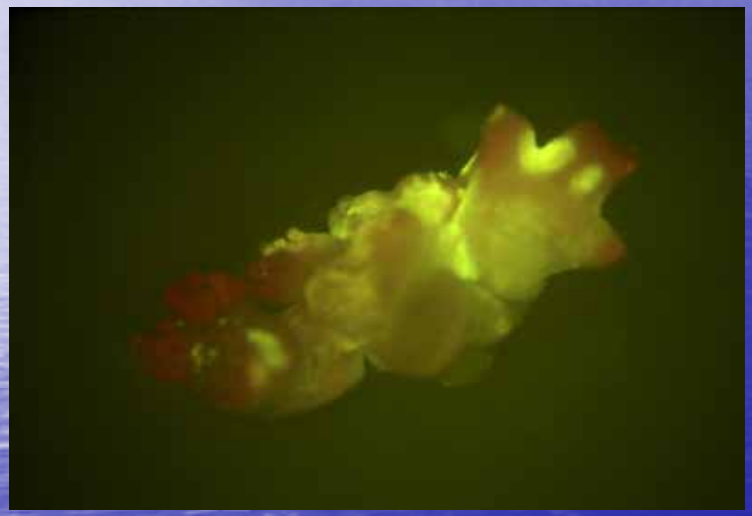
A transgenic crop plant contains a gene or genes which have been artificially inserted into the genome of the plant instead of the plant acquiring them through pollination

TRADITIONAL VS BIOTECH GENE MODIFICATION



Plant Transformation Research Center

Services, Training and Research



Citrus

Lily

Rice

Tissue-specific and Developmental Regulation of a *Citrus sinensis* Terminal Flower promoter-*GUS* Gene fusion in Tomato

Xuyen Thi Ngo[1], Javier Narváez-Vásquez[2], Lynn J. Pillitteri[3], Janet Giles[1], Benjamin J. Becerra[2], Carol J. Lovatt[2], and Martha L. Orozco-Cárdenas[1]
[1]Plant Transformation Research Center, University of California, Riverside, CA 92521
[2]Department of Botany and Plant Sciences, University of California, Riverside, CA 92521
[3]Present address: Department of Biology, University of Washington, Seattle, WA 98195

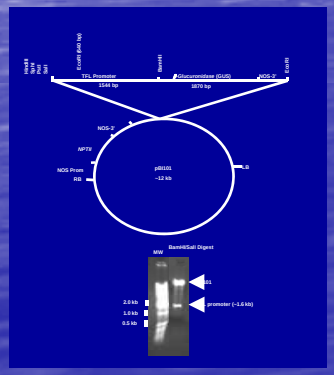
ABSTRACT

TERMINAL FLOWER1 (*TFL1*) is an important determinant of floral timing in *Arabidopsis* and other herbaceous species. A *TFL* gene promoter was isolated from the perennial tree crop 'Washington' navel orange (*Citrus sinensis* L. Osbeck). A plant expression vector was constructed containing 1544 bp of the *TFL* promoter fused upstream of the reporter gene β-*glucuronidase* (*GUS*) for functional analyses of the spatial and developmental expression of *CsTFL* in transgenic tomato plants (*Solanum lycopersicon*, cv. microtom). *GUS* was highly expressed in the four floral organs, green and ripening fruit, and in immature seeds, the later of which was not observed in *C. sinensis*. GUS expression was also detected in the shoot apex and stems of young seedlings, particularly in the vascular tissue, but not in the leaves. These results indicate that the *CsTFL* gene promoter displayed a tissue-specific and developmentally regulated pattern of expression in tomato similar to citrus.

INTRODUCTION

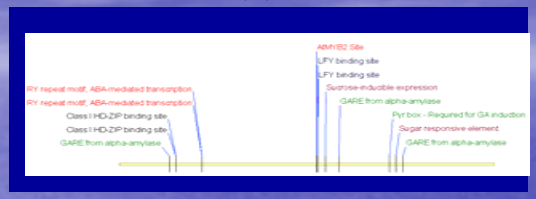
The most critical step in fruit crop production is the transition from the juvenile phase to the adult phase, when the plants become reproductively competent. The juvenile phase of woody perennials is significantly longer than that of herbaceous annuals or biennials, lasting 5 to 13 years in citrus, depending on the cultivar, and 20 to 30 years for other fruit tree crops and forest tree species (Davies and Albrigo, 1994; Meilan, 1997). The long juvenile phase is a constraint for traditional or transgenic breeding practices. In addition, although reproductively competent in the adult phase, most plants require an environmental cue to transition to reproductive development; however, a few initiate flowering autonomously after attaining a certain age or stage of development regardless of their environment. Using the model plant *Arabidopsis thaliana*, much of the genetic and physiological complexity of flowering has been elucidated (Jack, 2004). Alterations in the timing or location of expression of many flowering-related genes result in changes in vegetative phase length and/or flowering time. Among these genes, *TERMINAL FLOWER* (*TFL*) has been implicated in the regulation of flowering onset via maintenance of shoot apex indeterminacy (Pillitteri et al., 2004b; Hanzawa et al., 2005). Many aspects of the floral induction differ between the model plant *Arabidopsis*, an herbaceous annual, and woody perennial trees (Pillitteri et al., 2004a,b). Therefore, investigation of floral development gene regulation in economically important tree crops remains limited. To investigate the molecular mechanisms underlying juvenility and flower production in *Citrus sinensis*, the *CsTFL* gene promoter (1544 bp) was cloned upstream of the reporter *uidA* (*GUS*) gene, and tomato plants were transformed with the *CsTFL::GUS* cassette. Insertion and expression of the transgene was confirmed by genomic PCR, DNA and RNA gel blot analyses of the *GUS* gene. The *T0* and *T1* transgenic lines were characterized for tissue specificity and developmental regulation of GUS expression using the histochemical assay.

CsTFL::GUS REPORTER GENE CONSTRUCT



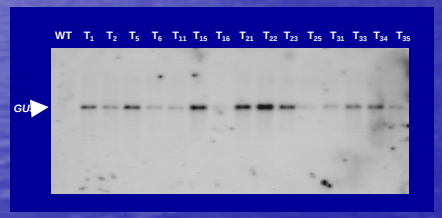
Citrus sinensis TERMINAL FLOWER (*TFL*) gene promoter (1544 bp) was cloned upstream of the *uidA* (*GUS*) gene in the binary vector pBI101 for *Agrobacterium*-mediated transformation of tomato (Narváez-Vásquez et al., 2007).

PUTATIVE REGULATORY ELEMENTS IN THE *CsTFL* PROMOTER



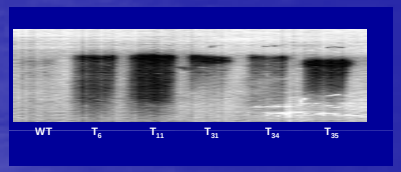
In silico analysis of the *CsTFL* gene promoter showing potential *cis*-regulatory elements. Most relevant putative regulatory motifs showing 100% homology are shown. Light-inducible elements and one auxin element were not included for simplification. Response elements are color coded by category: Red = Drought-inducible and ABA-dependent, Green = GA response elements (originally found in alpha-amylase in barley aleurone), Purple=inducible by sucrose, black = binding site for Class I HD-ZIP transcription factors, and blue=leafy binding sites. The *Arabidopsis* promoter has essentially the same elements, except that there is only one GARE in 1.6kb of the Arabidopsis *TFL* promoter and 3 in the citrus TFL promoter.

DNA GEL BLOT ANALYSIS OF TOMATO PLANTS TRANSFORMED WITH THE *TFL::GUS* CHIMERIC GENE CONSTRUCT



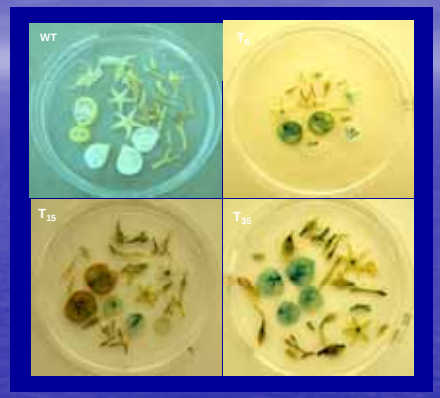
Tomato genomic DNA was extracted from leaves of kanamycin-resistant plants (primary transformants, T0 generation) using the DNAzol (Invitrogen), following the manufacturer's protocol. About 10 µg of genomic DNA was digested with EcoRI, fractionated on an agarose gel, and transferred onto a Hybond-N+ nylon membrane (Millipore). The presence of the *TFL::GUS* transgene was determined by hybridization with a ^{32}P-radiolabeled DNA fragment PCR-amplified from the *GUS* gene of the pBI101 binary vector, using the sense primer 5'-GTGGGTCAATAATCAGGAAG-3' and the antisense primer 5'-CCAATCCAGTCCATTAATGC-3'. Membranes were washed once with 2X SSPE for 20 min at room temperature; 2 times with 2X SSPE, 1% SDS, for 15-30 min at 65-°C; and then exposed for 3 days to a X-ray film as described (Narváez-Vásquez et al., 2007). Fifteen independent transformants (T_0) were confirmed for the presence of the transgene.

EXPRESSION OF *CsTFL::GUS* GENE IN TRANSGENIC TOMATO PLANTS



Total RNA was extracted from flowers of wild type (WT) and five independent transgenic plants using TRIZOL reagent as per the manufacture's protocol (GIBCO BRL life technologies). A ^{32}P-labeled PCR-amplified *GUS* gene fragment was used as probe in gel blot analysis for hybridization. Membranes were washed once with 2X SSPE for 20 min at room temperature, 2 times with 2X SSPE, 1% SDS, for 15-30 min at 65-C and then exposed 15-32 h to a X-ray film as described (Orozco-Cárdenas et al., 2001). Each lane corresponds to an independently transformed line.

HISTOCHEMICAL ANALYSIS OF GUS EXPRESSION IN DIFFERENT TISSUES OF T0 TRANSGENIC PLANTS



Stems, leaves, floral organs, green and ripening fruits from a wild type (WT) untransformed plant and different primary transformants (T0) were incubated in a solution containing 0.5 mg/ml 5-bromo-4-chloro-3-indolyl glucuronide (GUS substrate), 0.1 M Na_2HPO_4, pH 7.0, 10 mM EDTA, 0.5 mM potassium ferricyanide/ferrocyanide , and 0.06% Triton X-100 at 37°C for 16 h. The staining solution was removed, and the samples were cleared of chlorophyll by sequential changes of 75% and 95% ethanol (Jefferson, 1987).

GUS EXPRESSION IN T1 TRANSGENIC SEEDLINGS

Transgenic seedlings (T1progeny) grown on kanamycin-selective medium for two weeks, were used for the histochemical analysis of GUS expression, as explained above. Most seedlings showed GUS staining mainly in the shoot apex, the upper central (vascular tissue) of stems and the mid veins of developing leaves. Occasionally, GUS activity was also observed in the roots.

RESULTS AND DISCUSSION

Little is known about the regulation of *TFL* gene expression in evergreen and deciduous tree fruit crops. *CsTFL* has a clear role in maintenance of citrus juvenility (Pillitteri et al., 2004a,b). Studies comparing Arabidopsis with species such as rice, poplar, and tomato have highlighted the extensive conservation of floral regulation pathways among angiosperms (Jack, 2004). Among these, tomato may be a better model plant in which to investigate the regulation of citrus floral genes. Tomato and citrus share the same sympodal growth habit, in which the shoot apical meristem is terminated and future growth continues from the upper most lateral bud causing a typical zig-zag stem pattern. Furthermore, both citrus and tomato produce fruits that are berries. A functional ortholog of *TFL* in tomato is expressed in leaf and flower primordia, and controls vegetative and reproductive shoots alternation (Pnueli et al., 1998). Therefore, it was expected that regulation of GUS expression by the *CsTFL* promoter in tomato may occur through the binding of transcriptional regulators to putative response elements in the promoter as in citrus.

Tomato plants transformed with *CsTFL::GUS* gene exhibited relatively high levels of GUS histochemical staining in the cells of the four floral organs, green and ripening fruits, and in immature seeds. *GUS* activity was also detected in stems of young transgenic seedlings, particularly in the shoot apex and the vascular tissue towards young developing leaves. These results are in agreement with a previous study on the regulation of the *TFL* gene in citrus plants (Pillitteri et al., 2004a). In those studies, *CsTFL* transcripts were detected by RT-PCR in vegetative and floral tissues. *CsTFL* mRNAs were found in stems of juvenile plants, but not in roots, stems, or leaves of adult plants. *CsTFL* transcripts were also detected in all floral organs, including sepals, petals, stamen and carpels (Pillitteri et al., 2004a). However, the specific cell types or tissues, in which *CsTFL* gene is transcribed is not known. The expression of the *CsTFL* gene in citrus fruits has not been investigated. Further studies are necessary to determine the precise location of *CsTFL* gene expression, since it has been recently proposed that *TFL1* mRNA and/or its protein may be mobile signals controlling shoot meristem identity in Arabidopsis (Conti and Bradley, 2007).

So far the results obtained in this study indicate that the *CsTFL* gene promoter drives a tissue-specific and developmentally regulated pattern of expression in tomato as well as in Citrus. Future work will include experiments to characterize the expression patterns of the *CsTFL::GUS* gene in homozygous transgenic tomato plants subjected to low temperature, water-deficit stress, and GA_3 treatments. Such studies will contribute to the overall understanding of the genetic and physiological control of flowering in citrus and other woody perennials. Furthermore, studies can be performed to dissect the role of putative *cis*-regulatory elements present in the *CsTFL* gene promoter and their possible interactions with potential transcription factors. Results from these experiments will enable a focused analysis of homologous promoters in different crop species, for application in increasing crop productivity in those species.

Conti, L., and Bradley D. (2007). TERMINAL FLOWER1 is a mobile signal controlling *Arabidopsis* architecture. Plant Cell 19:767-778.

Davies, F.S. and Albrigo L.G. (1994). Citrus. CAB International, Wallingford, UK.

Hanzawa, Y., Money, T and. Bradley D. (2005). A single amino acid converts a repressor to an activator of flowering. Proc. Natl. Acad. Sci. USA 102:7748-7753.

Jack, T. 2004. Molecular and genetic mechanisms of floral control. Plant Cell 16:S1–S17. Meilan, R. (1997). Floral induction in woody angiosperms. New For. 14:179–202.

Jefferson, R.A. (1987) Assaying chimeric genes in plants: The GUS gene fusion system. Plant Molecular Biology Reporter 5: 387-405

Narváez-Vásquez, J., Orozco-Cárdenas, M.L. and Ryan C.A. (2007). Systemic wound signaling in tomato leaves is cooperatively regulated by multiple plant peptides. Plant Mol. Biol. 65, 711-718.

Orozco-Cárdenas, M.L. Narváez-Vásquez, J., and Ryan C.A. (2001). Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin and methyl jasmonate. Plant Cell 11:179-191

Pillitteri, L.J., Lovatt, C.J. and Walling L.L. (2004a). Isolation and characterization of a *TERMINAL FLOWER* homolog and its correlation with juvenility in citrus. Plant Phys. 135:1540–1551.

Pillitteri, L.J., Lovatt, C.J. and. Walling L.L. 2004b. Isolation and characterization of *LEAFY* and *APETALA1* homologues from *Citrus sinensis* L. Osbeck 'Washington'. J. Amer. Soc. Hort. Sci. 129:846–856.

Pnueli, L., Carmen-Goren, L., Hareven, D., Gutfinger, T., Alvarez, J., Ganal, M., Zamir, D. and Lifschitz, E. (1998). The *SELF-PRUNING* gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of CEN and TFL1. Development 125:1979-1989.

American-Vietnamese Management Consortium, Inc.

UCR VISITING SCIENTIST SUPPORT

- Official Visiting Scientist Appointment
- Use of Desk, Laboratory Space and Facilities/Equipment
- Computers & Internet
- Library (access to all UC Libraries)
- Local Travel
- Insurance

Potential Future Collaborations

- Asian Greening Disease
- Plant Transformation
- Molecular Biology
- Nematode Resistance
- Biological Control
- International Programs

TIPS TO SUCESSFUL INTERNATIONAL PARTNERSHIPS

- Develop Official Document – MOU to Leverage your Grant Applications
- Meet & Work with your University Administration & become familiar with Programs
- Attend Professional Meetings
- Meet the Leaders in your Profession
- Work Hard with your Mentors/Advsiors
- Publication of Research Results

My UCR Partnership Experience

- Pushchino Biological Research Center-1992
- Pushchino State University, Russia-1994
- Ministry of Agriculture of China-1998
- Moldova State University-1999
- Hanoi University of Agriculture-2000

UCR Extension International Programs

•Gangnam-University of California Riverside International Education Center - Korea

Integration of Science and Education

- Czechoslovakia AS with USNAS Support
- Pushchino Biological Research AS Institute, Russia with USAID Support
- Moldova NFFM, MSU, ASMIZ with ACID-VOCA, USSD, NAS Support

RESEARCH CAREER HIGHLIGHTS

Seymour Dean Van Gundy
PhD 1957, University of Wisconsin
Emeritus Professor & Dean, UCR
1957-2008

Fellow: AAAS, SON, APS

Honorary Member: SON 1997

Honorary Professor 2000: Linyi Teachers University, China

Outstanding Emeritus Faculty 2005-06, UCR

EC Metal for Outstanding Contributions, 2005

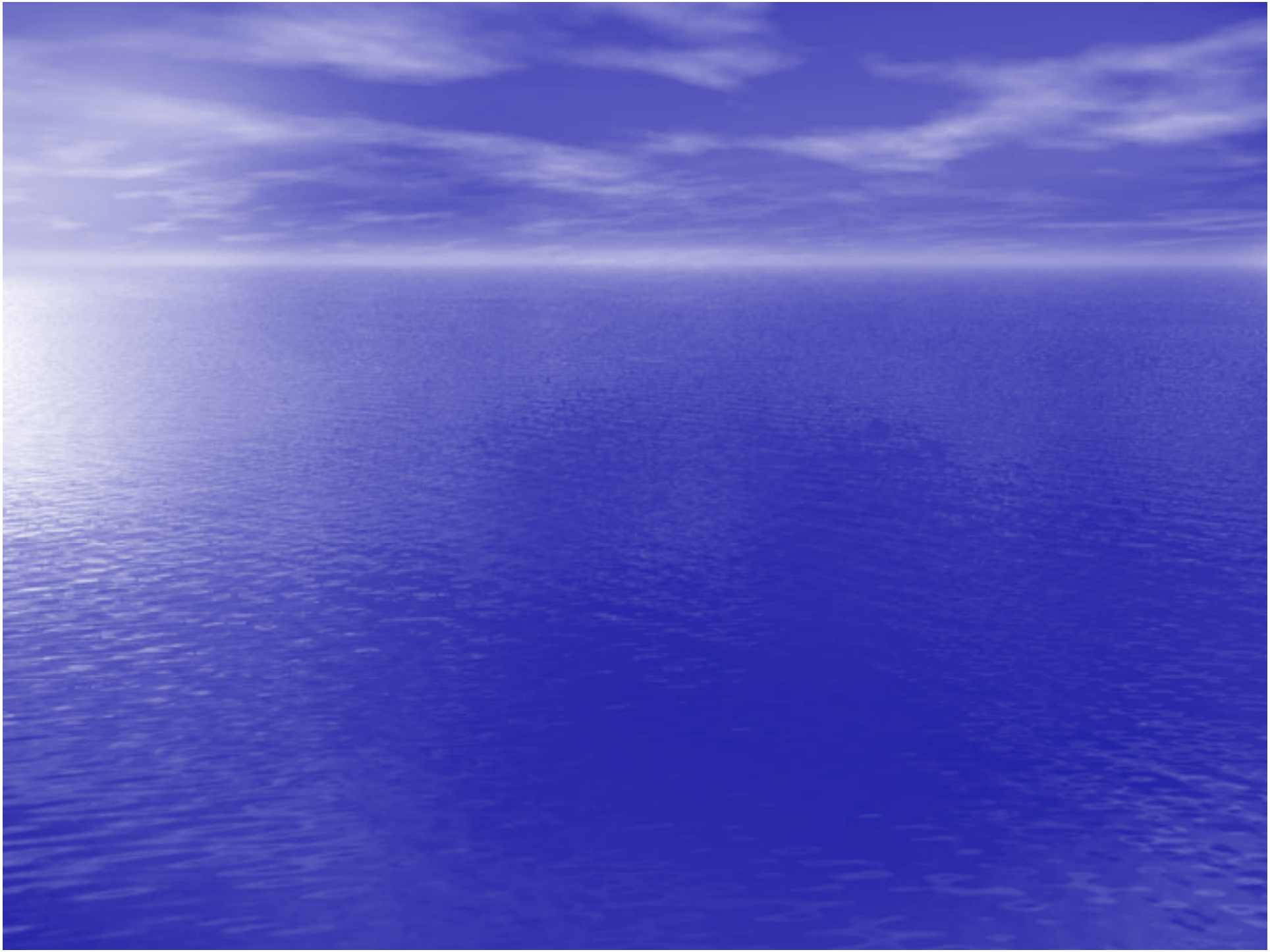
Honorary Member Acad.Sc. Moldova, 2006

Total Publications: 220

International Research

- CSIRO – Horticultural Institute, Australia
- Univ. of Cairo – Dept Zoology & Nematology
- French Oil Research Institute – South Amer.
- Hanoi Agricultural Univ. – Vietnam
- Can Tho Univ. – Vietnam
- CIANO/Rockefeller Foundation – Mexico
- MA/China Agric. University – China
- ASM Institute Zoology – Moldova
- Institute of Plant Protection – Moldova
- MSU Science Departments -Moldova

Science Service

- Editor-Chief, Journal of Nematology, 1967-72
- Vice President & President SON, 1972-74
- Editorial Board, Revue de Nematologie, 1977-86
- State of California Appointments
  - State Parks Board, 1992-2004
  - Citrus Research Board, 1998-present
  - Water Quality Control Bd, 2000-present
- FAS Reviewer IREX Moldova Applications, 1999-04
- Vice Chairman 2nd International Conference on Ecological Chemistry, Chisinau 2001-02

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