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IN THIS ISSUE:

Fusarium Graminearum, the Head Scab Fungus: An Update on Genetics and Genomics

by Frances Trail

Mycotoxin contamination of food and feed is one of the most important agricultural and health problems worldwide. *Fusarium and Aspergillus* species are some of the best studied of the mycotoxigenic fungi and occupy a similar ecological niche. These fungi are good saprophytes but can cause disease in plants, insects, and animals (including humans). They are naturally resistant to many fungicides, and indeed, fungicide treatments can cause increases in mycotoxin contamination of grains (D'Mello et al., 1998). Crop varieties resistant to these fungi have been difficult to develop. The physiological basis for the broad host range has not been elucidated, although a recent study by St. Leger et al. (2000) has begun to address this question in several *Aspergillus* species. The worldwide importance of these pathogens, and the difficulty in providing effective means of control is due to the lack of an understanding of their biology and the basis of their non-specific pathogenicity.

Fusarium graminearum (teleomorph: *Gibberella zeae*) causes head scab, or head blight of wheat, barley, and oats, and foot and crown rot of corn. Recent scab outbreaks in Asia, Canada, Europe, South America and the United States highlight the increased threat this disease poses to food supplies worldwide. The major impact of *F. graminearum* is due to the production by the fungus of the trichothecene mycotoxin deoxynivalenol (DON) upon infection of the developing grain. DON is a potent protein synthesis inhibitor and effects humans and animals that eat contaminated grain. The polyketide zearalenone is a second mycotoxin produced by *G. zeae* and has estrogenic activity in mammals. Between 1991 and 1996, three billion dollars were lost in the United States due to contamination of the winter and spring wheat and barley crop by DON (US Wheat and Barley Scab Initiative: Scab Initiative News, 1998).

Scab can be caused by four species of *Fusarium*: *F. graminearum*, *F. culmorum*, *F. avenaceum*, and *F. crookwellense*. Only the first two produce DON. In the United States, Canada, and continental Europe, *F. graminearum* is the most important scab-causing organism. The life cycle of *F. graminearum* is intimately tied to the host life cycle. The airborne ascospores, produced by perithecia formed on crop debris, are the primary inoculum for colonization of developing wheat heads (the most susceptible stage). A study of the development of perithecia has recently been published (Trail and Common, 2000). Conidia are

vital for colonization of vegetative tissue that later becomes crop debris.

Genetics

F. graminearum is readily adaptable to laboratory study. (Caveat: strain-to-strain variability is high for the following features.) Large numbers of conidia can be generated in liquid carboxymethylcellulose culture. These propagules can be germinated overnight for protoplasting or used ungerminated for inoculation and mutagenesis. Sexual reproduction of *F. graminearum* can be induced in a petri dish, where a lawn of perithecia will appear within days of induction and develop synchronously, at least with a cooperative strain (Klittich and Leslie, 1988). *F. graminearum* is homothallic, but can be outcrossed (Bowden and Leslie, 1999). S.-H. Yun, O. Yoder, and G. Turgeon (Novartis Agricultural Discovery Institute [NADI], San Diego, CA), and D. Brown and A. Desjardins (USDA, Peoria, Illinois) are attempting to create a heterothallic strain by manipulation of the mating type genes.

The molecular genetics of trichothecene production in *F. graminearum* and *F. sporotrichioides* is the best studied of any aspect of *F. graminearum* biology. Analysis of pathway mutants led to the identification of the trichothecene gene cluster (Hohn and Desjardins, 1992, Hohn et al., 1995; McCormick et al., 1996; Alexander et al., 1998). Ten genes involved in regulation and biosynthesis have been characterized. One of these, *tri6*, is a transcriptional activator (Hohn et al., 1999). A trichothecene efflux pump that may be involved in self-protection has been identified in *F. sporotrichioides* and might be useful for the engineering of trichothecene resistant grains (Alexander et al., 1999).

Because of its worldwide distribution, there has recently been much research activity to investigate the genetic diversity of the scab pathogen. RAPDs have been used for species identification and to study variation in field populations of *F. graminearum* and to distinguish it from several other *Fusarium* species (Schilling et al. 1996; Ouellet and Seifert, 1993). R. Bowden, J. Leslie and K. Zeller (Kansas State University) have used AFLP fingerprinting to show that the population in the Midwestern United States is diverse, but well-mixed, with no evidence of subdivision. They have also — in collaboration with J. Jurgenson (University of Northern Iowa) — created a linkage map with 1029 AFLP markers in nine linkage groups. In collaboration with N. Alexander and R. Plattner (USDA-ARS, Peoria), they have mapped the trichothecene gene cluster. D. Geiser (Pennsylvania State University) is composing a database of AFLP patterns that can be searched for matching strains. In collaboration with G. Kuldau (Pennsylvania State University), he will assemble profiles of large numbers of isolates of *F. graminearum* and other Fusaria.

Desjardins et al. (2000) documented the occurrence of several mycotoxin-producing *Fusarium* spp. in wheat and maize samples in Nepal. P. Nicholson (John Innes Centre, Norwich, UK, personal communication) has DNA evidence for population substructure within the Asian population and between the Asian and European/USA populations. Within the Asian population there appears to be a potential link between host of origin and subgroup. The global structure of the populations of *F. graminearum* were studied by O'Donnell et al. (2000), who identified seven distinct populations that correspond to geographic regions. They suggest that host-mediated speciation and physical isolation play a role in the evolution of these distinct populations. Understanding the diversity of this organism has implications for control and for the regulation of global transport of contaminated grain.

Trichothecene-nonproducing mutants of *F. graminearum* have reduced virulence in field trials on both wheat (Desjardins et al., 1996) and maize (Harris et al., 1999). Therefore, the mycotoxin DON contributes, at least in part, to the virulence of the pathogen. Pathogenicity assays have been developed for plant and animal systems. These assays are serving as a screen for pathogenicity-minus mutants in several labs. Pritsch et al. (2000) studied the progress of infection and early defense response gene expression in infected wheat spikes. Sumai 3, a cultivar with some resistance, showed expression of some pathogenesis-related genes for an extended time. *F. graminearum* is highly pathogenic to wax moth caterpillars, and the system is in use as a model for invasive fungal infection of humans (A. Diener and F. Ausubel,

Massachusetts General Hospital, Boston, personal communication).

Mutants

Adams et al. (1987) used UV mutagenesis to isolate auxotrophic strains of *F. graminearum*. In the last two years, workers in my laboratory have applied the technique of insertional mutagenesis to develop over 5000 randomly tagged mutants (C. Andries and F. Trail, unpublished results). We have isolated several mutants arrested in perithecium development and ascospore discharge and are isolating the genes by plasmid rescue. Transformation frequencies are relatively low for *F. graminearum* and the expense of protoplasting (not to mention the continuous battle to keep a steady source of protoplasting enzymes) makes large-scale transformation expensive. Several laboratories are now developing electroporation and *Agrobacterium*-mediated transformation systems for *F. graminearum* (A. Diener and H.C. Kistler, USDA-ARS, University of Minnesota, St. Paul, personal communication; F. Trail, unpublished). For a large scale scab genomics project (see below), specific disruption of large numbers of genes will be essential. An affordable and effective "bulk" transformation system is therefore needed to inactivate and thus functionally identify these genes.

Expressed sequence tags (ESTs) are being generated in two projects in the public sector. Approximately 600 ESTs have been sequenced to date by the laboratory of Y-H. Lee (Seoul National University). Funding was obtained for the generation and sequencing of 10,000 ESTs (F. Trail, H.C. Kistler, J.-R. Xu, Purdue University, O. Anderson, USDA, Albany CA). Public access to the ESTs will be available through the US Wheat and Barley Scab Initiative web-page (http://www.scabusa.org) by the end of this year. The rapid progress in genomics of two hosts, wheat and corn, will enhance the studies of host-pathogen interactions. A gene whose expression changes due to various treatments may provide a target for the development of new antifungal agents. Such a target may have medical as well as agricultural benefits.

From the AFLP map down to the ESTs, construction of a detailed genomic map is important. Construction of a BAC library has been initiated (J.R. Xu and O. Anderson, personal communication). The BAC library will be used to generate contigs for scaffolding future sequencing information and for linking to the AFLP map. It is likely that extensive genomic sequence is available in industry, but access to this information by researchers at public institutions is limited.

Biology-based control

With the use of genetic tools, novel and effective approaches will be developed for control of scab disease, and may shed light on human disease as well. Currently, studies on the mechanism of DON inhibition of protein synthesis in eukaryotes have identified the site of action as the 60S ribosomal protein L3. L. Harris and S. Gleddie (Agriculture & Agri-Food Canada, Ottawa, Ontario, personal communication) have altered a single amino acid codon in a rice cDNA encoding L3. Transgenic tobacco plants expressing the modified protein have increased growth rates in the presence of DON. Transfer of the modified gene to wheat and maize has recently been completed.

In May, 2000, the *International Symposium on Wheat Improvement for Scab Resistance* was held in Suzhou, China, and workers from all over the world shared information on breeding for resistance and host-pathogen interactions, emphasizing the international urgency of the problem. It is hoped that more information on the genetics and biology of this system will lead to novel approaches to control, as has the L3 study, and that these will be successfully integrated into disease management strategies worldwide.

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FROM THE PRESIDENT…

The Future of Teaching Molecular Plant-Microbe Interactions?

Newly emerging technologies not only enhance our research but now enable us to teach higher quality, interactive courses. In the fall 1999 semester, **Marty Dickman** (University of Nebraska), **Tom Wolpert** (Oregon State University) and I (Kansas State University) collaborated to teach Molecular Plant-Microbe Interactions, a graduate level course, using new interactive technologies made possible by the high-speed, high-capacity Internet 2. The Internet 2 can send more than 2.4 billion bits of information per second, a much faster rate than many phone modems that operate at 56,000 bits per second. The huge capacity of the Internet 2 makes it an ideal medium for sending high-quality video.

For many years, we have been concerned that in our small (and shrinking) class sizes, our students were missing out on the kinds of exciting exchanges that shaped our own graduate careers. Using the Internet 2 as our medium, our dream was to link our classes at the three universities into one high quality, fully interactive, real-time class. Each classroom contained microphones, cameras and video monitors. For example, in my classroom, I had three monitors, two showing the UNL and OSU classrooms, and one displaying the visual aides I was using. Initially, both students and professors were shy of the cameras, but it was amazing how quickly we adapted to talking to our colleagues on the television screens as if they were in the same room.

Our goal was to expose the students to the different expertise and philosophies of the three instructors while at the same time "meeting" and interacting with their future colleagues at the other universities. The class was a lecture/discussion format, with the three professors sharing responsibilities for presenting lectures and leading discussions. To help stimulate discussions, Tom, Marty, and I were present during each lecture or discussion. Guest lectures by Alan

Collmer (Cornell), Scot Hulbert (Kansas State), and Jian-Min Zhou (Kansas State) provided a greater breadth of expertise to the students. A common website provided a venue for posting notices, handouts and reading lists as well as for group threaded discussions.

At the outset, Tom, Marty, and I agreed that the class discussion sessions were critical for the students' learning experience, so having real-time discussion capabilities with no delays or gaps in conversations was given the highest priority—and was most taxing to the technologies employed. The broadcast video engineers and the computer and network specialists worked for 6 months before the start of the class, and were literally testing, experimenting, and writing software the whole semester to achieve the high quality we demanded. This was a true collaboration between the engineers, computer specialists, and instructors. And it worked. It was amazing how the technology became transparent during the heat of a discussion. After one particularly stimulating discussion where the students from all three campuses participated freely in the discussion, one engineer commented, we have landed.

While the course was not without its bugs, in general, the students and instructors agreed that the experiment was worth the effort, and that the technology offers great possibilities for teaching in the future. How we use that technology to provide high quality learning experiences for our students or to advance our research fields are only limited by our imaginations.

Jan Leach

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International Agricultural Research: Famine in the Midst of Plenty

by Luis Sequiera

Financial support for the scientific and technological development of Third World countries has been one of the cornerstones of U.S. foreign policy for decades. This policy is based on the sound concept that the political and financial stability of these countries is important to our own economic progress. Stable, prosperous nations become the best customers for our agricultural and industrial products. Thus, providing Third World countries with the means to acquire and adapt new technologies is a mutually beneficial policy and should continue as an important objective of the U.S. Agency for International Development (USAID), the U.S. Department of Agriculture (USDA) and other government agencies, as well as private foundations.

For the past 10 years however, cutbacks in USAID's support for R & D programs have had a serious impact on international efforts to provide food stability in countries where most of the world's poor live. Since it is in these countries where population pressures and nutritional needs are most severe and will continue to exacerbate, many scientists view, with alarm, the continuing reduction in the U.S. contribution to agricultural research programs abroad. At a time when advances in the field of communication make it possible for plant scientists worldwide to remain abreast of new technologies, the resources needed to apply these technologies in developing nations continues to decline.

A good example is the severe reduction in the USAID's contribution to the Consultative Group on International Agricultural Research (CGIAR). This organization funnels support from governments

and private foundations to 16 international centers that carry out research for the improvement of important food crops, the study of tropical soils, the design of better irrigation systems, the potential of local forestry, etc., all programs aimed at resolving the pressing demands for food, fiber, and shelter in many Third World countries. The CGIAR system emerged from the extraordinary success of the so-called Green Revolution that significantly increased production of wheat, rice, and maize in developing countries during the l960's.

The transfer of agricultural technology to Third World countries has been so effective that food production has kept up with increases in population (except in sub-Saharan countries in Africa) for the past 30 years. With a world population that is expected to double by the year 2050, it is evident that research must continue to provide the technologies required to increase both the quantity and the quality of food in tropical countries, while protecting their soil, water, and forest resources. The newer techniques of genetic engineering, sustainable management of agricultural systems, and marketing, etc., offer a potential solution. But R & D does not come free.

From 1981 to 1985, U.S. Government contributions accounted for 33% of the CGIAR budget; by 1999, that contribution had been reduced to less than 10% of the $328 million annual budget of that organization. This drop, coupled with a significant reduction in the contributions by private foundations, has caused sharp program cuts at most of the centers and particularly those projects in marginal areas where environmental problems are most severe. Only increased contributions to CGIAR by the Japanese government (now the primary contributor among individual nations), as well as by some European countries and the World Bank, have allowed continuation of the primary research programs in recent years.

The CGIAR example is but one of many that illustrate the increasing disengagement of the U.S. government from vital scientific, technical, and training support for developing nations. The decline in U.S. investment in international agricultural research mirrors the general scaling back in international assistance. The reductions in support to CGIAR reflect the USAID's larger budgetary problems, spurred by the end of the Cold War. In FY 1996 for example, the contributions to CGIAR were down approximately 20% from FY 1995 levels. But this took place in the midst of a 40% reduction in USAID's non-earmarked funds. The implications of these reductions reflect on the expected role of the U.S. in providing food and economic security to the poorest nations in the world.

It is ironic that the reduction in contributions by USAID, USDA, and other agencies that support international scientific research came in the midst of a buoyant economy in the U.S. and continued government support for increased globalization of trade. This decline in support threatens the gains that have been made in crop improvement over the past 50 years. Admittedly, there have been some bright notes in the international arena for plant sciences in recent years, such as the rice biotechnology program of the Rockefeller Foundation (now terminated) and the strong support for the international rice genome project by the National Science Foundation and, more recently, the US Department of Agriculture.

The reasons for the lack of support for international agriculture are not difficult to find. The end of the Cold War changed the focus in the U.S. Congress for foreign assistance programs. Donor "fatigue" among private foundations is another reason. The Directors of the Rockefeller Foundation, for example, felt that after 50 years of strong and highly successful investments in international agriculture, it was time to move into other areas, such as AIDS and malaria research. Finally, there is a pervasive feeling that the very high rates of return of the initial investments in research on rice and wheat at IRRI and CIMMYT would not be realized at other CGIAR centers.

It is unfortunate that the decline in support of international agricultural research has come at a time when unprecedented advances in biotechnology offer the best solution for many of the problems that face the crops that are important in the developing world.

Most developing countries lack the infrastructure and personnel required to support their own biotechnology programs, yet the ability to grow more food without further degradation to the

natural resources of developing nations is dependent on the acquisition of modern technologies.

Members of IS-MPMI should view with concern the reductions in appropriations for international agricultural research. The Society should urge Congress to support new and effective partnerships with private industry, foundations, and financial institutions to meet the challenge of sustainable food production in the next century.

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Proteomic Research and Rhizobium

by Barry G. Rolfe

The future of functional genomics

In his recent book John Maddox (1998) pointed out that "the harvest of understanding in genetics won in the past 20 years has outdone even the expectations of the early enthusiasts" ... but "there are several technical problems crying out for solution. How are groups of related genes regulated in concert?" My response is that there are several new technologies that now enable us to approach these issues with a new enthusiasm. These new technologies are able to simultaneously display many hundreds of changes to the presence or levels of cellular components. In the developing field of functional genomics, one approach emphasizes nucleic acid research, while the newly emerging field of proteomics deals with the analysis of whole genomes at the functional protein level by describing the protein complement expressed by the genome of an organism, tissue or dedifferentiated cell (Wilkins et al., 1995). The significance of these procedures is that the researcher can rapidly study global regulation of whole genomes in response to various stimuli. These new areas of analysis are underpinning current molecular genetics and are setting not only the pace of activity, but also the standards of scholarship that the major journals will in time use to assess publication acceptability.

Proteome studies

Proteomics is the study of protein properties (expression level, post-translational modification, interactions, etc.) on a large scale to obtain a global, integrated view of cellular processes, cell networks at the protein level, and disease processes. Proteome studies have developed from, and are dependent upon, the core technology of two-dimensional polyacrylamide gel electrophoresis (2-DE) for the separation of proteins from complex protein mixtures. This field has rapidly grown in importance because of two basic questions posed by all of the genome-sequencing projects: (a) what are the functions of all the gene products? and, (b) what is the importance of different post-translation modifications of the gene product? Three interlocking areas of research are providing a major shift in the way we approach cellular analyses: The first is the actual or pending availability of entire genomic sequences; the second is the ability to separate and display thousands of individual protein components by 2-D electrophoresis to

provide a global "snapshot" of metabolic activity under given conditions, and the third is the development of spectrometry techniques in which either peptide masses or direct protein sequence can be obtained from an individual protein spot.

Mass Spectrometry

Mass spectrometry (MS) has become the method of choice for identifying proteins separated by 2-DE and for protein identification and characterization of post-translational modifications. This technique can determine the mass of the molecule and, using tandem MS, generate sequence information. The data can then be used to search conceptual translations of various genomic and EST databases to identify the protein and link it to the nucleic acid sequence. This methodology is developing so fast and becoming so effective for functional genomics programs that a research organization without adequate current MS technology for peptide analysis may have to consider closing down many of its molecular genetics programs over the next decade.

Proteomic analysis and Rhizobium

We have shown that 2-DE can be used to analyze genome structure and global changes of gene expression in both *Rhizobium leguminosarum* bv. trifolii and *Sinorhizobium meliloti* (Guerreiro et al., 1997, 1998, 1999; Guerreiro et al., 2000; Rolfe et al., 2000; Nateria et al., 2000; Chen et al., 2000 a,b;) and *Rhizobium* sp. NGR234 (Worland et al., 1999).

***Rhizobium leguminosarum* bv. trifolii strain ANU843**

Image analysis of 2-DE protein gels of this bacterium revealed approximately 2000 proteins which represents about 36% of the estimated coding capacity of the strain (Guerreiro et al., 1997). A 2-DE protein database has been constructed for strain ANU843 based on both sequencing and amino acid composition analysis of more than 100 constitutively expressed proteins. To add to this database, a series of plasmid-cured derivatives of strain ANU843, which has five mega-plasmids ranging from 180 kbp to ~700 kbp, were examined by proteome analysis (Guerreiro et al., 1998). Even a loss of up to 1 Mbp from these plasmid-cured strains contributed only a small proportion of the gene products of the 2000 proteins visualized on the gels under the conditions used. The differences observed upon plasmid curing were protein loss, up and down-regulation of specific proteins, and the synthesis of some novel proteins, which have been sequenced and identified (Guerreiro et al., 1998). Additional information on the global regulation within *R. l.* bv. trifolii was gained from an analysis of the *exo* mutant ANU437. This mutant contains a Tn5 insertion in the *pssA* gene, which causes a defect in the isoprenylphosphate glucosephosphotransferase function and a nearly complete block in exopolysaccharide synthesis. 2-DE analysis showed that 23 proteins were either newly-induced or up-regulated indicating that EPS production in *R. leguminosarum* involves complex regulatory networks (Guerreiro et al., 2000).

Sinorhizobium meliloti

A proteome reference map has been initiated for *S. meliloti* strain 1021 (Guerreiro et al., 1999) using 2-DE. When early exponential phase cells were compared to late exponential phase cells a minimum of 52 reproducible changes in protein expression levels were detected. The contribution made by plasmid-encoded functions was studied (Chen et al., 2000a). The pSyma-plasmid, which is 1.4 Mb, contains the nodulation and nitrogen fixation genes and has an estimated coding potential of 1400 proteins. However, 60 protein spot differences were detected between the parent strain and its pSyma-cured derivative. These differences were due to both up- and down-regulation and to the lack of some proteins. These studies indicate a complex interplay is occurring between the plasmid replicons and the remaining replicons of a cell.

Other *Rhizobium* proteomic analysis studies include: (a) extractable proteins from *S. meliloti* strains AK631 and EK698 (a Tn5-induced -deficient mutant of AK631), grown with or without the addition of the plant signal luteolin. This investigation has led to the proposal that the NolR repressor is a global regulatory protein that responds to environmental factors to fine tune intracellular metabolism; (b) an analysis of the gene products that are differentially present in the

symbiotic and the non-symbiotic states of *S. meliloti* (Natera et al., 2000). These investigations provide insights into the diversity of *Rhizobium* metabolic pathways and how two symbiotic partners alter their respective metabolisms during the plant-microbe interaction.

Bradyrhizobium japonicum

Investigations have started on the use of 2-DE analysis of *B. japonicum* to classify proteins into functional groups with respect to the regulation of micro- and anaerobically- induced genes (Dainese-Hatt et al., 1999). Similar techniques also have been used to identify novel proteins associated with the peribacteroid membrane of soybean nodules induced after inoculation with *B. japonicum* (Panter et al., 2000).

***Rhizobium* and Biotechnology**

Farmers have been inoculating legumes with *Rhizobium* strains since 1895, which means that billions of bacterial cells have been released into the global environment over the last 104 years. There has never been any report of a negative human, animal or plant health/disease response due to this extensive bacterial release. Because of its long history of use, *Rhizobium* is a reasonable microbe to use as a delivery system for new agricultural biotechnology products. It is important that bacteria that are designed for commercial biotechnological applications be as well understood as possible. Today, this requires knowledge of both the genomics and the proteomics of the particular strain.

Rhizobium could be used as the inoculating bacterium for:

1. Biological nitrogen fixation with legumes and non-legume crop plants.

2. Biological control of plant diseases (e.g., as a possible replacement of *Agrobacterium tumefaciens* or *Pseudomonas* strains used to control take-all and root rot of wheat. Modified *Rhizobium* might be preferable because these other two microbes have been associated with plant disease or human health problems.

3. Bioremediation: cleaning up toxic waste dumps (using the bacterium to remove or render harmless dangerous pollutants and hazardous waste; conversion of toxic materials into benign substances; as "biosorption" systems to chelate mercury, copper, cadmium, uranium and cobalt). (*Rhizobium* already contains many of the necessary genes for such a role).

4. Soil modification and sustainable ecological practices: soils inoculated with strains that produce excess exopolysaccharide to alter soil structure and soil particle sizes. *Rhizobium* strains that produce excess exopolysaccharide have been shown to work.

5. Indoor agriculture (hydroponics), in which food and fiber is grown in giant bacterial vats.

6. Bioleaching. Bacteria can be used by the mining industry to extract cobalt, iron, nickel, copper and manganese from low-grade ores.

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MPMI Names New Senior Editor for Plant Responses

Thanks to **Jeff Dangl** for excellent service and welcome to **Jane Glazebrook** as the New Senior Editor for Plant Responses.

As of July 2000, Jeff Dangl will retire as the Senior Editor after 3 years of service for *MPMI* in the Plant Response area. Thank you very much Jeff for bringing your excellent values and tremendous insight to this position.

The new Senior Editor for the area of Plant Responses for *MPMI* will be Jane Glazebrook, Senior Staff Scientist, Novartis Agricultural Discovery Institute, Inc., San Diego, CA. Jane received a B.Sc. degree in Biochemistry from Case Western Reserve University in 1985. Her graduate work with **Graham Walker** concerning nodulation by *Sinorhizobium meliloti* led to a doctoral degree in Biology from the Massachusetts Institute of Technology in 1991. From 1991 to 1995, she was a post-doctoral fellow in **Fred Ausubel's** laboratory, where she began her work on genetic dissection of plant defense responses using *Arabidopsis thaliana*. For much of this time, she was supported by an NSF Plant Sciences Postdoctoral Fellowship. From 1995 to 1998, she was an assistant professor at the Center for Agricultural Biotechnology of the University of Maryland. Jane joined the Novartis Agricultural Discovery Institute, Inc. in 1998. Research in her group is aimed at elucidating the signal transduction network controlling activation of plant defense responses after pathogen attack, and at understanding the contribution of specific defense mechanisms to resistance to particular pathogens. She previously served as an Associate Editor of *MPMI*.

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MEETINGS AND EVENTS...

2000

17th North American Conference on Symbiotic Nitrogen Fixation
July 23-28, 2000, Laval University, Quebec, Canada
For more information contact: Hani Antoun, RSVS Pavillon, Charles-Eugéne Marchand Université Laval, Québec Canada GIK 7P4. Telephone: 418.656.2131, Fax: 418.656.7176, E-mail: antoun@rsvs.ulaval.ca

4th European Nitrogen Fixation Conference
September 16-20, 2000, Sevilla, Spain
Contact: Profs. A.J. Palomares and J. Olivares Dept. Microbiologia, Facultad de Farmacia, Universidad de Sevilla, 41071 Sevilla, Spain, Fax: +34.954556924, E-mail: 4thenfc@cica.es
Website: www.eez.csic.es/4thenfc/

4th International Rice Genetics Symposium
October 22-27, 2000, IRRI, Los Banos, The Phillippines
Contact Dr. G.S. Khush at E-mail: g.khush@cgiar.org

International Symposium on Durable Disease Resistance:
Key to Sustainable Agriculture
November 28 - December 1, 2000, Wageningen,The Netherlands
Contact: Dr. J.E. Parlevliet, Plant Breeding, Wageningen UR, PO Box 386, NL 6700 AJ Wageningen, The Netherlands Fax: +31.317 483457, E-mail: jan.parlevliet@users.pv.wau.nl
Website: www.spg.wau.nl/pv/symposium.htm

2001

Fungal Metabolites: The Good, Bad, and Deadly
April 22-27, 2001, University of Wales, Swansea, United Kingdom
Preliminary sessions include: "Fungi as a Source of Novel Bioactive Compounds," "Development of Tools and Methods to Study Metabolites," "Exploitation of Bioactive Metabolites," "Mycotoxins," "Risk Assessment of Fungal Biological Control Agents," poster session, and network discussions (an opportunity to identify partners for future collaborative research). Contact: Dr. Tariq M. Butt, University of Wales Swansea, School of Biological Sciences, Singleton Park, SWANSEA, SA2 8PP UK Phone: +44.792 295374 Fax: +44.1792 295447 E-mail: t.butt@swansea.ac.uk

Third International Conference on Mycorrhizas (ICOM3)
July 8-13, 2001, Adelaide Convention Centre, Adelaide, Australia
Contact: Professor Sally Smith, Department of Soil and Water, Waite Campus, University of Adelaide, PMB 1, Glen Osmond, South Australia 5064 Phone: +61.08 8303 7351 Fax: +61.08 8383 6511 E-mail:sally.smith@adelaide.edu.au Website: www.waite.adelaide.edu.au/soil_water/3icom.html

IS-MPMI Meeting
July 10-15, 2001, Madison, Wisconsin USA
The 2003 meeting will be in St. Petersburg, Russia

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ISMPMI REPORTER DEADLINE

Deadline for submitting items for the next issue is September 8, 2000.

Submission of materials as electronic files, either on disk or as e-mail attachments, will speed processing. Please submit black-and-white or color photos generated from negatives. If your image was created digitally, please submit a laser print of hte image and a disk containing the electronic graphics file (.tif and .eps formats are preferred).

For more information on submitting electonic images contact Kayleen Larson at klarson@uslink.net.

Send items to:

Jonathan D. Walton
Michigan State Univ.
DOE Plant Research Lab
East Lansing, MI 48824
Phone: (517) 353-4885
FAX: (517) 353-9168
E-mail: walton@pilot.msu.edu

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SPECIAL PROGRAMS…

Delaware Biotechnology Institute/DuPont Corporation Joint Initiative in Ph.D. Training in Plant Biology and Biotechnology

As part of the graduate program in Plant Biology at the University of Delaware, the Delaware Biotechnology Institute offers a unique Ph.D. training in Plant Molecular Biology and related skills including biotechnology in collaboration with DuPont. Incoming students do one rotation at the university, followed by one rotation in a DuPont lab. Students can choose to complete their Ph.D. thesis at either institution. All students will have the opportunity to see how science is approached in both industry and academia. Coursework, chosen from offerings at the University of Delaware, will be individually tailored to students' needs. Admission is open to students of all

nationalities. Further information, a list of the participating University of Delaware faculty and DuPont participating scientists, and application are available online at www.udel.edu/plants/index.html.

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WELCOME NEW MEMBERS

April - June 2000

Kevin P. Fort
University of California, Davis
Plant Pathology Department

C. Valerie Oke
University of Pittsburgh
Biological Sciences Department

Piet M. Boonekamp
Plant Research International
Biointeractions & Plant Health
Wageningen, Netherlands

Shashi B. Sharma
Rutgers Univ
Waksman Ins

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PEOPLE

Knogge joins faculty at University of Adelaide

After working for 15 years as a group leader in the Department of Biochemistry at the Max-Planck-Institute for Plant Breeding Research in Cologne, Germany, Wolfgang Knogge has started a new position as Senior Lecturer in the Department of Plant Science at the University of Adelaide, Australia. The focus of his research will remain the molecular basis of the interaction between the fungal pathogen *Rhynchosporium secalis* and its host plant barley.

One of the topics his new group will work on is the identification (through insertion mutagenesis), isolation and characterization of fungal pathogenicity and virulence genes. For this work a Ph.D. scholarship is available. Please see the job ads in this issue of the *Reporter* for more information.

Prof. Dr. Jozef S. Schell to retire in July, 2000

Dr. Schell, Director at the Max-Planck-Institut für Züchtungsforschung, Cologne, Germany, and Professor at the Collège de France, Paris, has announced that he will retire this July. Dr. Schell's extraordinary international reputation is based on his more than 600 publications in numerous fields of micro- and plant biology. His seminal contributions to our understanding of plant/microbe interactions are known to all IS-MPMI members.

Dr. Schell studied Zoology in Gent, Belgium and completed his Ph.D. in Microbiology (Comparative Biochemistry) in Gent and Utrecht (The Netherlands). He rose to Full Professor and Director of the Laboratory of General Genetics at the Rijksuniversiteit in Gent and taught at the Free University, Brussels, before becoming director of the European Molecular Biology Organization, Department of Genetic Principles of Plant Breeding, Max-Planck-Institut für Züchtungsforschung, Köln (Cologne)-Vogelsan(Germany) in 1978.

He is also Honorary Professor at the University of Cologne and Professor of Plant Molecular Biology, Collège de France, Paris. Dr. Schell has served on the editorial boards of many journals and as a member of many scientific advisory boards and councils of national and international scientific institutions. He is a member of the Deutsche Akademie der Naturforscher Leopoldina, Halle (Germany); U.S. National Academy of Sciences; Nordrhein-Westfälische Akademie der Wissenschaften (Germany); Indian National Science Academy; Academia Europaea, London; the Royal Swedish Academy, Stockholm; Koninklijke Academie voor Wetenschappen, Letteren en Schone Kunsten von Belgie, Brussels; Academy of Arts and Sciences, Cambridge (USA); and Hungarian Academy of Sciences, Budapest. Dr. Schell has received more than 20 honorary doctorates and scientific prizes from institutions around the world.

From your colleagues in the IS-MPMI, all best wishes on the occasion of your retirement.

Dr. Keisuke Kohmoto retires.

Dr. K. Kohmoto, a member of the IS-MPMI, retired from the professorship of plant pathology at Tottori University, Japan, in March, 2000.

Dr. Kohmoto received his bachelor's degree from Tottori University in 1957 and his M.S. degree from Kyoto University in 1959. In 1960, he joined Nippon Sode Company, where he discovered a new fungicide, thiophanate methyl (Topsin M), which has been used worldwide for many years. For this discovery, he received the Prime Minister's Award from the Japanese Association for Invention in 1976. In 1968, he returned to Tottori University as assistant and subsequently associate and full professor. He received his Dr. Agr. degree from Nagoya University in 1976. Throughout his distinguished career, Dr. Kohmoto focused on the study of host-selective toxins from different pathotypes of the cosmopolitan pathogen and saprophyte Alternaria alternata. He isolated and characterized many new host-selective toxins, including ACR- and ACT-toxins from the citrus brown spot pathogen. He worked with the late Dr. Robert Scheffer at Michigan State University from 1977-1978. Dr. Kohmoto published over 130 scientific papers. In 1988 he shared the Distinguished Award from the Japanese Society of Agricultural Sciences as well as the 25th Agricultural Science Award of Yomiuri Newspapers with the late Professor S. Nishimura of Nagoya University. Dr. Kohomoto was elected a fellow of the American Phytopathological Society in 1995. His colleagues in the IS-MPMI wish him a joyful and rewarding retirement.

1999 Theses Completed at the Graduate School Experimental Plant Sciences.

The Graduate School Experimental Plant Sciences (EPS) is a collaborative research and teaching institution of Wageningen University (WU), Leiden University (LU), Catholic University Nijmegen (KUN), and Utrecht University (UU). EPS was founded in 1992 in order to organize and

coordinate the fundamental and strategic plant research of the collaborating universities, aiming at the development of new principles in crop breeding, crop protection and crop growth for sustainable agriculture and horticulture. EPS also facilitates the training and teaching of Ph.D. students in these fields by organizing courses, seminars and summer schools.

A. Goverse

Cyst nematode-induced changes in plant development. Prof.dr. J. Bakker (promoter); dr. J. Helder, dr. A. Schots (co-promoters), WU, Wageningen, 15 October 1999, 95 pp.

Cyst nematodes are obligatory plant parasites and infective stages of these nematodes induce the formation of a feeding site in a plant root, a so-called syncytium. Secretions from the cyst nematode trigger a highly directed process of plant cell redifferentiation and this phenomenon was studied in detail in this thesis. Firstly, the effect of naturally-indu-ced secretions from infective juveniles of the potato cyst nematode *Globodera rostochiensis* on tobacco leaf protoplasts was investigated. A strong stimulation of protoplast division was found and low molecular weight peptide(s) (< 3 kDa) were shown to be responsible for the observed effect. Remarkably, nematode secretions also co-stimulated mitogenesis in human peripheral blood mononuclear cells (PBMC). Secondly, the effect of plant hormones on the ability of the nematode to induce the formation of a syncytium was investigated. Various experimental approaches including the use of hormone mutants from tomato and *Arabidopsis* strongly suggested that syncytium proliferation is guided by a radial auxin gradient in the plant root. The role of auxin and the cross-talk between auxin and ethylene in feeding cell induction and morphogenesis are discussed.

R.A. Griep

Development of recombinant antibody technology for application in plant pathogen diagnosis. Prof.dr. W.B. van Muiswinkel (promoter); dr. A. Schots (co-promoter), WU, Wageningen, 17 March 1999, 109 pp.

The thesis describes the applicability of the phage display technique to select plant pathogen-specific monoclonal antibodies (MAbs) from combinatorial antibody libraries. The retrieved MAbs are so specific that they can be used as diagnostic tools in sensitive immunoassays for the detection and identification of plant pathogens. Thus, antibodies have been selected against beet necrotic yellow vein virus, tomato spotted wilt virus, and *Ralstonia solanacaerum*. In addition, expression vectors were developed to fuse antibodies with alkaline phosphatase and the green fluorescent protein for use in ELISA and immunofluorescence, respectively. Test results from laboratories that have applied the recombinant MAbs were evaluated.

D.T.J. Kasteel

Structure, morphogenesis and function of tubular structures induced by cowpea mosaic virus. Prof.dr. R.W. Goldbach (promoter); dr. J.W.M. van Lent (co-promoter), WU, Wageningen, 10 September 1999, 71 pp.

Cowpea Mosaic Virus (CPMV) was the first plant virus demon-strated to 'move' from cell to cell through tubular structures in virion-form. The tubular structures are assembled into (heavily) modified plasmodesmata and are shown to contain only the (48 kDa) movement protein (MP) of the virus, but no other viral or plant-encoded constituents. These MP-containing tubules are also formed on the plasma membrane of protoplasts, devoid of plasmodesmata, and even on insect cells, demonstrating that the MP specifically trafficks to the cell surface but does not need a pre-existing plasmodesma to copolymerise. Furthermore, it is shown that two other, non-related plant viruses, Alfalfa Mosaic Virus and Brome Mosaic Virus, also form MP-containing tubules in plasmodesmata to allow systemic spread. These results underline that the 'tubule-guided' movement mechanism, as first demonstrated for CPMV, is a more general and widely spread mechanism among plant viruses than previously thought.

M. Kikkert

Role of the envelope glycoproteins in the infection cycle of tomato spotted wilt virus. Prof.dr. R.W. Goldbach (promoter); dr. R.J.M. Kormelink (co-promoter), WU, Wageningen, 18 June 1999, 117 pp.

Tomato Spotted Wilt Virus (TSWV) particles have a lipid envelope containing two virus-coded glycoproteins, G1 and G2. Applying a newly developed single cell (protoplast) infection system, allowing synchronous and high frequency TSWV infection, the maturation pathway of the virus in plant cells could be largely unraveled. Strikingly, the virus nucleocapsids, containing the genomic RNAs, do not bud into the lumen of the Golgi, but are 'enwrapped' by Golgi vesicles, resulting in doubly-enveloped particles as intermediates. Heterologous expression of (mutated versions of) the glycoproteins in animal cells showed that these proteins contain trafficking signals which are also recognised in an animal cell background. This fits with the fact that TSWV replicates both in plants (hosts) and in insects (vectors). The dual maturation pathway of TSWV in both plant and animal cells allows comparative studies in the future to define similarities and differences in the maturation of glycoproteins among plants and animals.

R. Laugé

Extracellular proteins of the tomato pathogen *Cladosporium fulvum*; a role in pathogenicity and avirulence.Prof.dr. P.J.G.M. de Wit (promoter); dr. M.H.A.J. Joosten, dr.H.W.J. van den Broek (co-promoters), WU, Wageningen, 3 March1999, 93 pp.

In contrast to mammals, little is known about the defense mechanisms operating in plants after challenge by pathogens. Many plant-pathogen interactions comply with the gene-for-gene concept, in which host resistance is based on cell death-associated recognition of molecules from the intruding pathogen. *Cladosporium fulvum* is a fungal pathogen of tomato (*Lycopersicon esculentum*), which exclusively colonizes the leaf apoplast. Resistance of tomato operates through specific recognition of extracellular proteins produced by the fungus.

Within the genus of tomato individual plants exist that respond with HR to one or more of the seven proteins secreted by *C. fulvum* that have been purified. Studies on the inheritance of HR-associated recognition confirmed that in each case a single dominant gene is involved. Moreover, a survey for recognitional specificities among various accessions of *L. pimpinellifolium* indicated that the ability to respond to the proteins is randomly distributed among the population. Furthermore, it was found that one extracellular protein is specifically recognized by *Nicotiana paniculata*, a species that is not a host for *C. fulvum*. It is concluded that plants display a versatile recognition system for 'foreign' proteins. The random distribution of recognitional specificities among plant populations, together with the high mutation rate of pathogens, provide a basis for the complex gene-for-gene relationships frequently observed in pathosystems.

S. Arpaia

Transgenic resistance of eggplants to the colorado potato beetle. Prof.dr. L.M. Schoonhoven (promoter); dr. J.J.A. van Loon (co-promoter), WU, Wageningen, 16 April 1999, 128 pp.

The thesis deals with the interactions between eggplants (*Solanum melongena* L.) and the Colorado potato beetle (*Leptinotarsa decemlineata* Say), a serious pest of eggplants, potato and tomato. Eggplants were genetically transformed with a mutated Cry 3B protein toxin gene derived from the bacterium *Bacillus thuringiensis*. Resistance was quantified using behavioural and life history parameters of larval and adult beetles and field evaluations. No detrimental effects on non-target arthropods were apparent. Using simulation modeling to predict possible adaptation of the beetle it was demonstrated that resistance will be durable only by integrating resistant varieties with other pest management tactics.

S.A. Hogenhout

The molecular basis of the interactions between luteoviruses and their aphid vectors. Prof.dr. R.W. Goldbach (promoter); dr. J.F.J.M. van den Heuvel (PRI; co-promoter), WU, Wageningen,

15 June 1999, 119 pp.

Using a newly developed "virus overlay" technique the (excreted) chaperonine protein GroEl of the endosymbiontic bacterium *Buchnera* sp. was identified as the major virus-binding protein during persistence of luteoviruses in the aphid vector. The presence of this protein was shown to be critical for persistence and therefore successful transmission. Using synthetic peptides and systematic mutagenesis, it could be demonstrated that specific amino acids in the equatorial domain of GroEl bind to the N-terminal region in the read-through domain of the viral CP. The results not only shed light on the molecular basis of the persistence of plant viruses in their aphid vectors but also open new ways to combat plant virus spread by specific interference with the protein-protein interactions involved.

(S.A.C.M. van Wees

Rhizobacteria-mediated induced systemic resistance in *Arabi-dopsis*: signal transduction and expression. Prof.dr. L.C. van Loon (promoter); dr. C.M.J. Pieterse (co-promoter), UU, Utrecht, 30 April 1999, 137 pp.

Specific non-pathogenic, root-colonizing *Pseudomonas* bacteria stimulate plants to develop an induced systemic resistance (ISR) against various types of pathogens. In *Arabidopsis*, induction of this enhanced defensive capacity is both rhizobacterial strain- and plant ecotype-specific. ISR proved to differ from phenotypically similar systemic acquired resistance (SAR), which is induced by necrotizing pathogens, depends on salicylic acid (SA) as a signalling molecule, and is associated with induction of pathogenesis-related proteins. ISR did not act through SA but required responsiveness of the plant to jasmonic acid and ethylene (in that order), and was not associated with major changes in gene expression. Simultaneous activation of ISR and SAR resulted in an additive enhancement of induced protection. These results offer considerable potential for integrating both forms of induced resistance for crop protection.

E.A. klein Gebbinck

Synthesis of model compounds derived from natural clerodane insect antifeedants Prof.dr. Æ. de Groot (promoter); dr. B.J.M. Jansen (co-promoter), WU, Wageningen, 22 September 1999, 283 pp.

A series of perhydrofuro[2,3b]furan compounds with different functional groups at C-2 were prepared in order to study the effect of C-2 functionalization upon the antifeedant activity of these modelcompounds for clerodin. The cyclohexyl substituent was chosen at C-5 to replace the decalin fragment that is present in the natural products, but also some other C-5 substituted model compounds were synthesized and tested. The C-20 hydroxy group in the substructure of azadirachtin is believed to be important for its antifeedant activity, and therefore a series of model compounds, based on the 3a-hy- droxyttrahydro-furo[2,3b]furan substructure were prepared and compared with those of the corresponding 3aH-perhydro-furo[2,3b]furans. The antifeedant activity, tested for *Pieris brassicae* larvae, of all model compounds was moderate and the tests support the conclusion that the presence of a 3a-hydroxy group in the furofuran ring system has no significant effect on the antifeedant activity.

The introduction of a hydroxy group at C-13 of the clerodane skeleton is proposed as a potential strategy to increase the antifeedant activity of simple analogs. This idea was applied to clerodane analogs with furan or butenolide type sidechains and syntheses were developed for several types of 3-alkyl-substituted butenolides, 3-alkyl-substituted-3-hy-droxybutenolides and 3-hydroxytetrahydrofuran derivatives. The antifeedant activity was tested on fifth instar larvae of *P. brassicae*.

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EMPLOYMENT

Ph.D. Scholarship

The Department of Plant Science at the University of Adelaide, Australia has a Ph.D. scholarship available for work on the identification (through insertion mutagenesis), isolation and characterization of fungal pathogenicity and virulence genes. The position will be available starting in July. Applications should be sent to Dr. Wolfgang Knogge, Department of Plant Science University of Adelaide, Waite Campus, Glen Osmond, SA 5064, Australia

Postdoctoral Position

Signal transduction in the *Rhizobium*-legume symbiosis. Postdoctoral position available immediately to study the signal transduction pathways involved in the formation of legume nodules in response to rhizobial infection. The research will focus on characterization of putative receptor proteins and identification of downstream pathways leading to gene expression. We envision taking a broad approach to this problem involving biochemistry, molecular biology and functional genomics. This position is funded by a recently awarded grant from the U.S. Dept. of Energy. Candidates with a strong background in biochemistry will be preferred. Training in molecular biology would also be a plus but is not required. Send/email/FAX curriculum vitae and three letters of reference to: Dr. Gary Stacey, Center for Legume Research, M409 Walters Life Science Bldg., The University of Tennessee, Knoxville, TN, 37996-0845, USA. FAX: 615-974-4007 email: gstacey@utk.edu. You can learn more about the laboratory by visiting our website at URL http://www.bio.utk.edu/microbio/staceylab/nodulate.html. The University of Tennessee is an Equal Opportunity/ Affirmative Action Employer.

Postdoctoral Fellowship Available

A position is available for an applicant who has a Ph.D. in biochemistry, genetics, cell biology, pathology or molecular biology.The successful applicant will carry out molecular analysis of ap1- and hrap-transgenic tobacco and *Arabidopsis*. These two genes encode proteins, called plant HR interferins, which were identified and isolated from sweet pepper. Transgenic plants expressing either of these genes are more resistant to most bacterial pathogens. The goal is to reveal the molecular mechanism of this enhanced resistance. Funding is currently available from the National Science Council, Republic of China. Send CV and three letters of reference to: Dr. Teng-yung FENG, Institute of Botany, Academia Sinica, Taipei, Taiwan 11529. E-mail bofeng@gate.sinica.edu.tw.

Postdoctoral and Ph.D. Student Positions

Six postdoctoral and four Ph.D. student positions in functional genomics are available as part of an EU-funded Research Training Network that will focus on *Lotus japonicus* as a model legume to study mutualistic symbioses. Fundamental aspects of nitrogen-fixing and mycorrhizal symbioses will be studied in a multidisciplinary collaboration between nine research groups: Dr. M. Udvardi (Max Planck Institute of Molecular Plant Physiology, Germany); Dr M. Chiurazzi (Consiglio Nazionale delle Ricerche, Naples, Italy); Prof. S. Katinakis (Agricultural University of Athens, Greece); Prof. A. Marquez (Universidad de Sevilla, Spain); Dr. M. Parnicke (John Innes Centre, UK); Dr. L. Rosendahl (Riose National Laboratory, Denmark); Prof H.P. Spaink (Leiden University, The Netherlands); Dr. J. Stougaard (University of Aarhus, Denmark); Dr. K.J. Webb (Institute of Grassland and Environmental Research, UK). Participating organizations are equal

opportunity employers. For further details about these positions refer to the following web page: http://www.mpimp-golm.mpg.de/lotus/ Or contact: udvardi@mpimp-golm.mpg.de.

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