IS-MPMI *Reporter*

International Society for Molecular Plant-Microbe Interactions
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Research and Training Programs

Research Consortia Maximize Resources and Scientific Progress By Bringing Together Researchers With Common Interests

In this issue we continue our coverage of research consortia relevant to studies of molecular plant-microbe interactions. The research consortia described in this issue are catalyzing basic studies of plant disease resistance, microbial ecology and the Medicago truncatula-Rhizobium meliloti interaction. Technology transfer and K-12 education are also mandates of the NSF Science and Technology Centers described herein.

CEPRAP
Center For Engineering Plants For Resistance Against Pathogens

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Background of CEPRAP

The U.S. National Science Foundation established its Science and Technology Centers (S&TCs) program in 1987 to "fund important basic research and education activities and to encourage technology transfer and innovative approaches to interdisciplinary problems." In particular, the charge to the centers was to identify and establish research leadership in new areas of fundamental significance that had potential for commercial application of either the technologies developed or the research information gained. In practice this generally meant a research thrust that was multidisciplinary, long term, high risk and outside the perview of single investigator grants. Given these prerequisites, 24 S&TCs were established in 1989 and 1991, in a broad range of research areas, each with an intended life of 11 years. The Center for Engineering Plants for Resistance against Pathogens (CEPRAP) at the University of California, Davis was funded in the second round of competition for S & TC funding as the only S&TC in the plant sciences beginning in fiscal year 1991. NSF support of CEPRAP brought together a multidisciplinary team of University and industry scientists in a collaborative effort to understand the molecular basis of plant response to pathogens and to develop novel molecular strategies to control plant diseases. As an S&TC, CEPRAP has an education and outreach program targeted to under represented minority K-12 students and also is concerned with technology transfer to other agricultural biotechnology industries.

Facilitation of technology transfer at CEPRAP was initiated by enlisting interaction with four corporations that had ongoing research and commercial interest in engineered resistance in plants to pathogens. The original corporate associates of CEPRAP were Calgene, Ciba Geigy, Rogers Sandoz Seeds, and ICI Seeds. The goal of this concerted action is to enable key players within the European Union to interact within a common framework of proposal actions, for symposia and workshops concerning the use of marker genes and reporter genes in microbial ecology. This cooperation will be particularly valuable for relay of information between the academic groups and the regulatory authorities.

CEPRAP Research Goals

Two general research goals were proposed in the original grant; (i) to investigate the mechanisms by which fungi, viruses, bacteria and nematodes infect susceptible plants and by which plants resist infection and (ii) to create plants bearing new genes for resistance against plant pathogens using recombinant DNA technologies.

As is consistent with the center concept, CEPRAP was established to function as a single research laboratory with approximately 25-30 research and support scientists, with a common budget for both permanent equipment and expendable resources. These researchers work an interdisciplinary fashion on parallel research paths. The goal was to promote continual interactions among the scientists who embrace specialties in genetics, biochemistry, cellbiology, tissue culture and plant pathology. The center has, on site, core facilities for plant transformation, automated DNA sequencing, microscopy, plant and animal cell culture, and fluorescence activated cell sorting(FACS). There is a focus on a single plant genetic system, the tomato. However, both tobacco and Arabidopsis are used in several contexts. The tomato was chosen because of the well characterized genetic system, availability of extensive wild germplasm, tractability of transformation, and because it has an extensive array of economically important pathogens representing the viruses, bacteria, fungi and nematodes.

Understanding the genetics and biochemistry of recognition between pathogen and plant, chemical signaling events, and the subsequent biochemical reactions of the plant and pathogen underlies both CEPRAP's basic research effort and some of CEPRAP's approaches to engineering resistance against pathogens. CEPRAP

research also is aimed at selecting anti-pathogen factors, from such sources as combinatorial libraries, that may be incorporated into synthetic genes for resistance. CEPRAP has proceeded with parallel investigations of several pathogen-plant systems that have identified an array of significant pathogen and plant molecules, along with accompanying genes that are involved in recognition, signaling, and the development of resistance responses and symptoms.

Examples of CEPRAP Research

An early focus at CEPRAP was on the identification and role of developmentally-regulated host genes in disease susceptibility. This led to the discovery of a functional paradigm of apoptosis (programmed cell death, PCD) in pathogen-plant interactions and in normal plant development. The existence of striking functional similarities between the programmed cell death of animal cells and plant cells was not widely anticipated. Apoptosis, and the genes that control it, plays a central role in normal development and in both degenerative diseases and cancer in animals. In plants, programmed cell death-regimes clearly occur at specific points during development and were speculated to be triggered in response to pathogens but the existence of a genetic program, functionally analogous to the apoptotic process in animals, was not known.

In situ analysis at CEPRAP of cells dying during normal development in both root caps and leaf tracheary elements showed characteristic apoptotic features as did cells induced to die by treatment with disease related factors including host-selective phytotoxins, elicitors of the hypersensitive reaction, and by the avirulence protein AvrPto. If apoptosis is a factor in pathogen-plant interactions, as we suspect, then both host and pathogen genes that influence PCD are potential targets for novel approaches to engineering resistance, mediating susceptibility, or even developing new pharmacological controls of disease.

At CEPRAP, parallel studies of apoptosis in plants and animals began with a collaboration with biologists at the University of Nebraska, Lincoln, to compare the biochemical and morphological changes in cells undergoing fungal toxin-induced death. Time and concentration dependent stereotypic hallmarks of apoptosis were observed both in tomato cells and in African green monkey kidney CV-1 cells treated with sphinganine analog mycotoxins (fumonisins and AAL toxins). Continuation of this integrated research approach at CEPRAP with both animal and plant systems has lead to the detection of a number of putative plant analogs of animal apoptosis genes and a signaling pathway to apoptosis involving ceramide-related second messengers similar to that now attracting wide interest among animal biologists.

These studies now are focused on isolating and characterizing functional homologs of animal genes that regulate apoptosis, signaling pathways in plant response to pathogens, and developing single cell technologies for cloning and characterizing disease-related apoptotic genes. The single cells, plant and animal, are used as transient expression assay systems and, with the aid of FACS, sorted and collected for biochemical analysis.

Recently, work at CEPRAP and elsewhere, using a yeast two-hybrid system, revealed binding between the bacterial avirulence gene, AvrPto of *Pseudomonas syringae*, and the Pto resistance gene in tomato. The resistance response of Pto-bearing tomato to AvrPto-bearing bacteria is characterized by a hypersensitive response (HR). Binding was reduced by those mutations in either AvrPto or Pto that also reduced the HR in the intact plant. These results suggest the Pto-mediated resistance results from a direct interaction of the AvrPto and Pto proteins, rather than a less direct interaction, for example between the products of reactions catalyzed by these protein(s). The binding observed in the yeast two-hybrid system was specific and represented the first evidence for an direct interaction between an R gene and an avr gene as predicted by Flor's gene-for-gene

hypothesis. These results are now the basis for extended structure-function studies among the gene products, elucidation of the downstream signaling pathways that may interface with apoptotic pathways, and for engineering approaches to create novel specificities in plant-microbe interactions.

Root-knot nematodes (Meloidigyne species) cause over a $100 billion in damage per year world wide and infect over a thousand crop plants, including tobacco, tomato, peppers, eggplants, carrot, cassava, coffee, grape, lettuce, walnut, beans, cowpeas, peach, almond, cucumbers, melons, cotton, alfalfa, and upland rice. The Mi1 gene of tomato confers resistance against most isolates of several species of Meloidigyne. CEPRAP researchers have localized Mi1 to a 60 kb region of the tomato genome by analysis of recombinants. An approximately 55kb bacterial artificial chromosome clone, spanning most of this region, was identified and the entire clone was sequenced. Analysis of the 55 kb insert revealed three regions with strong similarities to known resistance genes. Genes in this region are being identified by transformation of tomato for complementation, at CEPRAP and elsewhere.

Organization And Operation of CEPRAP

Most of the co-principal investigators of CEPRAP are appointed in academic departments of the College of Agricultural and Environmental Sciences at UC Davis. Two are located at UC Berkeley, one at UC Riverside, and one at Calgene, Inc.

CEPRAP has three research thrusts with emphasis on recognition, signaling, and genetically-engineered resistance, each with a group leader. Research topics of the one-to-three projects within each thrust are host and pathogen enzymes and receptors, genes, gene regulation, signal transduction, programmed cell death, transfer of resistance genes and chimeric resistance genes into susceptible species, and the design of synthetic genes for resistance. Each thrust and each project is advanced by a group consisting of CEPRAP co-principal investigators, postdoctoral associates, graduate and undergraduate students and technicians.

Outreach

CEPRAP's outreach program promotes education in several areas of modern plant biology and biotechnology, targeting K-12 students, their teachers, and community college students. The graphically illustrated color version of CEPRAP-developed interactive educational software, Germ Wars, is used in approximately 600 schools throughout the country. Although the programs are aimed at K-12 students, they also are available to the general public free of charge, for example at Disney's Epcot Center. The first two modules in the Germ Wars series, available on a CD-ROM, focus on microbes and how they affect people and plants.

To assist teachers in exploring the subject of biotechnology, CEPRAP has created an extensive biotechnology experiments kit that is available to teachers for use in their classroom. Using the kit, accompanying materials, and teacher training provided by CEPRAP, local students perform experiments such as restriction enzyme analysis and bacterial transformation. A new microbe kit loan program for middle school students is currently being developed. Experiments deal with a simulated crop epidemic and with other aspects of microbe-plant interactions.

One-on-one interactions also are a vital aspect of the outreach program. Through annual internship programs, CEPRAP reaches science teachers, high school students, and has developed linkages with students at D-Q University, an accredited Native American community college and middle schools in the U.S. Southeast.

Additional information on CEPRAP is available from our home page at http://www.ceprap.ucdavis.edu.

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CME
Center For Microbial Ecology

The Center for Microbial Ecology (CME) at Michigan State University was established in February 1989 by the National Science Foundation, as one of 11 new Science and Technology Centers. These Centers conduct basic research on complex problems that are of large scale, long duration and that require special facilities or collaborative arrangements. The CME is devoted to the basic understanding of microorganisms and their function in their natural and human-made environments, and has brought together scientists from the various disciplines important to microbial ecology. It is directed by James M. Tiedje.

Overview of the CME's structure and activities.

The intellectual focus of the Center for Microbial Ecology is to understand factors that influence the

competitiveness, diversity and function of microorganisms in their natural and managed habitats. This knowledge is important because microorganisms have major roles in determining global warming, ground water quality, plant and animal health, and organic matter cycling. Further, microorganisms are important to the biotechnology industry and include organisms developed to degrade hazardous chemicals, for the production of pharmaceuticals and for biocatalysis. To ensure a sustainable biosphere and to exploit these microbial processes for economic gain, we must develop a comprehensive understanding of microbial diversity, microbial processes and microbial interactions. This can best be achieved through a multidisciplinary research effort such as that of the Center.

The fundamental research done by the Center is organized into four thrust groups and one Research Initiative; namely Community Analysis, Population Dynamics and Evolution, Microbial Adaptation, Polyphasic Taxonomy, and Bioremediation. The thrust groups and the research initiative are comprised of interdisciplinary teams drawn from 35 faculty with expertise in the areas of microbial ecology and physiology; engineering; molecular biology and genetics; biochemistry; chemistry and environmental chemistry; mathematics and computer science; and ecology and evolutionary biology. The research is conducted by 70 graduate students and postdoctoral scientists in 15 academic departments and through collaborations with other U.S. and foreign institutions.

Community Analysis - Studies the ecological patterns of genotypic and phenotypic characters found in microbial communities that reflect the outcome of competitiveness and diversity and ultimately influence the dynamics of microbial processes in the environment.

Population Dynamics and Evolution - Conducts studies on genetic mutability and competitiveness to understand better the factors that contribute to evolutionary success in microorganisms.

Microbial Adaptation - Seeks to understand the genotypic and phenotypic characteristics that influence the ability of microbial populations to adapt to stress and other changes in the environment.

Polyphasic Taxonomy - Addresses the national need for additional and integrated information on microbial biodiversity through the development and use of methods and databases to understand better the extent and utility of microbial biological diversity and seeks to explore, identify, and characterize novel microbial diversity.

Bioremediation Research Initiative -- Research in the Bioremediation Initiative is directed toward enhancing the bioremediation of environmental pollutants, developing practical bioremediation processes, and characterizing populations of microorganisms able to degrade environmental contaminants.

The CME faculty and outside collaborators responsible for carrying out the research in these thrust groups are listed below:

Community Analysis (Ecological Patterns)	**Population Dynamics and Evolution (Evolutionary Processes)**	**Microbial Adaptation (phenotypic and Genotypic Traits)**	**Polyphasic Taxonomy Core (Approaches and Techniques)**	**Biormeditation**	
Kay Gross James Tiedje Michael Klug	Richard Lenski	Michael Tomashow	John Breznak James Tiedje	James Tiedje Craig Criddle	**Thrust Group Leader**
Robert Hickey			Thomas Schmidt	Mike Klug Bill Punch	

Thomas Schmidt C.A. Reddy Craig Criddle Anil Jain Frans de Bruijn	Robert Hausinger Julius Jackson Anthony Jarosz Dennis Fulbright	Frans de Bruijn Richard Lenski Thomas Schmidt	Niels Larsen Sakti Pramanik John Holt George Garrity Ross Overbeek	Robert Hickey Robert Hausinger Peter Adriaens Steven Boyd C. A. Reddy Sharon Anderson	**Core Faculty**
Philip Robertson Frank Dazzo John McGrath Peter Adriaens Eldor Paul	Michael Bagdasarian Larry Snyder William Holben Dan Dykhuizen	C. Peter Wolk Wendy Champness Rawle Hollingsworth John McGrath	Joanne Whallon Herman Hughes Michael Klug Mark Worden Anil Jain Carl Woese Gary Olsen	Peggy Ostrom Tom Voice Susan Masten Richard Wiggert	**Contributing Faculty**
Ecology Computer Science Microbial Genetics Soil Science Microbial Ecology Environ. Engineer	Population Biology Biochemistry Mathematics Microbial Genetics Microbial Ecology Molecular Biology	Biochemistry Molecular Biology Population Biology Microbial Ecology Chem. Engineering Microbial Genetics	Computer Science Molecular Biology Microbial Ecology Microbial Genetics	Computer Science Enviorn.Engineering Geology Microbial Genetics Microbial Ecology Biochemistry Soil Science	**Expertise**

The interdisciplinary teams of scientists and engineers involved in these projects have made several important contributions to the field of microbial ecology. For example, they have developed and used methods to measure diversity within microbial communities and to assess changes in the structure and function of bioreactor and soil communities that occur in response to perturbations or changes in environmental conditions, thus providing insight to improved management of various microbial processes. Studies on microbial processes involved in the degradation of pollutants have led to new technologies for environmental remediation and new understanding of how to enhance the rates of naturally occurring processes. Research to understand the evolution of catabolic pathways has led to surprising discoveries concerning the global distribution of specific genes and organisms, as well as the high degree of genetic diversity and functional redundancy that can exist in a microbial community. Finally, this research has begun to unravel the complex issue of how various microbial characters contribute to the competitive abilities of microbial populations. There are several specific cases in which this knowledge has been used to develop new technologies for environmental remediation, improve strategies for waste management and to better understand the fates of chemicals in the environment, all of which will lead to improved environmental quality.

International research.

The Center has ongoing collaborations with scientists from Japan, Russia, Norway, Belgium, Germany and Canada, including two major long-standing institutional collaborations with Japan and Russia. Moreover, an agreement with the Research Center for Molecular Microbiology (RCMM), one of the South Korean science and technology centers was recently signed.

Japan. In 1996 the five year international collaborative project with Japan under JRDC's International ERATO Program was completed. Three postdoctoral scientists from the NSF Center for Microbial Ecology conducted research in Japan during the past year. In return, three scientists from Japan conducted research at CME. The research findings of the project were presented at a symposium entitled "Microbial Evolution and Biodegradation" held in Tokyo, Japan in February 1996.

Russia. Two unique opportunities arose from collaborations with scientists at the Russian Academy of Sciences. First, was the opportunity to study microorganisms in permafrost soil cores from the Kolyma region near the East Siberian Sea, in collaboration with the Laboratory of Cryopedology. The second collaboration, with the Institute of Physiology and Biochemistry of Microorganisms, provides an opportunity to study the biochemistry and

genetic basis of chloroaromatic compound metabolism. A third collaboration with the Institute of Microbiology in Moscow has also been initiated. This collaboration is focused on carbon and methane cycling in the massive tiaga wetlands. Six Russians will have worked at CME in 1996 under this NSF-INT/STC funded program.

Korea. The Korean collaboration is with a multi-institutional center with similarities to the STC Program/CME and is funded by the Korean Science and Engineering Foundation. RCMM is headquartered at Seoul National University. Their multidisciplinary Center has strong expertise in actinomycete taxonomy, molecular response to environmental stress, microbial biodegradation and molecular community and nutrient cycling studies in lakes. Our collaboration is focused on short-term exchanges for targeted collaborative projects. The first exchanges are expected to occur in the summer of 1997.

In addition to its research focus, the Center has major programs in Industrial Outreach, Graduate Education and Educational Outreach designed to create new interdisciplinary knowledge, increase scientific literacy, help to develop a well-trained workforce knowledgeable in microbial ecology, and to facilitate the dissemination of knowledge to the industrial and governmental sectors.

Industrial Outreach.

Through the Industrial Outreach Program of the Center, strong linkages have been formed with corporations in many business sectors as well as state and federal agencies. These linkages have resulted in several joint research projects, attracted extramural funding for research, and resulted in the licensing of technology developed at the Center. Perhaps of greatest importance is that these linkages have provided an efficient means to transfer knowledge developed through the Center's research that can be applied to meet specific needs related to the use of microbial processes. In response to the interest expressed by the industrial and governmental sectors, these linkages have focused primarily on (a) the development and demonstration of innovative waste treatment or environmental remediation technologies that require the management of naturally occurring or engineered biological processes; and (b) understanding physicochemical and biological factors that influence the fate of pollutants in the environment.

Education of undergraduate- and graduate students and educational outreach.

The multidisciplinary nature of the CME has attracted many highly motivated undergraduate-, as well as graduate students. A number of MSU undergraduate students are carrying out research projects in laboratories of CME faculty members and are making significant contributions to the publications being generated. In addition, links have been established with other colleges; for example, thirteen minority students from Historically Black Colleges and Universities and the University of Puerto Rico participated in the Research Experience for Undergraduates program at the Center last year.

The CME supports approximately 25 Ph.D. students, who carry out research in the different thrust groups; these students are usually affiliated with the MSU Microbiology department, and, together with the CME postdoctoral fellows constitute the driving force behind the CME research efforts, including the international collaborations. The interdisciplinary thrust group approach in the CME strongly benefits the education of the students, and much better prepares them for the job market lying ahead.

The CME educational outreach program has been instrumental in passing on knowledge to the larger community, especially K-12 schools and their students. The development of the CME- Detroit Urban Systemic

Initiative/Michigan Statewide Systemic Initiative Interlink (STC-DUSI/MSSI INTERLINK) collaboration is a recent addition to the Educational Outreach program, "The Unseen World". Statewide, a postdoctoral fellow will be assisting MSSI in coordinating the sharing and disseminating of information between other regional K-12 programs. In Detroit, the primary goal is to assist with development and implementation of hands-on science materials.

Part of this initiative is a direct outgrowth of the "Science in the City" program in Detroit. CME assistance will include supplemental hands-on project kit development and science teacher training on an individual basis. For example, during the summer of 1995 one middle school and two high school teachers from Detroit Public Schools came to the CME to participate in the Minority Research Apprenticeship program.

For detailed information about the CME and its activities, see the CME Web page at http://www.cme.msu.edu/CME/

International Research Efforts on *Medicago truncatula*: a model for legume biology

Legumes are the system of choice for study of both rhizobial and mycorrhizal associations, as well as other plant-microbe interactions and aspects of plant biology that are not readily studied in non-legume systems. However, genetic analysis of these processes is difficult in the major crop legume species, including pea, alfalfa and soybean, due to features such as tetrapolidy, large genomes with abundant repetitive DNA, and inefficient methods for transformation and regeneration. Therefore, legume researchers have sought a legume species, with attributes of a simple genetic system, that could serve as a model for legume biology. One such species is the diploid alfalfa relative *Medicago truncatula* (Barker et al., 1990; Cook et al, 1997).

Key attributes of *M. truncatula* include its diploid and autogamous nature, small genome (500 mbp/1C), rapid reproductive cycle (2-3 weeks for vernalized seed), numerous genetically distinct plant ecotypes, and a well characterized bacterial symbiont, *Rhizobium meliloti*. Moreover, *M. truncatula* researchers have been developing key enabling technologies including efficient methods for transformation and regeneration, simple protocols for artificial hybridization, expressed sequence tag and bacterial artificial chromosome libraries, genetic maps, germplasm resources, and numerous characterized plant mutants.

Although first proposed as a model for molecular-genetic studies in the late 1980s, *M. truncatula* has been an important agricultural species for nearly three decades and has been the subject of more than 100 agronomy publications between 1960 and 1990. *M. truncatula's* agronomic value derives from its drought and salinity tolerance and its ability to grow over a wide range of soil and environmental conditions. In dry regions of Australia, *M. truncatula* is cultivated in rotation with cereals, while in the Mediterranean basin, *M. truncatula* is used as a forage for livestock.

More recently, the increasing emphasis on *M. truncatula* as an experimental system for basic plant biology has led to a proliferation in the number of laboratories involved in the cloning and characterization of plant genes and mutants, as well as the development of important resources for molecular-genetic analysis. This common interest in *M. truncatula* as a model system provides the foundation of numerous formal and informal research collaborations, and has prompted researchers to organize a first meeting devoted exclusively to *M. truncatula* biology (July 18-10, 1997, in Paris, France). The meeting is expected to attract more than seventy researchers from seven countries.

The research summaries given below provide a broad overview of *M. truncatula* research involving more than twenty-five principle investigators from Australia, France, Switzerland, and the United States. The topics covered by these investigators frequently involve plant-microbe interactions and related aspects of plant cellular and developmental biology. Examples include:

- Rhizobium-legume interactions
- mycorrhizal interactions
- plant-pathogen interactions
- plant-insect interactions
- signal transduction
- genetics and population biology
- phosphate and iron transport
- gene regulation
- isoflavonoid metabolism

The University of Newcastle, Department of Biological Sciences, Newcastle, Australia (contributed by Ray Rose)

My laboratory's interest is in genetic manipulation of *Medicago truncatula* in terms of basic research (transgenics, cell fusion and genetic mechanisms of regeneration) and also in its application to agronomic characters and nitrogen fixation. Our recent research on genetic manipulation has built on previous work demonstrating regeneration of *M. truncatula* from mesophyll protoplasts using the highly regenerable seed line Jemalong 2HA and an agarose droplet technique (with Kim Nolan). It is possible to fuse these cells with protoplasts from other *Medicago* species and produce asymmetric hybrids as demonstrated by molecular analysis of the regenerants (with Dacheng Tian). This work is continuing to study its utility and molecular genetics. Jemalong and Jemalong 2HA are near isogenic lines that differ in their regeneration efficiencies. We will use these lines to analyze gene expression in relation to regenerability via organogenesis and somatic embryogenesis. In a second project, we have transformed M. truncatula with the AMV coat protein gene. In collaboration with John Randle's lab (University of Adelaide) we have determined that plants expressing this gene are virus tolerant. Other collaborative research with transgenic *M.truncatula* relates to Bt genes directed against Sitona weevil (which grazes nodules), herbicide resistance and high sulfur proteins.

Laboratoire de Biologie Moleculaire des Relations Plantes-Microrganismes, INRA-CNRS, Toulouse, France (Contributed by Thierry Huguet)

The general objectives of the Toulouse laboratory regarding *M. truncatula* deal with molecular and genetic studies to analyse plant responses during the *Rhizobium meliloti* symbiosis. Our plant material for general purposes is the line A17, derived from *M. truncatula* cultivar Jemalong.

The P. Gamas and J. Cullimore research group is studying early symbiotic responses of *M. truncatula*. In collaboration with the group of R. Ranjeva (CNRS, Toulouse University, France), a first theme is the search, using biochemical and molecular biology methods, for receptors to lipooligosaccharides (Nod factors) synthesized by *Rhizobium*. A second theme is the identification and characterization of new genes expressed during various steps of nodulation. In addition, the characterization and study of regulation of symbiotic expression of *M. truncatula* glutamine synthetase genes is under way in collaboration with H. Carvahlo

(University of Porto, Portugal).

T. Huguet's group is collaborating with the J.M. Prospéri group (INRA, Montpellier, France) to establish a genetic map of *M. truncatula* based on crosses between natural populations and a set of F7 recombinant inbred lines. Another objective is the characterization and genetic mapping of genes for symbiotic variation revealed by inoculation of natural populations of *M. truncatula* with a collection of wild type *R.meliloti* strains. Lastly, the group is engaged in the genetic mapping and positional cloning of symbiotic mutations (Nod- and Nod++) of *M. truncatula* in collaboration with the G. Duc group (INRA, Dijon, France).

D. Barker and E.P. Journet's group is studying plant genes (early nodulins) which are expressed during the earliest steps of the legume/Rhizobium interaction and in particular in response to *Rhizobium* Nod factors. Transgenic *M. truncatula* expressing a number of early nodulins promoter/Gus fusions have been constructed in order to study spatio-temporal gene expression in detail. A pharmacological approach is being used in conjunction with transgenic lines to study Nod factor signal transduction. In collaboration with the Gianninazzi group (INRA, Dijon, France), the group is engaged in the study of early nodulin expression during the mycorrhizal symbiosis.

The J. Dénarié and C. Rosenberg group is engaged, in collaboration with D. Cook's group (Texas A&M University, USA), in the identification of *M. truncatula* mutants affected for early response during nodulation. These mutants will be characterized by cellular, molecular and genetic techniques. Genes involved in nod factor perception and transduction will be cloned by positional cloning. Another approach is to screen *M. truncatula* natural populations, in collaboration with J.M. Prospéri's group (INRA, Montpellier, France), to identify natural alleles differing in their response to Nod factors modified in some of their structural motifs (sulfatation, acetylation, acylation...).

Station de Genetique et d'Amelioration des Plantes, INRA, Montpellier, France (Contributed by J.M. Prosperi)

The aim of the INRA Montpellier Genetic Resources and Lucerne Plant Breeding Laboratory is to select cultivars for rangeland improvement or forage crops in Mediterranean regions. Priority is given to two species: *Medicago sativa* and *Medicago truncatula*. Based on surveys of natural populations (more than 3000 natural populations were collected since 1985) using agronomic and biomolecular tools (electrophoresis, RAPD, RFLP...), the research is focused on the study of the diversity maintained within natural populations, and on the mechanisms involved in the maintenance of this diversity. Genetic diversity differs according to the variability observed: neutral or selected genes with fixed or variable selection pressures. Indeed, distribution of genetic diversity depends on the scale of observation: between populations from several ecological environments, between populations within a given ecological area; between different plants from one natural population... To address these issues, we focus on the diploid model species, *M. truncatula* Gaertn with extension on the tetraploid *M. sativa*. For *M. truncatula*: A high level of polymorphism was observed both between and within natural populations for both neutral markers (RAPD and isozymes) and morphological traits. A strong level of distortion of PCR-derived markers appeared when parental genotypes from different populations were crossed. We suggest that parental genotypes have diverged for sometime and that this level of distortion is related to the genome size difference measured between the two parental genotypes. Objectives of this research include obtaining recombinant lines from 6 intra-specific crosses (based on Single-Seed-Descent method), constructing a genetic map (T. Huguet et al 1996), studying the segregation of important agronomic traits (cold tolerance, nitrogen fixation efficiency) and identifying QTLs involved in such traits.

Station de Genetique et d'Amelioration des Plantes, INRA, Dijon, France (Contributed by Muriel Sagan)

The laboratory of Genetics and Plant Breeding in Dijon (France) is involved in identifying plant symbiotic genes. Our recent research efforts on M. truncatula have emphasized identification of symbiotic mutants after gamma-ray mutagenesis. We have identified eighteen nodulation and mycorrhizal mutants derived from *M. truncatula* cv Jemalong. Our current research involves identification of additional nodulation and mycorrhizal mutants (more than 30 mutant lines are now available), as well as the classical genetic analysis of these mutants. To date we have characterized six mutants, each representing distinct genes, where the mutant phenotypes are Nod-Myc- (1), Nod+Fix- (4) and Nod++ (1), respectively. We are also involved in cytogenetic studies for physical mapping of genes by in situ hybridization, and we have established a collaboration with the laboratory of A. Kondorosi in Gif-sur-Yvette (France) to develop a transposon mutagenesis system in *M. truncatula*.

Institut des Sciences Vegetales, CNRS, Gif-sur-Yvette, France (Contributed by Pascal Ratet)

The group of A. Kondoraosi is involved in developing new tools for genetic and molecular studies of the M. *truncatula-R. meliloti* interaction. We have improved the regeneration capacity of *M. truncatula* genotype 108 and established efficient transformation protocols that allow routine production of transgenic plants. These transgenic plants are used to study: (1) early recognition mechanism(s) between *M. truncatula and R. meliloti*, (2) early events of nodule organogenesis, (3) the role of nodulin Enod40 in nodule organogenesis, (4) cell cycle control during nodule organogenesis, and (5) defense reactions during the symbiosis. In addition, we have introduced En/Spm derived constructs into *M. truncatula*. Transgenic lines carrying an active En/Spm transposon will be used for a mutagenesis program. The use of transposon tagged mutants may allow the identification and characterization of genes involved in *M. truncatula-R. meliloti* recognition as well as in processes for nodule organogenesis.

Botanisches Institut der Universitat Basel, Basel, Switzerland (Contributed by Regina Vogeli-Lange)

Ongoing research programs at the University of Basel, Switzerland, center on *M. truncatula* as a host plant for symbiotic interactions with arbuscular mycorrhizal fungi. Regina Voegeli-Lange focuses on the identification and characterization of novel plant genes regulated during mycorrhiza formation. Several promising candidates have been isolated by mRNA differential display and are currently being investigated in more detail. Many of the clones appear to be derived from rare transcripts and represent previously unknown genes. Since analysis of nodulation mutants predicts a certain overlap in gene expression during nodulation and mycorrhization, it will be of particular interest to determine whether the newly identified mycorrhiza genes are similarly regulated in the symbiosis with rhizobia.

In successful symbiotic interactions, plant defense responses often are suppressed or not elicited. Surprisingly, ectomycorrhizal fungi release elicitors which induce defense responses in cells of host plants. Peter Salzer found that secreted plant chitinases are able to inactivate these elicitors. Thus, plant chitinases may be necessary for basic compatibility between the two partners. In a direct approach, Salzer is attempting to knock out genes coding for apoplastic chitinases in roots of *M. truncatula* and wants to study the effect of reduced chitinase activity in host plants on the induction of defense responses during mycorrhiza formation.

University of California-Santa Barbara, Department of Molecular Biology, USA (Contributed by Jim

Cooper)

Most of the work the Cooper lab is focused on understanding the structure, regulation and function of the family of plant extracellular matrix proteins, called proline-rich proteins (PRPs), that are thought to form covalently crosslinked structural networks in plant cell walls. Clones encoding several members of the PRP family were characterized and used to show that the PRP gene family is tightly regulated during both root and nodule development. Antibodies raised against synthetic PRP peptide domains are currently being used to describe the patterns of PRP deposition in developing roots and nodules. Confocal microscopy experiments demonstrate that PRPs are primarily localized in the intercellular spaces between root cortical cells and in the developing vascular tissues in seedling roots. In differentiating xylem vessels, the spatial pattern of PRP deposition presages the eventual pattern of lignin deposition. Using affinity chromatography, we isolated anti-PRP antibodies that recognize only nodule-specific PRPs, and these are now being used to investigate nodule infection by both wild-type and infection-deficient *Rhizobium* mutants. All of our data indicate that an important early response of host root cells to symbiotic signal molecules is reorganization of host cell wall architecture.

Drexel University, Department of Bioscience and Biotechnology, Philadelphia, USA (Contributed by Rebecca Dickstein)

My lab is investigating the function and role of plant genes associated with the development of nitrogen fixing root nodules in both *M. truncatula* and alfalfa. We are focusing on the Enod8 gene, as well as several other genes expressed in empty as well as invaded root nodules. Enod8 is similar to a number of other plant genes whose transcript levels increase upon exposure to elicitors or upon wounding. It is also similar to several bacterial and one identified plant gene encoding lipolytic enzymes. We have raised antisera to alfalfa Enod8 and are using it to characterize the Enod8 protein for immunocytochemical localization studies, and to help us purify the Enod8 protein from both root nodules and from *E. coli* expressing Enod8. To address questions about Enod8's regulation, we are using RT-PCR to evaluate Enod8 expression in Medicago roots spot inoculated with *R. meliloti*. We have also cloned genomic Enod8 genes from *M. truncatula*, with the goal of identifying the important cis-regulatory promoter elements. We have obtained several genomic clones already from an existing genomic library, but unfortunately, these do not contain sufficient Enod8 5' flanking region. We are currently having a new *M. truncatula* genomic library made in a lambda vector, which we will screen for Enod8. We are also making Enod8 antisense constructs, which we will transform into *M. truncatula*, to address the question of whether Enod8 is required for nodule development.

USDA-ARS Children's Nutrition Center, Houston, USA (Contributed by Mike Grusak)

My group is investigating the processes of root iron acquisition and whole-plant iron partitioning in crop plants. Our goal is to identify the molecular mechanisms that could be manipulated to enhance iron content in edible organs, and thus have an impact on iron deficiency-induced anemia in humans. This nutritional disease is a serious problem, particularly in developing countries, where plant foods are the main source of dietary nutrients. Our studies to date have focused on two unique *Pisum sativum* mutants that can hyperaccumulate iron in their vegetative tissues; one mutant also has the capacity to transport excess iron to its developing seeds. We have shown that a root plasmalemma-localized iron(III) reductase system is the rate limiting process in root iron acquisition, and that phloem iron transport to seeds requires a chelator for phloem loading. Recently, we have begun studies with *M. truncatula* in order to characterize the iron physiology of this species and to devise a screen for new iron-hyperaccumulating mutants (i.e. exhibiting iron toxicity symptoms). Studies with wild type

plants have demonstrated that *M. truncatula* exhibits the normal root processes associated with iron acquisition in legumes and thus should be a useful model plant for studying higher plant iron physiology.

Complex Carbohydrate Research Center, University of Georgia, USA (Contributed by Michael Hahn)

The group of Michael Hahn and Francois Côté are using *M. truncatula* as a model system to understand the response(s) of plant cells to oligosaccharide signals originating from the mycelial walls of phytopathogenic oomycetes (e.g., *Phytophthora* spp.). Specifically, we seek to identify and characterize in *M. truncatula* the molecular components of the signal transduction pathway whose activation by glucan elicitors leads to the biosynthesis and accumulation of antimicrobial phytoalexins. Our experimental approaches are a combination of biochemical, molecular genetic, and ultrastructural techniques. At the biochemical level, we have shown that *M. truncatula* has a specific, high affinity binding site for glucan elicitors and we are attempting to isolate and characterize the protein(s) responsible for the elicitor-binding activity. In parallel, we are seeking to identify components of the signal transduction pathway by screening for *M. truncatula* mutants that are defective in their ability to accumulate phytoalexins in response to glucan elicitors. Mutants isolated by this screen are expected to include those with defects in: 1) structural genes encoding signal transduction pathway components; 2) enzymes involved in biosynthesis of the pterocarpan phytoalexins; and 3) regulatory genes interacting with any of these components. Lastly, we are examining the interaction of *M. truncatula* with the alfalfa pathogen *Phytophthora megasperma* f.sp. *medicaginis* at the light and electron microscopic level (in collaboration with Dr. C. W. Mims) to learn more about the spatial and temporal characteristics of the plant's responses to infection.

The Samuel Roberts Noble Foundation, Oklahoma, USA (Contributed by Maria Harrison)

Current research in the Harrison lab includes molecular and genetic analyses of the arbuscular mycorrhizal symbiosis formed between *M. truncatula* and members of the Glomalean fungi. The aim of the research is to gain insight into the molecular mechanisms underlying formation and functioning of the symbiosis with emphasis on two areas, (1) the signaling processes that occur between the two partners to permit development of a functional symbiosis and (2) phosphate transport in the symbiosis. Molecular analyses have resulted in the cloning of a number of mycorrhizal induced or mycorrhizal repressed cDNA clones including three cDNA's encoding phosphate transporters, one from a mycorrhizal fungus, *Glomus versiforme* and two from *M. truncatula*. These are currently being used to investigate aspects of phosphate uptake in the symbiosis. A genetic approach to identify genes essential for the symbiosis is also being pursued and five *M. truncatula* mutants unable to form a complete mycorrhizal symbiosis have been identified. These were identified from a set of 31 nodulation mutants previously isolated in D. Cook's lab (Texas A&M University). Further analysis of these mutants including details of the phenotypes and genetic analysis are in progress.

University of North Carolina-Chapel Hill, Biology Department, USA (Contributed by Jason Reed)

We wish to identify host requirements for rhizobial invasion of nodules through genetic studies of the model legume *M. truncatula* and its symbiotic partner *R. meliloti*. Rhizobia invade nodules as they are forming through tubes called infection threads. Previous studies of the *R. meliloti*/alfalfa (*Medicago sativa*) symbiosis have shown that in order to invade nodules successfully, the bacteria must make one of three different exopolysaccharides called EPS I, EPS II, and KPS. These exopolysaccharides may function as signals to the plant, or may have some other function. We are searching for plant genes involved in nodule invasion by assaying natural ecotypes of the model legume *M. truncatula* for variation in ability to support nodulation by bacterial

strains making one or another of these exopolysaccharides; by screening for mutants of the Jemalong cultivar of *M. truncatula* that are less permissive to nodule invasion by bacteria making only EPS I; and by screening for mutants of Jemalong that are more permissive to nodulation by bacteria making only non-functional exopolysaccharides. We expect these studies to reveal plant genes important for exopolysaccharide recognition, or for nodule invasion more generally.

Texas A&M University, Department of Plant Pathology and Microbiology, and Department of Biology, USA (Contributed by Doug Cook and Kathryn VandenBosch, respectively)

The Cook lab is pursuing two strategies to elucidate mechanisms for host control of nodulation. First, we are examining regulation of the *Rhizobium*-induced peroxidase gene rip1, and second, we are characterizing plant mutants with defects in early nodule development. We anticipate that these two strategies will overlap, as plant genes and proteins required for correct regulation of early nodulin genes may also be targets in our mutant hunts. Initial screens identified over 100 putative symbiotic mutants, thirty of which have been confirmed as heritable. Six of these mutants segregate as single gene mutations in different loci. Currently we are using double mutant analysis to determine gene interactions and construct a frame-work genetic pathway for nodulation. We are interested in positional cloning of the responsible genes and we have produced mapping populations for two distinct supernodulation mutants and a single nod-myc- mutant. In related work, we have constructed a megabase-sized BAC DNA library that contains five genome equivalents. Our primary collaborations are with the laboratories of Kate VandenBosch, Maria Harrison and Jean Dénarié.

Kate VandenBosch's group, in collaboration with Doug Cook, uses cellular and genetic approaches to study root development and infection by *Rhizobium meliloti* in *M. truncatula*,. The first theme is arrest of infections. We are analyzing mutants that have reduced numbers of infections and/or which arrest infections in the root epidermis. In some mutants, we have found defense responses such as induction of isoflavanoid phytoalexin biosynthesis. We are investigating changes in the extracellular matrix during infection and infection arrest. Work with Marcia Kielisewski (Ohio University) investigates cross-linking and other modifications of structural cell wall proteins, while collaborative work with Cook studies function of a *Rhizobium*-induced cell wall peroxidase. A second line of inquiry examines non-nodulating mutants that show defects in polar growth of root hairs. We expect this work will elucidate shared aspects of root and symbiotic development, including control of exocytosis, cytoskeletal function, and phytohormone responses.

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For further information about this consortium, contact: Dr. Douglas Cook at: drc1653@acs.tamu.edu

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Program for the Biology of Filamentous Fungi Texas A&M University

In many of the catalogues of living organisms, fungi occupy one of the four kingdoms of eukaryotes along with plants, animals, and protists. Their biological distinctiveness is matched by their economic importance. They are the most numerous causal agents of plant disease, and economically, the most important. They are primary

degraders of complex organic molecules, symbionts of higher and lower plants, and pathogens of humans and other animals. Our lives would be very different, and less enjoyable, without their role in food fermentation, and as one of our most commonly eaten vegetables. Their secondary metabolites and enzymes are the products of large pharmaceutical and chemical fermentation industries. And they give us great joy when we walk through damp woods and see their bright colors and myriad forms.

The importance of fungi has not been matched by research efforts to understand their biology and to either enhance their economic importance or reduce their adverse economic impacts. Recognizing the importance of fungi and the potential for future research developments, the Program for the Biology of Filamentous Fungi was organized at Texas A&M University.

This program was originally organized by a group of faculty from two departments and has since expanded to include faculty from three departments. Internal funding provided the opportunity for the program to sponsor an annual symposium on a pertinent topic and to provide funding to support the research assistantships of both graduate and undergraduate students. This seed money was followed by a successful NSF graduate student training grant with institutional matching support used to maintain the undergraduate program and the annual symposium.

There are currently 12 faculty members associated with this program. These faculty and members of their laboratories meet monthly for informal seminars by students, post-docs, etc. with more than 30 people usually in attendance. The annual symposium is timed to correspond to the peak wild-flower season of Texas to provide a beautiful backdrop to a combination of formal presentations, informal discussions, and relaxation. This agenda provides a maximum opportunity for interaction between the four invited speakers and other participants. Post-doctoral students are encouraged to present talks about their research during the two formal half-day sessions. Since the symposium is not advertised the numbers of individuals involved remain small to assure optimum interaction between students and participants.

The NSF training grant provides a very competitive fellowship to graduate students who are admitted into the program. All students rotate through different laboratories before selecting a laboratory and department for their degree. The graduate degrees that can be offered through this program include M.S. and Ph.D. in Plant Pathology, Biology, Genetics, Toxicology, and Biochemistry. Faculty research strengths are in the areas of fungal secondary metabolism, fungal gene regulation, fungal development, population biology, fungal viruses, molecular basis of fungal virulence, informatics, biological control of fungi, and fungal ecology. A sequencing center to initiate the sequencing of a fungal genome is currently being established. For further information contact: Neal Van Alfen at vanalfen@ppserver.tamu.edu

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American Phytopathological Society
1997 Annual Meeting

This year's APS Annual Meeting will have something to offer virtually everyone in the molecular plant sciences. IS-MPMI members may want to take note of these planned events in particular:

Colloquia:

New Perspectives on Phytopathogenic Mollicutes (Sponsored by the APS Bacteriology Committee)

Plant Viruses as Tools (Sponsored by the Virology Committee)
A Holistic View of Plant-Nematode Interactions (Sponsored by the Nematology Committee)

Discussion Sessions:

Plant Virus Synergies (Sponsored by the Virology Committee)

Symposia:

Transgenic Plants(Sponsored by the Councilor's Forum)
Horizontal Gene Transfer (Sponsored by the Genetics Committee)
Fusarium Development and Pathogenesis: Nectria haematococca as a Model System (Sponsored by the Biochemistry, Physiology and Molecular Biology Committee)

Contributed Paper/Poster Sessions in the Following Categories:

Bacteria - genetics, molecular biology, cell biology
Fungi - genetics, molecular biology, cell biology
Host-parasite relations - biochemistry, molecular biology, cell biology
Nematodes - Genetics, molecular biology, cell biologyPhytoplasmas, spiroplasmas, fastidious prokaryotes
Viruses - genetics, molecular biology

Complete program information and registration materials can be found at APS's web site located at: http://www.scisoc.org/ or by contacting Betty Pfeifer at APS Headquarters, 3340 Pilot Knob Rd., St. Paul, MN 55121 Phone 612-454-7250 Fax 612-454-0766.

IS-MPMI PUBLICATION

Biology of Plant-Microbe Interactions

Edited by Gary Stacey, Beth Mullin, and Peter M. Gresshoff

This collection of studies is an overview of recent advances in the intriguing field of molecular plant-microbe interactions research. Each chapter was contributed by participants in the Eighth International Congress on Molecular Plant-Microbe Interactions which was held in Knoxville, Tennessee, USA in July 1996.

This book should prove useful for researchers, scholars, and students interested in the most recent advances in the study of plant-microbe interactions. The chapters are written by world authorities on information that has been gathered during the last two years using modern molecular methods.

These studies draw on a wide variety of scientific disciplines, including biology, chemistry, biochemistry, and cell biology. Topics covered include molecular studies of plant symbioses, *Agrobacterium*, bacterial and fungal pathogens, microbial signals and signal transduction pathways, biocontrol, and applications to biotechnology. A section focusing on emerging areas of research on plant-microbe interactions is also included.
1997; 6" x 9"; hardbound; 608 pages, 16 black and white photographs, 88 figures, and 22tables; ISBN: 0-9654625-0-1

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A Note From The Editors

We invite you to submit your material for publication in the *IS-MPMI Reporter*. We also welcome your suggestions and ideas for future articles.

The IS-MPMI Reporter 1997 schedule is:

Summer issue (includes member directory and post meeting highlights)
Articles due: August 1
Mail date: August 30

Fall/Winter Issue
Articles due: December 1
Mail due: December 30

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