MOLECULAR STRATEGIES FOR CROP PROTECTION

Charles J. Arntzen and Clarence A. Ryan, Organizers

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Plant Responses to Wounding or Pest Invasion

J1 TISSUE-SPECIFIC RESPONSES TO RECOGNITION OF SELF AND NON-SELF. Adrienne E. Clarke, Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Parkville, Victoria 3052, Australia

Two systems in which specific responses to cell-cell recognition occur will be compared.

1. Arrest of growth of self-pollen tubes by the pistil of plants in the family Solanaceae which have gametophytic self-incompatibility systems. This process is controlled by S-genes with multiple alleles. Arrest of pollen tube growth occurs when the haploid pollen carries an S-allele identical with one of the S-alleles carried by the diploid tissues of the pistil. cDNA clones encoding proteins from styles which segregate with particular S-alleles have been used in in situ hybridization experiments to show the tissue specificity and developmental regulation of expression of the S-gene. A class of proteoglycans, the arabinogalactan proteins (AGPs), are also tissue-specific and developmentally regulated. The amount of AGP in the stigma increases (25%) in response to pollination; this increase occurs irrespective of whether the pollination is compatible or incompatible. Changes in the wall composition of the pollen tube also occur in response to pollination.
2. Arrest of growth of Phytophthora vignae hyphae in the tissues of resistant cultivars of cowpea. This process is controlled by a single gene (R-gene) in the host plant which confers resistance to particular races of the fungus. Arrest of growth of fungal hyphae in the host tissues occurs if the host plant expresses the R-gene. cDNA clones encoding proteins from epicotyls which are expressed at particular times in response to infection have been used in in situ hybridization experiments to show tissue specific responses to infection. The nature and level of AGPs in particular tissues also change in response to infection. Changes in wall composition can be followed by fluorescence microscopy and electron microscopy. These changes, particularly formation of callose, a (1→3)-β-glucan, are specific for certain cell types within a particular tissue.

J2 ACTIVATION OF PLANT DEFENSE GENES BY WOUND SIGNALS, C.A. Ryan, G. An, R.W. Thornburg, J. Lee, E. Fox and G. Pearce. Institute of Biological Chemistry, Washington State University, Pullman, WA 99164.

Genes encoding two families of plant proteinase inhibitors, Potato Inhibitor I, Family and Potato Inhibitor II Family, are expressed developmentally in tomato fruit and potato tubers and/or are wound-induced in both tomato and potato leaves. Both inhibitor genes are members of small multigene families. Several genes coding for the two inhibitors have been isolated from potato and tomato genomic libraries and characterized. Promoter regions from tomato Inhibitor I and potato Inhibitor II genes, after fusion with the CAT gene, were used to transform tobacco plants. In leaves of transformed tobacco plants, the Inhibitor II promoter resulted in a wound-induced CAT expression while the Inhibitor I promoter resulted in a developmentally regulated CAT expression. (Supported in part by grants from N.S.F. and U.S.D.A. Competitive Grants Program).

J3 SOME MOLECULAR DETAILS OF PLANT CELL WALL GLYCOPROTEINS, J.E. Varner, Department of Biology, Washington University, St. Louis, MO 63130

The hydroxyproline-rich glycoprotein (HRGP) content of plant cell walls increases in response to red light, tissue culture, ethylene, wounding and bacterial, fungal and viral infection. The principal (perhaps the only) HRGP component of the dicot cell wall is extensin - a molecule with many repeats of -SerHypHypHypHyp- in which the Sers and Hyps have characteristic galactosylation and arabinosylation patterns respectively. With the availability of the complete derived sequence of one carrot extensin gene, the partial derived sequences of several tomato and petunia extensin cDNAs, the partial derived sequence of one tomato extensin gene and the amino acid sequences of the most abundant peptides of two tomato extensins it is clear that the extensins consist of -SerHyp$_4$s- separated by amino acids with functional groups on their side chains. Threonine, histidine, lysine and tyrosine are the most abundant. These side chains provide opportunities for several kinds of electrovalent and covalent interaction with other wall components. In addition the high isoelectric point of extensin (~ 11) allows it to be a non-specific bacterial agglutinin. Details of how carrot roots respond to wounding, how bean cells in suspension culture respond to elicitors, how bean hypocotyls respond to infection and how tomato stems respond to wounding will be presented along with discussion and speculation about how the properties of extensin suit it for a role as a defense protein.

Determinants of Fungal Pathogenicity

J4 GENETIC DETERMINANTS OF PATHOGENICITY AND HOST SPECIFICITY IN PYRICULARIA, Forrest G. Chumley, Kenneth Parsons, and Barbara Valent, Central Research and Development Department, E.I. De Nemours and Co., Inc., Wilmington, DE 19898

The Pyricularia are filamentous, haploid, heterothallic Ascomycetes that cause serious disease on many grass species. In the past, two species of the fungus have been distinguished, based on host range differences. Strains that infect rice, causing rice blast disease, have been known as P. oryzae, while strains that infect other grasses have been known as P. grisea. However, P. grisea and P. oryzae are interfertile and morphologically indistinguishable, and they probably represent a single species. While the species shows a broad host range, individual field isolates are capable of infecting only one or a few grasses. Rice pathogens exhibit further restrictions in host specificity with regard to infectivity on different cultivars of rice. The objective of our research has been to conduct a genetic analysis of host-pathogen interactions in this system, with a major emphasis on the mechanisms that govern host species and cultivar specificity. In order to achieve this objective, we have pursued a systematic breeding program designed to develop fertile laboratory strains that retain interesting differences in pathogenicity and host specificity. The results of this program will be discussed with reference to genes that govern rice pathogenicity and cultivar specificity. We have isolated many mutants of Pyricularia with altered morphological or nutritional properties. Mutants that are defective in pigment production are especially interesting because they are unable to infect any host. The results of studies of the genetics and cell biology of pigment mutants will be presented. We will also discuss efforts to initiate a screen for fungal mutants that are blocked in the infection process or in the elicitation of the hypersensitive response of rice cultivars. Finally, we will discuss efforts to clone genes that govern pathogenicity and host specificity using appropriate strains of Pyricularia as recipients in transformation experiments with Pyricularia gene banks.

J5 FUNGAL TOXINS AS DISEASE DETERMINANTS, Larry D. Dunkle, USDA-ARS, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

In numerous fungi, pathogenicity (the ability to cause disease) and virulence (a quantitative measure of pathogenicity) are associated with the ability of the pathogen to produce phytotoxic metabolites. The most important genera in this group of fungi are Alternaria (on dicotylendonous hosts) and Helminthosporium (on graminaceous hosts). Several lines of evidence from genetic and biochemical studies indicate that toxin production by certain fungi is the determinant of pathogenicity (1). For example, on susceptible maize genotypes, race 2 of H. carbonum, a non-toxin-producing (tox^-) race, causes small, chlorotic leaf spots, and growth of the fungus ceases after only a few cells are colonized. Addition of the host-specific toxin (HC-toxin) produced by race 1 (tox^+) to the race 2 inoculum results in a large, necrotic lesion identical to the symptom caused by race 1 and enables extensive colonization and sporulation by the "non-pathogen". Crosses between compatible mating types of race 1 and race 2 of H. carbonum indicated that pathogenicity is inherited with toxin-producing ability. Interspecific crosses between H. carbonum and H. victoriae, a toxin-producing pathogen of oats, indicated that production of each toxin is determined by distinct, single genes (1).

The HC-toxin is a cyclic tetrapeptide containing an epoxy amino acid (aoe), which is required for toxic activity (2). Other fungi produce aoe-containing cyclic tetrapeptides that are biologically active on maize, but those fungi are non-pathogenic. Thus, characteristics in addition to toxin-producing ability are essential for pathogenicity. The genes determining those qualities may be considered "pathogenicity genes". For potential pathogens, e.g., those lacking the ability to produce toxins but having the other necessary qualities, addition of toxin or mutation to toxin production allows them to become pathogens of a toxin-sensitive host. Toxin-assisted pathogenesis is not effective with saprophytic fungi, which apparently do not possess the basic parasitic attributes, those determined by pathogenicity genes. Fungi in which a single gene determines pathogenicity may provide the simplest system to explore the function of such genes.

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J6 **HIGH AFFINITY IRON TRANSPORT IN USTILAGO MAYDIS.** **Sally A. Leong, Jun Wang, Allen Budde, David Holden, Thomas Kinscherf, and Timothy Smith, USDA-ARS, University of Wisconsin, Madison, WI 53706.**

The high affinity iron transport system of Ustilago maydis, the causative agent of corn smut, is being examined. The longterm goal of this work is to elucidate the structure and regulation of the genes involved in the process of biosynthesis and uptake of the siderophores of U. maydis and to assess the role of siderophore-mediated iron uptake in phytopathogenicity. U. maydis was confirmed to produce two hydroxamate siderophores ferrichrome and ferrichrome A. Using novel screening bioassays, four classes of mutants defective in siderophore production were isolated after N'-methyl-N'-nitro-N-nitrosoguanidine mutagenesis of haploid basidiospores. Of these, three classes were deficient in siderophore biogenesis. The fourth class was found to produce siderophore constitutively. Genetic analysis of one mutant, doubly defective in ferrichrome and ferrichrome A synthesis, has revealed that this lesion segregates as a single gene. The defect was corrected by supplementation of low iron media with δ-N-OH-L-ornithine suggesting that this mutant is unable to hydroxylate L-ornithine, a common precursor of ferrichrome and ferrichrome A. Efforts are also being made to develop tools for molecular genetic analysis of U. maydis. Progress on the development of a transformation system and a molecular karyotype will be described.

Controls of Fungal Pathogenicity

J7 HOST VS. NON-HOST RESISTANCE, Michele C. Heath, Botany Department, University of Toronto, Toronto, Ont. M5S 1A1, Canada.

To be a plant pathogen, a fungus usually must possess attributes that enable it to 1) enter the tissue, 2) obtain from the tissue those factors necessary to support growth and reproduction, and 3) combat the plant's constitutive or induced defense mechanisms. The lack of any of these attributes with respect to a given plant will result in plant resistance. Conceptually, two types of resistance towards a given plant pathogen can be recognized: host resistance that is expressed only by certain genotypes of an otherwise susceptible plant species, and non-host resistance that is exhibited by plant species that contain no susceptible genotypes. Studies with rust fungi suggest that compared with host resistance, non-host resistance is expressed earlier, may involve different mechanisms, and is more difficult for the fungus to overcome. The possible reasons for the latter phenomenon, and their significance in designing novel and durable forms of disease resistance, will be discussed.

J8 THE IMPORTANCE OF FUNGAL CYTOCHROME P-450 MONOOXYGENASE IN PHYTOALEXIN DETOXIFICATION AND PLANT PATHOGENESIS, Hans Van Etten and David E. Matthews, Plant Pathology Department, Cornell University, Ithaca, New York 14853

The synthesis of phytoalexins by plants in response to challenge by microorganisms is believed to be part of an active mechanism of disease resistance. Our research objective has been to elucidate the molecular basis by which a pathogen may overcome this resistance mechanism. The fungus Nectria haematococca causes a disease on pea even though the infected tissue synthesizes large amounts of the phytoalexin pisatin. Our studies indicate that virulent isolates of N. haematococca can grow in such tissue because they are able to detoxify pisatin. The enzyme pisatin demethylase, responsible for detoxifying pisatin, is a substrate-inducible microsomal cytochrome P-450. Conventional genetic studies have identified several different genes (PDA genes) coding for pisatin demethylation. Some of these genes code for low levels of pisatin demethylating activity and these genes do not enhance the virulence of the pathogen. Recently, a N. haematococca PDA gene has been isolated by expression in Aspergillus nidulans. Our current research effort is directed at characterizing this gene and using this information to determine whether the PDA genes associated with different levels of virulence encode different regulatory regions and/or different cytochrome P-450s.

Determinants of Bacterial Pathogenicity

J9 MOLECULAR BASIS OF NODULATION AND HOST RANGE IN THE RHIZOBIUM-LEGUME SYMBIOSIS, A.W.B. Johnston, L. Rossen, C. Shearman, I. Evans and J.A. Downie, Department of Genetics, John Innes Institute, Colney Lane, NORWICH NR4 7UH, UK

Bacteria of the genus Rhizobium have the unique ability of infecting legumes, inducing root nodules, and, within these nodules fixing atmospheric nitrogen. Our approach to the study of this interaction has been to identify and analyse the genes that Rhizobium possesses, which allow it to recognize and nodulate legumes, most of these studies having been done with R. leguminosarum, the species that nodulates peas.

Many of the genes that determine nodulation ability and the host-range specificity of this species are clustered in a small region of a large "symbiotic" plasmid, pRL1JI. DNA sequence analysis has revealed the presence of eight genes in this region which are required for the normal nodulation of peas. Mutations in some of the genes, nodA,B,C and D abolish nodulation ability whereas mutations in nodE,F,I and J delay and reduce nodule formation.

Translational fusions have been constructed in which genes in each of the transcriptional units in the nod region of pRL1JI were fused to the lacZ gene of E. coli cloned in a new wide host-range translational fusion vector. It was found that nodD is autoregulatory, being able to inhibit its own transcription. In addition, the presence of the intact nodD gene allowed activation of transcription of the nodABC and the nodFE transcriptional units but this induction required the presence of a compound in the root exudate of peas.

J10 VIRULENCE GENES IN PLANT-BACTERIA INTERACTIONS, T. Kosuge, T. Yamada, C. J. Palm, N. L. Glass, F. Roberto, Department of Plant Pathology, University of California, Davis, CA 95616

Microorganisms possess many attributes that relate to their pathogenicity on plants. The general term pathogenicity is defined here as the capacity of an organism to cause disease on a plant; virulence is one component of pathogenicity and concerns those attributes of a pathogen directly involved with formation and severity of symptoms caused on plants. Expression of virulence can only be detected by symptoms formed on a susceptible plant infected by the pathogen. Primary symptoms of a disease are the reactions of the host to the interactions between the pathogen's virulence factors and their targets in the plant. The interaction is affected by the environment as well as the developmental stages of the plant.

A virulence factor may be a pectate lyase produced by a soft rot bacterium or a secondary metabolite, such as a toxin or a phytohormone, produced by a plant pathogenic bacterium or a fungus. Since virulence can be lost without lethal effects on the pathogen, many if not most virulence factors may be secondary metabolites or enzymes such as pectate lyase which are associated with specialized metabolic pathways. Expression of virulence would be controlled by mechanisms specific to the type of metabolic pathway that is involved.

Phytohormone production is used as a model for studying virulence expression in the tumorigenic bacterium, Pseudomonas savastanoi. Synthesis of indoleacetic acid (IAA) is a basis for virulence in the interaction of the bacterium with its hosts, oleander and olive; IAA is produced by the reactions L-tryptophan --------→ indoleacetamide --------→ IAA. The enzymes and their genetic determinants are, respectively, tryptophan monooxygenase (iaaM) and indoleacetamide hydrolase (iaaH).

Factors controlling production and secretion of IAA therefore affect expression of virulence in the pathogen. The rate of IAA synthesis is regulated by feedback inhibition of tryptophan monooxygenase, and conversion to IAA-lysine affects free IAA pool size.

Genes concerned with IAA synthesis have been cloned and sequenced; mechanisms controlling expression of the IAA genes are being investigated. Based upon similarities in nucleotide sequences, genes similar to the IAA genes from P. savastanoi have been found in other plant pathogenic bacteria.

Genes Mediating Host-Bacterial Interactions

J11 REGULATION OF RHIZOBIUM INFECTION GENES. Sharon R. Long, Dept. of Biological Sciences, Stanford University, CA 94305.

Early stages of Rhizobium - Legume symbiotic interaction require activity of nodulation (nod) genes in the bacterium. In Rhizobium meliloti, the alfalfa symbiont, nodulation genes map over a 17+kb DNA segment linked closely to the nitrogenase (nif) genes. One cluster, including the common nod genes DABC, has been mapped and sequenced, and the protein products defined (1, 2, 3). NodA protein appears to be located in a membrane fraction in Rhizobium meliloti cells (3), a possibility which is being studied further by immunolocalization. The protein products of nodABC are not detectable in bacterial cells grown in ordinary laboratory media. This reflects the regulation of the nod genes, which was elucidated by studies using translational fusions of E. coli lacZ to R. meliloti nodD and nodC genes (4), and using antibody against nod gene protein products to follow their production in vivo. The gene fusion analyses revealed that nodD was expressed in cells grown in laboratory media, but that nodABC expression was very low. NodABC expression was stimulated dramatically by supplying R. meliloti cells with exudate from alfalfa or other legume plants. Furthermore, the induction of nodABC by plant exudate was dependent on expression of nodD: when nodD was supplied in trans to nodABC, and expressed at high level from its own (4), or from an exogenous promoter (3), the cells showed an exaggerated response to plant exudate. The active factor present in exudate appears to be a small molecular weight molecule of aromatic character; exudates with activity on R. meliloti have been obtained from many legumes, but to date activity has not been detected in non-legumes. The role of nodD may be in receiving or transducing the plant signal, and/or in transcriptional activation at the nodABC promoter. In R. meliloti, multiple DNA segments with sequence homology to nodD have been found, several of them being located in the vicinity of other symbiotic genes. At least one of these "nodD" segments appears to have a partial effect on nodABC expression. In addition, a new locus has been identified which has the effect, when carried in trans, of raising nodABC expression to a high level independent of plant exudate. The relationship of this new locus to nodD activity, and its position of the nod-nif regulation hierarchy, have been explored using mutation and gene fusions.

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J12 SIDEROPHORES: MOLECULAR BIOLOGY AND RHIZOSPHERE ACTIVITY. J. B. Neilands, Department of Biochemistry, University of California, Berkeley, CA 94720.

With a few notable exceptions, siderophore systems of iron assimilation have been shown to be widely distributed in the microbial world. The detection of siderophore activity in soil and the actual isolation of a specimen of schizokinen from this source (1) indicates that a possible role of these compounds in modulation of plant growth and disease cannot be neglected (2). The molecular genetics of the aerobactin operon of plasmid ColV-K30 of virulent strains of Escherichia coli is now known in some detail (3). Four genes code for biosynthesis of the siderophore and a fifth gene specifies the outer membrane receptor for ferric aerobactin. The complex is surrounded by ISl elements, which may enhance its recombinational mobility (4). The aerobactin operon is not restricted to ColV type plasmids but occurs commonly on the chromosome of clinical isolates of E. coli (5). Regulation by iron of expression of the aerobactin operon has been shown by analysis of specific RNA to take place directly at the transcriptional level(6). The fur (ferric uptake regulation) gene of enteric bacteria codes for a product found in the case of E. coli to be a 17 kDa polypeptide (7), the mode of action of which requires further elucidation. The isolation from low-iron grown Rhizobium meliloti of a structurally novel siderophore, rhizobactin (8), has stimulated the search for a perfectly general method for detection of siderophores and isolation of mutants affected in the synthesis or regulation of siderophore systems.

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Viral Replication in Plants

J13 THE ROLE OF RNA PROCESSING IN VIROID REPLICATION,
Andrea D. Branch, Bonnie J. Benenfeld, Dianne L. Rosen and Hugh D. Robertson. Laboratory of Genetics, Rockefeller University, New York, N. Y. 10021.

Viroids are the smallest microbial agents causing stress in plants. Their replication pathway forms a pivotal link between the host cell (which appears to provide all the factors required for viroid multiplication) and the ability of these minute RNA molecules to cause a puzzling variety of symptoms.

There is now general agreement that viroid replication proceeds via a rolling circle mechanism involving multimeric forms of both plus and minus strands. RNA processing reactions are required to generate the mature circular plus strands which are the final products of the replication cycle. Several lines of evidence suggest that immediate precursor to the circular form is a unit-length linear RNA with a 5' phosphate terminus and a 2', 3' cyclic phosphate moiety at its 3' end. Although the details of the cleavage reactions leading to this intermediate are still being worked out, it is likely that multimeric plus strands promote their own processing to a variable degree. Thus, it is worth seeking elements in their structure which might facilitate auto-catalytic cleavage. In this regard, it is interesting to note that we recently discovered a novel element of local tertiary structure in potato spindle tuber viroid. This element occurs in the most highly conserved region of the viroid RNA--a region considered essential for replication. Future experiments are aimed at defining how the central conserved region contributes to the numerous steps of the viroid replication cycle and, in particular, to determine whether it plays a specific role in the processing of viroid precursor molecules.

J14 REPLICASE AND REPLICATION: STRATEGIES FOR BROME MOSAIC VIRUS. T.C. Hall, T.W. Dreher and L. Marsh, Biology Department, Texas A&M University, College Station, TX 77843-3258

Despite the importance of RNA viruses as infectious agents in crop plants (and in animals, including man), remarkably little is known about the mechanisms of RNA replication in eukaryotes. Although rapid synthesis and accumulation of new viral RNA molecules in infected plants can be detected, very few laboratories have reported convincing evidence of viral RNA replication by extracts in vitro. We have shown that a membrane-associated enzyme fraction can be isolated from barley plants infected with brome mosaic virus (BMV) which, after treatment with micrococcal nuclease to remove traces of endogenous RNA, initiates synthesis of new (-) sense RNA on supplied template (1). Using in vitro transcription systems, we have shown (2) that transcripts of cDNA clones corresponding to wild type sequences (and containing the tRNA-like 3' terminus common to each of the four types of encapsidated BMV RNA molecules) are used by this enzyme to give (-) sense copies. Site-directed mutagenesis of the cDNA clones has been undertaken to identify regions within the 3' structure that are important in replicase recognition and initiation (2,3). The 3' structure of BMV RNA also serves as a template for tyrosine esterification, and the above studies have revealed that although replication and aminoacylation functions overlap, regions affecting one but not the other activity can be identified. This ability to distinguish regions exhibiting these two activities shows that aminoacylation is not obligatory to replicase activity in vitro. We are investigating whether this is also true in vivo, and whether a cooperative role is involved.

We have also used the in vitro transcription-replication system to identify the mechanism by which the subgenomic component (BMV RNA 4), that functions in viral coat protein synthesis, arises (4). Thus, recombinant DNA procedures are providing insight to viral functions that have been recalcitrant when studied by more classical methods. Understanding these functions can be expected to lead to novel approaches for prevention or cure of infections by RNA viruses.

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J15 REPLICATION AND EXPRESSION OF CAULIFLOWER MOSAIC VIRUS, T. Hohn, J.-M. Bonneville, J. Fütterer, J. Martinez-Izquierdo and M. Pietrzak, Friedrich Miescher-Institut, P.O. Box 2543, CH-4002 Basel, Switzerland.

Cauliflower mosaic virus, albeit a DNA virus, uses reverse transcriptase for genome replication and its replication cycle is related to the retroviruses, Hepatitis B virus and retrotransposons. Also CaMV ORFs IV,V and VI show similarities in organization, function and sequence to the "gag", "pol" and "env" genes of retroviruses. On the other hand, there are ORFs which are peculiar for CaMV and the expression of which seems to involve a "polycistronic messenger". Experiments using SP6-promoted transcripts from hybrid plasmids grown in E.coli show that indeed CaMV ORFs oriented in tandem are recognized by eukaryotic ribosomes and that the long and several ATG-codons-containing leader of the large CaMV transcript is no hindrance for translation. CaMV proteins are posttranslationally modified by protein cleavage and phosphorylation and the modification functions might be virus-coated.

Controlling Viral Diseases

J16 PROTEINASE INHIBITORS AND VIRAL REPLICATION, George Bruening, Fernando Ponz and Christopher B. Glascock, Department of Plant Pathology, University of California, Davis, CA 95616

Seedlings of line Arlington cowpea (Vigna unguiculata) fail to support the replication of cowpea mosaic virus (CPMV). In contrast to the seedlings, the protoplasts of the Arlington cowpea do support CPMV replication, but only to a very low level compared to protoplasts from the systemic CPMV host, cultivar Blackeye 5 cowpea. A proteinase inhibitor obtained from Arlington cowpea protoplasts is a candidate for the mediator of resistance of Arlington protoplasts to CPMV because it inhibited a CPMV proteinase in an in vitro assay (1). We now have extended our observations to extracts of Arlington cowpea leaves and found them to be a source not only of (1) proteinase inhibitor but also of two other candidate resistance factors: (2) an inhibitor of the translation of CPMV RNAs and (3) a proteinase that degrades CPMV polyproteins. We required that a reasonable candidate resistance factor (a) must be obtained in, at most, very reduced amounts from extracts of Blackeye 5 cowpeas, (b) must be present only in those progeny of Blackeye 5 and Arlington crosses that also show resistance to CPMV and (c) must act in in vitro assays on CPMV but not on a related virus that replicates efficiently in Arlington cowpeas: cowpea severe mosaic virus. Activities 2 and 3 met criterion (a) but not criteria (b) and (c). Activity 1 met criterion (a) and, less definitely, criterion (b). Thus the proteinase inhibitor of Arlington cowpeas remains as the most likely candidate as a, perhaps the, CPMV resistance factor not only of protoplasts but also of seedlings of the Arlington cowpea.

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J17 GEMINIVIRUS DISEASE MODELS: TOWARDS AN UNDERSTANDING OF THE ROLES OF CIS AND TRANS ELEMENTS IN TOMATO GOLDEN MOSIAC VIRUS INFECTION, S. G. Rogers, D. M. Bisaro*, G. Sunter*, W. Gardiner*, I. Chang*, R. B. Horsch, R. T. Fraley, L. Brand, and J. S. Elmer,* Department of Botany and Microbiology, Auburn University, Auburn, AL 36849 and Biological Sciences, Monsanto Company, 700 Chesterfield Village Parkway, St. Louis, MO 63198.

The geminiviruses are unique among eucaryotic viruses in both their bi-partite icosahedral virion structure and in the organization of the genome into single stranded circular DNAs. Tomato Golden Mosaic Virus (TGMV), a representative of the whitefly transmitted geminiviruses, has a genome comprised of two single stranded circular DNAs referred to as the A and B components. The A and B DNAs are each approximately 2.5 kb in size and share a 230 bp common region of nearly perfect homology that has been suggested to contain the origin of replication. Computer analysis of the sequences of these two DNAs has identified four open reading frames on the A component and two on the B component. The A component is believed to encode the coat protein based on homology with the coat protein gene of cassava latent virus, another two component geminivirus. A second class of geminiviruses, represented by maize streak virus, are leafhopper transmitted and contain one genome organized as a 2.7 kb single stranded circle. The occurance of two classes of geminiviruses, one of which has half the genetic information of the other, and the separation of the functions into two separate DNAs in the TGMV class, raises interesting questions concerning the possible genes and their roles in symptom causation and replication.

We have investigated the functions encoded by each of the components of TGMV using the Agrobacterium tumefaciens Ti plasmid gene transfer system. Tandem, direct repeats of each of the components were introduced into petunia and tobacco cells and plants were regenerated. The regenerates were normal in morphology and fertile. When the progeny of an A plant crossed with a B plant were examined, one quarter showed virus symptoms demonstrating that the integrated components were functional. Analysis of total DNA from the leaves of symptomatic progeny demonstrated the presence of intracellular virus DNA including single and double strand circular forms. Surprisingly, analysis of the tandem A insert parents also showed single and double strand circular forms that hybridized to A but not B specific probes. Plants containing tandem B inserts did not contain free circular forms. This result demonstrated that the A component encodes all of the virus functions necessary for release and replication of the virus DNA. Since the tandem A containing plants show no symptoms the B component must contribute functions necessary for symptom appearance. Experiments are now in progress to define the roles of the various B encoded proteins in symptom manifestation.

Biotechnology Applications: Creating Herbicide Resistance in Crop Plants

J18 DEVELOPING PLANT VARIETIES RESISTANT TO SULFONYLUREA HERBICIDES, R. S. Chaleff, S. A. Sebastian, T. B. Ray, C. J. Mauvais, and B. Mazur, Central Research and Development Department, Experimental Station, E. I. du Pont de Nemours & Co., Wilmington, DE 19898

Several experimental strategies are being employed to develop crop varieties resistant to sulfonylurea herbicides. Resistant tobacco mutants were first isolated by selection in cell culture. Genetic studies of regenerated plants revealed that resistance was semidominant and resulted from mutation of either of at least two unlinked nuclear genes. Mutants of both classes possessed an altered form of acetolactate synthase (ALS) that was less sensitive to inhibition by the herbicide than was the normal enzyme. Doubly mutant plants, which are at least 1000 times less sensitive to chlorsulfuron than are plants of the parental variety, were produced by selecting for an even higher level of resistance among singly mutant cell cultures. These second site mutations, which are genetically linked to the first mutation, increase the proportion of herbicide-resistant ALS activity in leaf extracts.

For species for which plant regeneration from cultured cells is difficult or has not yet been accomplished, mutation breeding offers an alternative method for isolating herbicide-tolerant mutants. This procedure has been used to isolate soybean mutants with increased tolerance for sulfonylurea herbicides. Unlike the mutations recovered in tobacco, the soybean mutations only increase herbicide tolerance between five and ten-fold, are recessive, and do not affect the herbicide sensitivity of ALS.

A molecular genetic approach to generating sulfonylurea herbicide-resistant plants is also being pursued. A DNA fragment encoding a polypeptide with a deduced amino acid sequence similar to the amino acid sequences of the yeast and Escherichia coli ALS enzymes was cloned from a genomic library of a herbicide-resistant tobacco mutant. This cloned fragment is now being inserted into appropriate vectors that will permit its transfer to sensitive plant cells.

J19 STRATEGIES UTILIZING BACTERIAL GENES AS HERBICIDE RESISTANCE DETERMINANTS IN PLANTS, David M. Stalker, Kevin McBride, Ronald E. Rose and Luca Comai, Calgene Inc. 1920 Fifth St., Davis, CA 95616
In plants, many herbicide resistance traits involve single dominant gene loci. This observation makes herbicide resistance an attractive trait for transfer to plants by genetic engineering technology. Herbicide resistance genes can be identified and characterized from a variety of organisms. We are attempting to utilize bacteria as sources for herbicide resistance genes. Resistance determinants can be obtained by 1) defining a herbicide target in bacteria and altering the target enzyme by mutagenesis to affect resistance to the herbicide; 2) isolating naturally occuring herbicide degrading bacteria and characterizing the enzymatic degradative mechanism. Aspects of the above two systems will be discussed as methods for tailoring bacterial genes to confer herbicide resistance to plants.

Natural Strategies for Insect Control (Joint)

J20 APPLICATIONS OF A BACILLUS THURINGIENSIS CRYSTAL PROTEIN FOR INSECT CONTROL, M. J. Adang, D. DeBoer, E. Firoozabady, J. D. Kemp[1], E. Murray, T. A. Rocheleau, K. Rashka, G. Staffeld, C. Stock, D. Sutton[1], and D. J. Merlo, Agrigenetics Corporation, Advanced Research Division, 5649 East Buckeye Road, Madison, WI 53716
A continuing challenge in controlling pest insects is the development of plants resistant or tolerant to insect attack. One approach to accomplishing this objective is to express proteins that are deleterious to insects in plants. Insect pathogens such as Bacillus thuringiensis provide a source for these toxin genes (1). We recently described the cloning and characterization of full-length and toxic fragments of the B. thuringiensis HD-73 crystal protein gene (2). Both the complete HD-73 gene and truncated genes encoding toxic fragments have been engineered behind the mannopine (ORF 24) promoter of pTi15955 (3, 4). These promoter/gene cassettes were cloned into a binary micro T-DNA vector containing a plant selectable marker and delivered into tobacco plants. We have observed significant levels of truncated B. thuringiensis peptides synthesized in callus and immature shoot tissue. Work is in progress to further evaluate the expression of this gene in tobacco and other plants.

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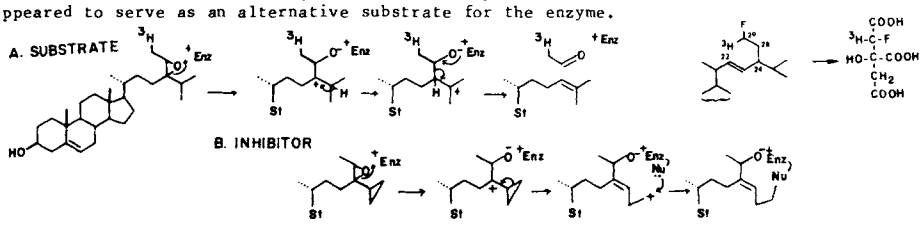
J21 MODIFIED PHYTOSTEROLS AS ENZYME-TARGETED PROINSECTICIDES AND SUICIDE SUBSTRATES, Glenn D. Prestwich, Department of Chemistry, State University of New York, Stony Brook, New York 11794-3400

A novel pro-insecticide, 29-fluorostigmasterol, causes mortality due to release of fluoroacetate during side chain dealkylation. The 29-^{3}H-labeled substrate was fed to third instar tobacco hornworms (Manduca sexta) and erythro-2-fluoro-2[2-^{3}H] citrate was isolated in 0.012% yield. The less toxic 29-fluoro-[29-^{3}H] sitosterol produced no labeled fluorocitrate, while a more toxic 16-^{3}H-labeled 16-fluorofatty acid gave 1% conversion to labeled fluorocitrate. This is the first direct chemical evidence for the fate of the two carbons removed during phytosterol dealkylation in an insect.

Fucosterol epoxide labeled with tritium in the C-29 methyl was synthesized and employed to develop a partition assay, allowing rapid determination of fucosterol epoxide lyase (FEL) activity in vitro in homogenates of insect tissues. [24-^{14}C]-Fucosterol epoxide provided a control substrate. The diastereomeric (24R,28R) and (24S,28S)-[29-^{3}H]-fucosterol epoxides were obtained via HPLC separation of their benzoate esters. Homogenates of the midgut and other tissues of larval M. sexta were assayed at pH 7.4 in 76 mM phosphate buffer at 37° C. The (24R,28R) diastereomer was metabolized at a rate at least 100 times that of the (24S,28S) isomer.

The FEL assay was used to evaluate many inhibitors, including a potential suicide substrate. The cyclopropyl analog of fucosterol epoxide, 26,27-cyclo-fucosterol-24,28-epoxide, was synthesized in ten steps from stigmasterol. Electrophile-induced epoxide opening of this analog by the insect enzyme FEL formally leads to a stable cyclopropyl carbinyl cation which can rearrange to a homoallylic cation, with subsequent capture by an active site nucleophile. Although cyclofucosterol epoxide did not seem to cause irreversible inhibition of FEL, it was the most potent competitive inhibitor examined and appeared to serve as an alternative substrate for the enzyme.



Biological Control of Insects and Pathogens

J22 MOLECULAR BIOLOGY IN BIOLOGICAL CONTROL OF SOILBORNE PATHOGENS, Ralph Baker, Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523

Molecular biology is in its infancy in the discipline of biological control of plant pathogens. There are some interesting examples, however, of the variety of approaches to such studies. A possible basis for recognition in the interaction between mycoparasites and their hosts through the mediation of lectins was advanced by Barak et al (1). The potential host range of a mycoparasite may be partially explained by the incapability of the antagonist to produce enzymes that could digest certain cell wall components of some plant pathogens (2). Again, elements, essential for successful infection by soilborne pathogens, can be limited by nutrient-acquiring metabolites produced by biocontrol agents (3). The limiting factor in genetic improvement of biocontrol agents, however, is identification of attributes which would increase their efficiency.

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J23 "ENHANCEMENT OF ROOT HEALTH AND PLANT GROWTH BY RHIZOBACTERIA" by R. James Cook, D. M. Weller, and L. S. Thomashow, USDA, ARS, Washington State Univ., Pullman, WA.

The presence of a complex community of nonpathogenic epiphytes and endophytes provides an important barrier to the establishment of pathogens on plants, and may well be the first line of defense used by plants against pathogens. Bacterial seed treatments using strains of fluorescent Pseudomonas spp., obtained from roots of healthy wheat plants grown in soil naturally infested with Pythium spp., Gaeumannomyces graminis, or both pathogens, and selected for their ability to inhibit one or both these pathogens in vitro, resulted in 10-25% greater yield of wheat when applied as seed treatments in field trials conducted since 1979. No plant-growth response to the bacteria could be demonstrated in pathogen-free soil. Root colonization is a two-phase process involving a) passive carriage of the bacteria with the advancing root tip and b) multiplication limited by the carrying capacity of the rhizosphere and competition with indigenous strains. The higher the ratio of introduced to indigenous colonists, the better the protection. Protection also depends on ability of the introduced strain to produce one or more siderophores, antibiotics, or both compounds. Mutants that lack ability to produce these compounds colonize but do not protect wheat roots. The suppressiveness of one strain effective against both pathogens is probably due to the production of a dimer of phenazine-1-carboxylate. Of 43 genera of fungi tested, G. graminis and Pythium species were the most sensitive to this antibiotic in vitro. Research is now focused on the genetics and ecology of the beneficial rhizobacteria, and on finding or developing superior strains of rhizobacteria and wheat germplasm more supportive of the bacteria.

J24 IMPROVEMENT OF THE EFFICACY OF VIRAL PESTICIDES THROUGH RECOMBINANT DNA TECHNOLOGY, Lois K. Miller, Department of Bacteriology and Biochemistry, University of Idaho, Moscow, ID 83843.

Baculoviruses can cause natural epizootic diseases in insects. Their pathogenicity, environmental stability and relative safety to nontarget organisms have led to their use as insect pest control agents (1). Although several baculoviruses have been developed for commercial or large-scale application as insecticides, their use remains limited. One factor contributing to their limited commercial success is the narrow host specificity of the viruses that requires the industrial production and marketing of numerous viruses to control the many different pest species. Another major factor contributing to their limited commercial use is the requirement that the viruses replicate extensively in the insect before morbidity occurs; conventional insect control programs employ contact chemical agents which generally have instantaneous effects on insect behavior. The ability to insert and express foreign genes in baculovirus genomes (2,3,4) opens many new frontiers in developing broader-ranged, faster-acting baculovirus pesticides (1,5).

The basis for improving baculovirus efficacy through recombinant DNA technology is baculovirus-mediated expression of a gene encoding a peptide or protein which can modify insect behavior. Such a gene might encode an insect-specific neurotoxin, an insect hormone or a hormone activator. When the gene is placed under the control of a promoter which is expressed early during the infection process, insect behavior may be abruptly altered. Recent model experiments show that if the gene encoding chloramphenicol acetyl transferase (CAT) is inserted into the baculovirus Autographa californica nuclear polyhedrosis virus (AcNPV) under the control of a broad range promoter, the Rous sarcoma virus long terminal repeat (RSV-LTR), the baculovirus-borne CAT gene is expressed in non-target insects (eg. flies and mosquitoes) as well as target lepidopteran species (5). Thus the effective host range of a recombinant baculovirus of this nature may be considerably expanded. Current effort is directed to cloning and expressing insect behavior-modifying genes. One of the attractive features of recombinant baculovirus pesticides is that a variety of genes, including the insect's own genes, may be the basis of the "active ingredient" of the pesticides.

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Plant Responses to Biological Stresses

J25 THE ACTIN MULTIGENE FAMILY IN PETUNIA: CHARACTERIZATION AND DIFFERENTIAL EXPRESSION OF ITS MEMBERS. Wm. Vance Baird and Richard B. Meagher, Genetics Dept., University of Georgia, Athens, GA 30602

The genes encoding actin, a ubiquitous protein in all eukaryotic cells, provide an excellent model system for studying the structure and tissue specific expression of a multigene family. In Petunia hybrida 'Mitchell', actin proteins are encoded by a large and complex multigene family. Southern blot analyses of genomic DNA indicates that although the members share various degrees of homology (cross-hybridization) certain members are present in one or two copies per haploid genome while others are present at 20 or more copies. Also it appears that most if not all of the 'Mitchell' actin genes are derived from the P. axillaris parent. Eight distinct clones were isolated from a phage lambda genomic library and characterized by restriction endonuclease digestion and filter hybridization to known heterologous actin clones (Dictyostelium, Drosophila, rat and soybean). Transcripts from the genomic clones were detected in the poly (A)$^+$ RNA fraction isolated from leaves, roots, stems and flowers by Northern analysis and by probing the restricted lambda clones with labeled RNA. Genes or gene pairs were characteristically expressed at different levels in each organ and one clone was not detected in any organ type. We are currently sequencing the clone most highly expressed in leaf tissues as well as constructing chimeric genes containing a reporter function linked to putative promoter and enhancer regions. Their regulation will be further characterized in transgenic Petunia plants.

J26 A NOVEL POLY-GLYCINE ENCODING GENE FROM PETUNIA, Carol M. Condit and Richard B. Meagher, University of Georgia, Genetics Department, Athens, Ga. 30605.

We have isolated and partially sequenced a novel single-copy gene, pGly, from a Mitchell petunia genomic DNA library which encodes a protein of very high glycine content. The 3' end of this gene contains a continuous open-reading-frame of 1068 bases. The predicted polypeptide contains 60% glycine and contains a number of small repeats of three, five and seven glycine residues usually interupted by only one amino acid. In addition, the predicted amino-acid sequence contains two large repeats of 29 and 44 amino-acids. These large repeats are present at the amino-acid level but not at the nucleotide level.

Using M-13 recombinant DNA probes representing opposite strands of the gene, we have confirmed that it is, indeed, the strand encoding the glycine-rich polypeptide which is expressed as RNA. PA$^+$-RNA isolated from young and old tissue contains two RNAs homologous to this gene at very high stringency (0.1XSSC, 90°C). These RNAs are approximately 1300 and 2200 bases in length and appear to be expressed in the same ratio in both types of tissue. However, the RNAs appear to be expressed at a 40-fold higher level in young tissue than in old tissue.

We propose that this gene encodes a structural protein which is one of the cell wall proteins of petunia.

J27 IMMUNIZATION IN CUCUMBER: IS A WOUND NECESSARY FOR THE INCREASED LIGNIFICATION OF IMMUNIZED TISSUES? R. A. Dean & J. Kuć, Univ. of Kentucky, Lexington, KY 40546.

Localized infection of cucumber plants with fungi, bacteria and viruses induces non-specific systemic protection (immunity) against disease caused by pathogens from these 3 classes. Peroxidase activity increases 3-fold in immunized tissues. Vacuum infiltration of ^{14}C t-cinnamic acid and ^{14}C phenylalanine into leaf discs resulted in a greater rate of incorporation into a lignin residue in immunized as compared to control leaves after challenge with Colletotrichum lagenarium. ^{14}C p-hydroxybenzaldehyde and ^{14}C vanillin, typical lignin oxidation products, were recovered from residues by reverse phase C-18 H.P.L.C. after nitrobenzene oxidation. Incorporation of ^{14}C t-cinnamic acid or ^{14}C phenylalanine is ca. 50% greater in unchallenged immunized leaf discs compared to control discs. Autoradiograms and extraction of the inner and outer regions of the leaf discs revealed that the ^{14}C is predominantly confined to the wounded outer edge. Further studies, which involved making small puncture wounds through drops of ^{14}C t-cinnamic acid placed on the leaf surface, confirmed the greater rate and extent of incorporation (ca. 50%) in wounded immunized tissues. When ^{14}C t-cinnamic acid was pulse-fed through the roots of small plants 16% more ^{14}C was incorporated into non-wounded immunized leaves than in control leaves. Wounding increased the incorporation by a further 10%. Yields of lignothioglycolic acid indicated more lignin (ca. 10%) present in unchallenged immunized leaves than in control leaves. These data suggest immunized leaves contain more lignin and have a greater ability to further lignify upon wounding/infection than control leaves.

J28 DISEASE RESISTANCE RESPONSE GENES IN PLANTS: THEIR EXPRESSION IN NON-HOST AND RACE-SPECIFIC RESISTANCE. Hadwiger, Lee, Catherine Daniels, Brian Fristensky, Wendy Wagoner. Washington State University, Pullman, WA 99164-6430.

Alaska peas express a non-host resistance response against Fusarium solani f. sp. phaseoli but are susceptible to Fusarium solani f. sp. pisi. Five other pea varieties express differential race-specific resistance to races 1, 2 and 3 of Psuedomonas syringae pv. pisi. In previous work we have cloned cDNAs corresponding to mRNAs which are induced in pea tissue as it resists F. solani f. sp. phaseoli. Treatments which interfere with the expression of these genes result in a "susceptible" phenotype. These clones were also used to monitor the accumulation of individual mRNA species in each of five pea varieties challenged with three races of Psuedomonas syringae pv. pisi. Northern blots indicate that phenotypic resistance correlates temporally with specific RNA accumulation and susceptibility correlates with depressed levels of, or absence of, a specific RNA. Not all race-pea variety northerns were representative of the plant-expressed resistance phenotype. We conclude that some of the same pea genes are expressed both in non-host and in race-specific resistance.

J29 HOW CHITOSAN, A DNA-COMPLEXING CARBOHYDRATE ACTIVATES GENES ASSOCIATED WITH DISEASE RESISTANCE IN PEAS. Hadwiger, L. A., Fristensky, B. W., D. F. Kendra, C. Daniels, W. Wagoner, Washington State Univesity, Pullman, WA 99164

Chitosan, β-1,4 linked glucosamine is a minor component of Fusarium solani f. sp. pisi (a pea pathogen) and f. sp. phaseoli (a bean pathogen, non-pathogen on peas) cell walls. Chitosan (or heptamer polymers of glucosamine) applied to pea endocarp tissue mimics f. sp. phaseoli inoculation (1) by inducing β-glucanase, chitinase, phenylalanine ammonia-lyase, and pisatin; (2) in inducing 20 major "disease resistance response proteins" and 5 specific pea genes whose homologous clones were screened from a cDNA library of disease resistance-response genes; (3) in inducing, in peas, complete disease resistance to f. sp. pisi. That the polycationic chitosan's action is DNA-specific has been indicated by DNA affinity and precipitation, changes in DNA melting temp curves and C.D. spectra, alterations in restriction digests, in vivo localization with cellular DNA, and by specific mutagenesis action in the Ames test. The importance of the 5 pea genes activated by chitosan in the resistance response is reflected in the observation that these genes are also activated in the race-specific resistance reactions (but not in susceptible reactions) in 5 pea varieties against 3 races of Psuedomonas syringae pv. pisi. Finally, the patented chitosan has recently been adapted and marketed as a commercial seed treatment for the protection and yield enhancement of wheat.

J30 ENGINEERING BACTERIAL CHITINASE GENES FOR CROP PROTECTION Jonathan Jones, J. Taylor, K. Grady, G. Mueller and T. Suslow. Advanced Genetic Sciences, 6701 San Pablo Ave, Oakland, Ca 94608

Many fungal plant pathogens contain chitin in their cell walls. Plants induce an endogenous chitinase activity in response to disease. We are isolating bacterial chitinase genes and expressing them in plant cells in order to test whether this will augment preexisting plant disease resistance mechanisms. This is a progress report.

1) We have isolated two chitinase genes from Serratia marcescens.
2) One of these genes has been sequenced.
3) This gene has been mutagenised by insertion of an NPT gene and this mutation has been recombined back into the S. marcescens chromosome.
4) The resulting mutants are impaired in their capacity to retard fungal growth proving that chitinase plays a role in the anti-fungal phenotype of the wild type bacteria.
5) We have expressed this gene in plants. Transformed calli synthesise immunologically cross-reactive material which comigrates with the mature form of the chitinase expressed in bacterial cells. This indicates that plant cells can authentically process the bacterial signal peptide.

J31 REGULATION OF SYNTHESIS AND LOCALIZATION OF PATHOGENESIS-RELATED PROTEINS OF TOBACCO
D.K. Klessig, D. Dixon, B. Nikolau, K. Voelkerding and J. Carr, Waksman Institute, Rutgers University, Piscataway, New Jersey 08854

The pathogenesis-related (PR) proteins of tobacco plants are induced in response to a variety of pathogenic and chemical agents. Although the function of these proteins is unknown, they are associated with resistance to multiplication and/or spread of tobacco mosaic virus. We have found that functional mRNAs encoding PR proteins are present only when synthesis of these proteins has been induced, suggesting that their synthesis is controlled in part at the level of mRNA accumulation. In addition PR proteins appear to be synthesized and processed in a manner analogous to proteins destined for the endoplasmic reticulum since: (i) the in vitro translation products synthesized in the wheat-germ cell-free system are slightly larger than the in vivo products, (ii) translation of PR mRNAs in the rabbit reticulocyte lysate system is blocked unless that system is supplemented with dog pancreas microsomes and (iii) mRNAs for PR proteins are associated predominantly with membrane-bound polysomes in vivo. This pathway of synthesis is consistent with our biochemical and immunomicroscopy studies which indicate that these proteins are extruded beyond the plasma membrane.

J32 RECOGNITION SIGNALS AND PLANT RESPONSES, Christopher J. Lamb, Plant Biology Laboratory, Salk Institute, P. O. Box 85800, San Diego, CA 92138

Treatment of suspension-cultured cells with fungal elicitors switches RNA synthesis by transcriptional activation of defense genes, in some cases within 5 min, leading to induction of mRNAs encoding enzymes of lignin and phytoalexin biosynthesis, as well as chitinase and hydroxyproline-rich glycoproteins. Transcriptional activation of defense genes is also observed in race: cultivar specific interactions between the fungus Colletotrichum lindemuthianum and hypocotyls of bean (Phaseolus vulgaris L.) with clear temporal differences in the pattern of mRNA induction between incompatible (host resistant) and compatible (host susceptible) interactions. Induction occurs in distant, hitherto uninfected tissue as well as directly infected tissue implying intercellular transmission of elicitation signals. Defense gene systems are highly polymorphic at the gene, RNA and protein levels, and strategies for identification of stress signals, receptors and signal transduction mechanisms involved in defense gene activation will be discussed in relation to emerging prospects for enhancement of disease resistance.

J33 Phosphoinositide Metabolism And Elicitor-Induced Coumarin Biosynthesis In Cultured Parsley Cells, Ulrich Matern, Hilke Wendorff and Heiner Strasser, University of Freiburg, D-7800 Freiburg, West Germany

Cultured parsley cells respond to elicitor treatment by a rapid change in uptake and intracellular distribution of inorganic phosphate, which is succeeded by accumulation of psoralens and a pyronocoumarin. Whereas the biosynthetic route from L-phenylalanine to the intermediate 7-hydroxycoumarin is still obscure, the path of its further conversion into psoralen via (+)marmesin has been elucidated. Microsomes prepared from elicitor-induced parsley cells catalyzed the cofactor-dependent formation of psoralen from synthetic [2-^{14}C]marmesin.

The change in phosphate uptake of cells observed immediately after addition of the elicitor suggested an effect on the plasmalemma organization. Upon examination of the parsley phospholipids, small amounts of phosphoinositides (PIP, PIP_2) were identified besides PC, PE and PI. Preliminary experiments revealed a transient increase in the synthesis of PIP shortly following the addition of elicitor to the cells. No effect of elicitor on the other phospholipids was found. Changes in phosphoinositide metabolism may be involved in the elicitor signal-transduction which eventually causes the accumulation of coumarins in parsley cells.

J34 TOXIC AND ANTIFUNGAL PROPERTIES OF SOUTHEAST ASIAN TREE RESINS. Adam C. Messer, Sharon L.W. Greenberg, David P. Richardson, Henry H. Hagedorn and Jerrold Meinwald. Departments of Entomology and Chemistry, Cornell University, Ithaca, New York, 14853-0999

Resins of the tropical trees Shorea javanica and Dipterocarpus kerrii (both Dipterocarpaceae) were tested for toxicity to termites, and fungal growth inhibition. Crude resins and some TLC fractions fed on filter papers to Zootermopsis angusticollis workers produced 50% mortality in 4-7 days. Preliminary results suggest that resins may affect the termite gut protozoa. Fresh resins of D. kerrii inhibited growth of Cladosporium cucumerinum on agar. Chemical analysis shows the resins contain many terpene fractions, and experiments are in progress to isolate and characterize the biologically active fractions.

J35 REGULATION OF RIBOSOMAL DNA EXPRESSION IN THE YEAST SACCHAROMYCES CEREVISIAE. R. Mestel, M. Yip, J. Holland, E. Wang and M. Holland, University of California, Davis, CA 95616.

Ribosomal DNA (rDNA) cistrons are tandemly organized in the yeast genome. The 18, 25 and 5.8S ribosomal RNAs are derived from the processing of a 35S precursor. A spacer region separates each unit of 35S information.

An in vitro RNA polymerase I-dependent transcription system has been developed. In this system, transcription initiates within the spacer, 2.2 kilobases upstream of the 35S start site. The same region of the spacer contains sequences which enhance the level of 35S expression 15-20 fold in vivo. The sequences responsible for the enhancing effect are being localized by deletion analysis. They appear to include sequences required for the in vitro-defined promoter activity.

J36 Immunocytochemical Localization of Xyloglucan, Rhamnogalacturonan I and Extensin in Plant Cell Walls. Patricia Johnston Moore, Joel P. Stafstrom, and L. Andrew Staehelin, Dept. of MCD Biology, University of Colorado, Boulder, CO 80309-0347

Plant cell walls serve several functions: they impart rigidity to the plant, provide a physical and chemical barrier between the cell and its environment, and regulate the size and shape of each cell. The cell wall is also where a pathogen initially contacts a plant and where the plant initially responds to the pathogen. Chemical studies have provided information on the biochemical composition of plant cell walls as well as detailed knowledge of individual cell wall molecules. In contrast, very little is known about the distribution of specific cell wall components around individual cells and throughout tissues. To address the problems, we have produced polyclonal antibodies against three cell wall matrix components, xyloglucan (XG), a hemicellulose, rhamnogalacturonan I (RGI), a pectic polysaccharide, and extensin, a cell wall glycoprotein. By using the antibodies as specific markers we have been able to localize these polymers on thin sections of different plant tissues. Our results reveal that each molecule has a unique distribution. XG is localized throughout the entire wall and middle lamella. RGI is restricted to the middle lamella and is especially evident in the tricellular junctions. Extensin, on the other hand, displays the opposite localization to RGI, being excluded from the middle lamella but present throughout the rest of the cell wall. These observations indicate that plant cell walls may have more distinct chemical (and functional?) domains than previously envisaged, and that current models of cell wall architecture will have to be re-examined. Supported by NIH grant GM 18639.

J37 CHARACTERISATION OF ACID PHOSPHATASE 1 FROM A NEMATODE RESISTANT TOMATO CULTIVAR, Elizabeth M. Paul and Valerie M. Williamson, ARCO Plant Cell Research Institute, Dublin, California 94568.

The nematode Meliodogyne incognita parasitises several crop species, causing root knot diseases. Resistance in tomato is conferred by a dominant gene Mi and was originally transferred to the cultivated tomato Lycopersicon esculentum from the wild species L. peruvianum. The nematode induces complex physiological and morphological changes in the host plant, and so a direct determination of the Mi gene product would be difficult. However, the gene coding for an electrophoretic variant of an acid phosphatase isozyme (APase 1^1) is closely linked to Mi. Therefore we plan to clone the gene Aps 1^1 and use the technique of 'chromosome walking' to obtain the resistance gene.

Several acid phosphatase isozymes which differ in electrophoretic mobility are present in tomato. APase 1^1 is only a small proportion of the total activity, as determined by a standard APase assay. We have developed a simple, rapid system to analyse APase isozymes using cellulose acetate electrophoresis. By using this system as an assay we have purified the enzyme APase 1^1 from tomato cell culture. The purification protocol and characterisation of APase 1^1 will be presented.

J38 ENZYMATIC SYNTHESIS OF THE PHYTOALEXIN PISATIN IN PEA. Carol L. Preisig, David E. Matthews, James A. Sweigard and Hans D. VanEtten, Cornell University, Ithaca, NY 14853.

The isoflavonoid phytoalexin pisatin is synthesized by Pisum sativum in response to microbial infection and certain other forms of stress. We have begun studies on the late steps of pisatin biosynthesis, to complement previous work on the regulation of enzymes which function earlier in the pathway. An enzyme which synthesizes pisatin by methylating the 3 hydroxyl of (+)6a-hydroxymaackiain (HMK) was extracted from stressed pea seedlings. The enzyme was specific for (+)HMK, methylating (+)maackiain, (-)HMK and (-)maackiain less than 5% as rapidly. These results support a previous proposal, based on incorporation of radiolabelled precursors in vivo, that the terminal step in the biosynthetic pathway for pisatin is methylation of (+)HMK. The methyltransferase was induced by fungal infection or by treatment with $CuCl_2$, although a low level of activity was also present in healthy seedlings. Copper chloride-induced methyltransferase was detected within 8 hours after treatment; it began to decline after 48 hours, but high levels were still present up to 120 hours.

J39 Photoactivation of cotton sesquiterpenoid pnytoalexins in DNA cleavage and enzyme, virus and bacteria inactivation. Tzeli Julia Sun, Ulrich Melcher and Margaret Essenberg, Biochemistry Department, Oklahoma State University, Oklahoma Agricultural Experiment Station, Stillwater OK 74078

Sesquiterpenoid phytoalexins accumulate in cotton leaves after inoculation with incompatible races of Xanthomonas campestris pv. malvacearum (1). It has been shown, by fluorescence-activated cell-sorting, fluorescence microscopy and quantitative extraction, that the local concentrations of phytoalexins at infection sites in leaves of highly resistant cotton lines reach inhibitory levels by the time the pathogen is inhibited (M. L. Pierce *et al.*; unpublished). The most inhibitory compound in this system is 2,7-dihydroxycadalene (DHC) (1). DHC for this work was chemically synthesized in our laboratory by a new pathway (R. D. Stipanovic and J. Steidl; unpublished). The inhibitory activity of the synthetic DHC was similar to that of the natural product. At 0.1 mM, a much lower concentration than the reported ED_{50}, DHC only partially inhibited multiplication of race 1 in the dark, but in the light ($2x10^4$ lux) this concentration was bactericidal. Light alone was only bacteriostatic. In the light DHC will induce nicks in plasmid pBR322. This was concluded from observing a decrease in closed circle and increase in the open circle and linear forms of the plasmids by agarose gel electrophoresis after the DHC plus light treatment. The extent of DNA nicking in the presence of DHC was dependent on the duration of exposure to light. If the DNA was denatured by either boiling or alkali treatment following the DHC plus light treatment, many fragments smaller than intact single strands were observed. This suggests that nicking of DNA occurred at random sites. When DNase I was incubated with the DHC under light, its catalytic activity was destroyed. Light alone had much less effect on plasmid structural integrity and on enzymic activity, DHC alone had no effect on either.

1) Essenberg, M. *et al.* 1982. Phytopathology 72: 1349-1356.

J40 THE EFFECT OF EXTRACELLULAR FUNGAL COMPONENTS ON PLANT MEMBRANE FUNCTIONS, Kim Rogers, Craig S. Tepper and Anne J. Anderson, Biology Department, Utah State University, Logan, Utah 84322-4500.

Plant cell necrosis occurs in both incompatible and compatible interactions of bean cultivars with races of Colletotrichum lindemuthianum. Factors of fungal origin may be involved in initiation of necrosis through causing membrane perturbation. Impaired plasmalemma and tonoplast function occurred upon treatment of bean protoplasts and vesicle preparations with extracellular mycelial components from the alpha race of C. lindemuthianum. The fungal components were extracted by 1.0 M NaCl treatment of intact mycelia and were fractionated by DEAE-Sephadex chromatography. Certain of these fractions inhibited proton transport in sealed vesicle preparations which were enriched in tonoplast membranes. Proton transport was measured using a quinacrine probe. Concentrations of 150 ug/ml carbohydrate and 150 ug/ml of protein caused a 50% reduction in transport of a vesicle preparation containing 150 ug/ml of protein. The DEAE-Sephadex fractions also caused rapid increase in plasmalemma permeability of bean stem protoplasts when assayed using fluorescein staining. A 50% loss in viability of 2×10^5 protoplasts/ml was observed upon treatment with 20 ug/ml carbohydrate and 20 ug/ml protein of the fungal components. It is possible that these membrane effects are important in lesion formation and in the resistant response of hypersensitivity.

J41 THE GENES ENCODING THE SMALL SUBUNIT OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE ARE TRANSCRIBED DIFFERENTIALLY IN PETUNIA LEAVES, Nilgun E. Tumer, Gail J. Tabor, Robert T. Fraley, and Dilip M. Shah, Monsanto Company, 700 Chesterfield Village Parkway, St. Louis, MO 63198

We have cloned five different genes for the small subunit of ribulose-1,5-bisphosphate carboxylase from petunia and examined their expression in petunia leaves. We detected transcripts specific for three of the five genomic clones by hybridizing first strand cDNA and ^{32}P labelled leaf poly A^+ RNA to DNA from different recombinant phage. Two of these genes ssu11A and ssu11B are linked in petunia genome and the third gene ssu8 is in another region of petunia nuclear DNA. We characterized ssu8 by nucleotide sequence analysis and compared its structural features to ssu11A. The two genes are 89% homologous at the nucleotide level. Unlike ssu11A which has two introns, ssu8 contains three introns. The first two introns are at identical positions, while the third intron in ssu 8 is located within the last exon of ssu11A. The transcript start sites of the two genes were characterized by S1-nuclease mapping. The 5' upstream sequences are 80% homologous while the 5' leader sequences of the two transcripts do not show any homology.

We examined the expression of these genes using gene specific oligonucleotide probes for ssu11A and ssu8. These probes were labelled with ^{32}P and hybridized with poly A^+ RNA from petunia leaves. Quantitation of the hybridization signals indicated that Petunia leaves contained 10-fold higher levels of RNA specific for ssu11A than for ssu 8.

J42 PHYSICAL AND FUNCTIONAL CHARACTERIZATION OF A GENE FROM POTATO ENCODING PROTEINASE INHIBITOR II, Lothar Willmitzer, Jose Sanchez-Serrano and Michael Keil, Max-Planck-Institut fur Zuch tungsforschung, 5 Koln 30 FRG

Differential screening of a cDNA library established from potato poly A+ RNA yielded two cDNA clones encoding proteinase inhibitor II. The amino acid sequence of the potato protein is 82% homologous to the corresponding protein from tomato. Several genomic clones have been isolated from a library established from a monohaploid potato line using the proteinase inhibitor II cDNA as a probe.

Results will be presented describing the structural analysis of these genes as well as first attempts towards a functional analysis by reintroducing different chimaeric genes back into potato and analysis of their expression.

J43 MECHANISMS FOR RESISTANCE OF RICE TO XANTHOMONAS CAMPESTRIS PV. ORYZAE: REINDUCIBLE PLUGGING OF WATER PORES. Jeff D. Wilson and Jan E. Leach, Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Scanning electron microscopy (SEM) of rice (Oryza sativa) leaves spray-inoculated with incompatible races of the bacterial blight pathogen (Xanthomonas campestris pv. oryzae) revealed that the bacteria are entrapped in an exudate which emanates from the hydathode water pores of the host (Mew et al., Phytopathology 74:635, 1984). The exudate was evident by 24 h after inoculation and eventually plugged the opening of the water pores. Compatible races of the pathogen did not induce water pore plugs and multiplied freely on the leaf surface. After 72 h the plugs induced in the incompatible interaction disappeared and the pores reopened. We will present SEM micrographs demonstrating that the loss of water pore plugs after bacterial entrapment frees the water pores to respond to a subsequent challenge by avirulent bacteria. Incompatible bacteria (PX086) were spray-inoculated onto leaves of a resistant rice cultivar (Cas 209) and within 24 h the water pores became plugged with exudate. After the pores had reopened (96 h), the leaves were resprayed with incompatible bacteria. Within 24 h after the second challenge, the water pores again became plugged suggesting a nonhypersensitive, reinducible resistance mechanism. Investigations are in progress to determine if live incompatible cells are required for plug induction, and further, if the first challenge conditions the water pores to respond with a resistant response to a subsequent challenge with compatible bacteria.

Interactions Between Fungal Pathogens and their Hosts

J44 MONOCLONAL ANTIBODIES SPECIFIC FOR PATHOGEN CARBOHYDRATE ANTIGENS -- CANDIDATES FOR AVIRULENCE DETERMINANTS, Arthur R. Ayers, Keith L. Wycoff and Ursula Hanfstingl, Harvard University, Cambridge, MA 02138.

Genetic studies of plants and their pathogens indicate that dominant alleles for resistance in hosts are complemented by corresponding dominant alleles for avirulence in pathogens. Products of these genes have not yet been identified. We have produced murine monoclonal antibodies (Mabs) to extracellular and wall antigens of the fungal soybean pathogen Phytophthora megasperma f.s. glycinea (Pmg) in an effort to identify antigenic determinants associated with particular avirulence genes. Sixty independent Mabs have been characterized by Western blots of Pmg extracellular glycoproteins and by competition assays with modified glycoproteins (TFMS, periodate, Smith degradation, a-mannosidase, endo-H). The Mabs are predominantly glyco-specific and have be grouped in at least six classes based on interactions with Pmg glycoproteins. Binding patterns in Western blots using various Mabs indicate that a Pmg protein may have a single or multiple types of glycomoieties. Races of Pmg with differing avirulence genes exhibit more characteristic patterns in the western analysis than in glycoprotein profiles stained for protein. At least one Mab exhibits enhanced binding to glycoproteins from races with a particular avirulence gene. In current studies we are enriching for avirulence-correlated antibodies by using an immunosuppressant (cyclophosphamide) to inhibit immunological response to antigens common to races with different avirulence genes.

J45 ISOLATION AND CHARACTERIZATION OF MUTANTS BLOCKED IN TRICHOTHECENE BIOSYNTHESIS, Marian N. Beremand, USDA, Northern regional Research Center, ARS, Peoria, IL 61604

The trichothecenes are a chemically related group of toxic sequiterpenoid secondary metabolites produced by certain species of Fusarium. Much remains to be discovered concerning their biosynthetic pathway, the regulatory controls which govern their production, and their physiological function. A mutational analysis has been initiated in order to study several aspects of trichothecene biogenesis and bioactivity. Mutants of F. sporotrichioides 3299, blocked or altered in the biosynthesis of the trichothecene, T2-toxin, have been generated by UV treatment and identified by a rapid screen utilizing monoclonal antibodies to T2. Three stable mutants have been isolated and chemically characterized. Two mutants accumulate diacetoxyscripenol, DAS, suggesting that they are defective in the conversion of DAS to neosolaniol. A third mutant appears to be blocked at an early step in the pathway as it produces greatly reduced levels of both DAS and T2. Study of these mutants and others will provide experimental evidence for the structure of intermediates, the order of the biosynthetic pathway, the source of intermediates, a means of identifying structural and regulatory genes and their products, and, potentially, a system for isolating specific genes via cloning by complementation. Finally, many Fusarium species that produce trichothecenes are plant pathogens. Mutants blocked in trichothecene production should reveal the role of trichothecenes in the pathogenesis of various plant diseases.

J46 DIFFERENTIATION SPECIFIC PROTEINS IN THE RUST FUNGI, Siriama Bhairi, Pauline Freve, and Richard C. Staples, Boyce Thompson Institute and Department of Plant Pathology, Cornell University, University, Tower Road, Ithaca, NY

The parasitic bean rust fungus, Uromyces appendiculatus, invades its host plant through the stomata. The germinating uredospores differentiate to form appressoria when their germ tube tips contact the stomatal guard cell. Differentiation can also be induced on artificial surfaces such as collodion membranes. Induction of germling differentiation on these collodion membranes resulted in the synthesis of three new proteins as revealed by 1-D PAGE.

We have now analyzed the pattern of protein synthesis during differentiation using two dimensional gel analyses. Eleven proteins were specifically synthesized during appressorium formation. Sixteen proteins that were present in nondifferentiated germlings were absent at the time of differentiation. Concentration of a number of proteins (about 21) decreased in the differentiated germlings when compared to the nondifferentiated germlings. Two-dimensional PAGE analyses of the products of in vitro translation of poly(A)+RNA isolated from nondifferentiated and differentiated germlings revealed at least two polypeptides that were specific to appressorium formation. This suggests that transcriptional controls may regulate the synthesis of some of the differentiation-specific proteins. Studies are underway to isolate differentiation specific genes using modified cascade hybridization.

J47 BIOSYNTHESIS AND METABOLISM OF PHYTOALEXINS IN SOYBEANS FOLLOWING WOUNDING OR INFECTION, M.K. Bhattacharyya and E.W.B. Ward, Research Centre, Agriculture Canada, University Sub Post Office, London, Ontario, Canada, N6A 5B7.

Rapid biosynthesis of daidzein and glyceollin I was demonstrated in wounded soybean hypocotyls pulse labelled with [^{14}C] phenylalanine, but only very small amounts accumulated. This evidence for rapid metabolism of the two compounds was supported by pulse-chase experiments and feeding [^{14}C] glyceollin I to wounds. Following inoculation of wounds with Phytophthora megasperma f. sp. glycinea biosynthesis was initially comparable to that in uninoculated wounds but declined after 11 h in the compatible and after 15 h in the incompatible interaction. Glyceollin I accumulated in both interactions but did so earlier and much more rapidly in the incompatible interaction. Thus infection caused a decrease in wound-induced biosynthesis but also an inhibition of metabolism. In contrast, in inoculated intact hypocotyls biosynthesis commenced earlier in the incompatible interaction and reached a rate more than three times that in the compatible interaction.There was some inhibition of glyceollin metabolism in the incompatible interaction and also, at later stages in the compatible interaction.

J48 MOLECULAR CLONING AND IN VIVO EXPRESSION OF A MUTIGENE FAMILY ENCODING CHITINASE FROM PHASEOLUS VULGARIS, Richard Broglie and Karen Broglie, Central Research & Development Dept., E. I. du Pont de Nemours Co., Wilmington, DE 19898

Disease resistance in higher plants is manifested by the accumulation of a number of host-synthesized polypeptides which are produced in response to pathogen attack. One of these putative defense proteins is the enzyme chitinase which catalyzes the hydrolysis of N-acetyl-O-glucosamine polymers of chitin, a major component of fungal cell walls. Recent studies show that the activity of this enzyme can be increased by exposure to exogenous ethylene. As a first step towards investigating the molecular mechanisms responsible for ethylene regulation of chitinase gene expression, we have isolated and characterized six chitinase clones from a cDNA library constructed with mRNA from ethylene-treated plants. DNA sequence analysis of a full-length mRNA copy (pCH18) indicates that it encodes a 27 residue amino terminal signal peptide as well as the 301 amino acid residues of the mature protein. Utilizing pCH18 as a hybridization probe, we have found that the increase in chitinase activity seen upon ethylene treatment is due to a 25-fold increase in steady state mRNA levels. Southern blot analysis revealed that chitinase is encoded by a small, multigene family consisting of four members, at least two of which are expressed.

J49 INHIBITOR PROTEIN OF POLYGALACTURONASE:PURIFICATION BY AFFINITY CHROMATHOGRAPHY.
F.Cervone[1],G.De Lorenzo[1],L.Degrà[1],G.Salvi[1] and M.Bergami[2],Plant Biology Dpt.[1] and Genetics and Molecular Biology Dpt.[2],University "La Sapienza"Rome-Italy.

Plant genetic manipulation makes now possible to transfer of genes into plants.Nevertheless it is not clear yet what kind of genes may improve plant productivity.The degradation of plant cell wall by polysaccharidases is a general mechanism by which phatogenic fungi colonize host plants.Among many polysaccharide-degrading enzymes,polygalacturonase (PG) has a critical primary role as it must act before different enzymes can attack other substrates.Furthermore it is the first polysaccharide-degrading enzyme secreted by certain pathogens cultured on isolated cell walls.Plants may counteract the action of PG with specific glycoproteins which inhibit its activity.We have developed a rapid and simple purification procedure which allows the preparation of 2 mg of PG-inhibiting protein in a single step.Our procedure exploited the high affinity that PG exhibits towards its inhibiting protein.100 ml of Phaseolus vulgaris extract were passed through a column containing 1g of Sepharose 4B to which 6mg of electrophoretically homogeneous PG from Aspergillus niger had been covalently linked.Proteins which remained absorbed to the column were eluted in 5 ml of PBS buffer contained all PG-inhibiting activity of the original plant extract.The fraction eluted whit PBS showed upon polyacrilamide gel electrophoresis only one major protein band which was at least 80%pure.Purified protein exhibited differential inhibitory activity against PG from A.niger,F.moniliforme,C.lindemuthianum.

J50 A Genetic System for Trichothecene Toxin Production in Gibberella pulicaris (Fusarium sambucinum). A. E. Desjardins and M. N. Beremand, Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL 61604

The trichothecenes are a family of sesquiterpenoid toxins which are produced by Fusarium and related fungi. Fusarium infection and trichothecene contamination of agricultural products have long been recognized as recurring public health problems. However, little is known about the biosynthesis and regulation of the trichothecene toxins or about their role in plant pathogenesis. We have recently developed novel methods to obtain the sexual stage, Gibberella pulicaris, in toxin-producing isolates of F. sambucinum. Segregation patterns among random ascospore progeny from parents differing in mating type, sex, pigmentation and toxin production are being examined. Preliminary results indicate that G. pulicaris is amenable to classical genetic analysis. This is the first documented genetic system in any trichothecene-producing species of Fusarium.

J51 **ISOLATION OF DNA SEQUENCES FROM COLLETOTRICHUM GLOEOSPORIOIDES PREFERENTIALLY EXPRESSED WHEN INDUCED BY CUTIN,** **Martin B. Dickman, and Suresh S. Patil, University of Hawaii, Dept. of Plant Pathology, Honolulu, Hawaii 96822**

Cutinase secreted by the fungus Colletotrichum gloeosporioides Penz., the causal agent of papaya anthracnose, is required for pathogen ingress in intact papaya tissue. Complete genomic libraries of this fungus have been constructed in bacteriophage 1059 and two derivatives, EMBL3 and EMBL4, for isolating and cloning the cutinase gene. Cutinase is an inducible enzyme subject to catabolite repression. Labelled cDNA probes were synthesized from poly(A)$^+$ RNA isolated from cultures of the pathogen grown either in dextrose or papaya cutin as the sole carbon sources. These probes were then used to screen the λ libraries using differential plaque hybridization. Recombinant phages which hybridized preferentially to the induced probe were nick translated and used as probes in Northern blot analysis of both mRNA populations. Signals have been obtained which hybridized to the poly(A)$^+$ RNA from cutin induced cultures. Positive clones are being screened for the presence of cutinase-specific sequences.

J52 MECHANISMS REGULATING THE PHYTOALEXIN DEFENSE RESPONSE IN SOYBEAN. J. Ebel, D. Grab, H. Mieth, W.E. Schmidt and M.R. Stäb, Biologisches Institut, Universität Freiburg, D-7800 Freiburg, FRG

We have used soybean (Glycine max) tissues and a β-glucan elicitor of a soybean pathogen (Phytophthora megasperma f.sp. glycinea) for studies of the induced synthesis of isoflavonoid phytoalexins, one type of inducible defense response of plants to invading microorganisms. Phytoalexin accumulation is correlated with transient increases in the activities of several enzymes of phytoalexin biosynthesis. The transient increase in chalcone synthase activity, the first enzyme specific for flavonoid/isoflavonoid biosynthesis, is preceded by a large and rapid enhancement in chalcone synthase mRNA activity and amount, suggesting that the phytoalexin defense response in soybean is regulated by temporary gene activation. Elicitor treatment of cultured soybean cells caused major changes in the population of total translatable mRNA which indicated large metabolic changes associated with phytoalexin synthesis and possibly other pathways of the challenged cells. Omission of Ca^{2+} from the cell culture medium or addition of Ca^{2+} antagonists abolished the elicitor-mediated phytoalexin response. Studies in vitro demonstrated that soybean membrane fractions contained β-glucan binding sites which could be important in the initial plant cell-elicitor interaction. (Supported by Deutsche Forschungsgemeinschaft.)

J53 BLOT ANALYSIS OF A PHYTOTOXIN FROM CULTURE OF STEMPHYLIUM BOTRYOSUM PATHOGENIC ON ALFALFA. Dana Kelly Heiny and David G. Gilchrist, Department of Plant Pathology, University of California, Davis, CA 95616.

Isolates of the cool-temperature biotype of Stemphylium botryosum Wallr., causal agent of Stemphylium leafspot of alfalfa, produce a phytotoxic molecule when cultured in a defined liquid medium. Culture filtrates purified by gel filtration, chromatofocusing and/or ion exchange cause necrosis resembling Stemphylium leafspot when injected into alfalfa leaflets. The molecular weight of the toxic component estimated by gel filtration is approximately 19,500. Sodium dodecylsulfate-polyacrylamide gel electrophoresis of the purified fractions with toxic activity revealed two major protein bands (M_r=19,500; 26,900) and a triplet of smaller bands (M_r=15,500). Immunogammaglobulins (IgG) produced in response to purified toxin identify the two major bands and the triplet on Western blots, as well as a series of high molecular mass bands not visualized by conventional protein staining methods. The IgG produced in response to either major band reacts with the two major bands and the triplet, but not the high mass band series. The potential of these antibodies to neutralize, quantify, and localize toxin in culture or infected tissue is under investigation.

J54 A RELATIONSHIP BETWEEN MELANIN BIOSYNTHESIS AND APPRESSORIAL PORE SIZE IN PYRICULARIA ORYZAE, Richard J. Howard, Du Pont Co., Experimental Station, Wilmington, DE 19898

Inhibition of the polyketide pathway for melanin biosynthesis by tricyclazole is known to prevent fungal ingress into protected plants but have no generally toxic effect on vegetative growth of the pathogen. The 'antipenetrant' aspect of tricyclazole activity might be related to the lack of melanin, normally localized in appressorial cell walls. This study was aimed at defining the antipenetrant effect by understanding the role of melanin in the penetration of host surface. Appressoria of seven strains of P. oryzae, differentiated on plastic or cellulose membrane, were studied by video-enhanced contrast differential interference contrast light microscopy and electron microscopy after freeze substitution. The strains included 3 Buf^+ wildtypes and 4 Buf^- mutants deficient in melanin biosynthesis. All strains differentiated appressoria in a similar manner. Mature appressoria were characterized by a pore, an area apparently devoid of cell wall, in the region of contact with the substrate. Two conspicuous effects of tricyclazole on appressoria were identified: changes in cell wall structure and increased appressorial pore size. This latter effect is reported here for the first time. The greatest increase in pore size, due to tricyclazole, was observed in strain P2 where the mean of 100 pore diameters (for each of treated and control samples) increased from 2.5 um to 5.7 um. However, a statistically significant increase occurred in every strain with the smallest increases among the Buf^- mutants. One possible interpretation of these results would support the idea that appressorium pore diameter is a critical factor in pathogenesis owing to the essential role of a mechanical component in the penetration process.

J55 ANALYSIS OF VARIATION IN BREMIA LACTUCAE. Scot H. Hulbert and Richard W. Michelmore, University of California, Davis, CA 95616, U.S.A.

Bremia lactucae is a diploid, Oomycete fungus which exhibits a clear gene-for-gene relationship with its host, Lactucae sativa (lettuce). In order to study the genomic organization and mechanisms of pathogenic variation, a detailed genetic map is being developed using restriction fragment length polymorphisms (RFLPs). Large numbers of RFLPs have been identified using genomic clones as probes. Cosegregation of RFLPs and virulence genes is being studied in sexual progenies.

Some lines of B. lactucae exhibit somatic instability of virulence phenotype. The genetic basis for these changes will be analyzed by examining the markers that flank the virulence genes responsible.

An extrachromosomal DNA element, approximately 1.2 Kb in size has been isolated from a satellite band on a CsCl gradient. Southern hybridization has demonstrated its presence in all of the six isolates of B. lactucae so far tested. The element has been cloned into pUC13. The cellular location and homologies with the nuclear or mitochondrial genomes is being investigated.

J56 CLASSICAL GENETICS OF LETTUCE DOWNY MILDEW. Terrence W. Ilott and Richard W. Michelmore, University of California, Davis, CA 95616, U.S.A.
Specificity in lettuce downy mildew (Lactuca sativa - Bremia lactucae) is conditioned by a gene-for-gene interaction. The L. sativa/B. lactucae system is one of the few plant-pathogen interactions in which both host and pathogen are amenable to fine genetic analysis. At least 22 interacting pairs of loci have been identified in host and pathogen. Resistance in the host and avirulence in the pathogen are determined by dominant alleles; hence it appears that incompatibility in this interaction is an active process involving the products of both the resistance and avirulence loci. A variety of interaction phenotypes occur, however, and genetic control of the interaction is likely to be more complex than a simple interpretation of the gene-for-gene theory would indicate.

In our present studies, genes in the pathogen have been demonstrated which modify or completely inhibit an avirulent response to a resistant host cultivar. Such effects may depend on the genetic background of the host or pathogen isolate. Incomplete dominance of avirulence genes has also been indicated. Crosses between pathogen isolates of different geographical origin have been used to investigate whether all genes determining virulence to a specific resistance gene map to the same locus; this has generally been assumed in gene-for-gene interactions but has rarely been rigorously tested. Crosses have also been made to investigate the possibility of linkage between avirulence genes in B. lactucae; several host resistance genes are tightly clustered. Understanding at the classical genetic level is a prerequisite to molecular studies of this interaction.

J57 DNA RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) ANALYSIS IN LETTUCE, Benoit S. Landry, Hei Leung and Richard W. Michelmore. University of California, Davis, CA 95616, USA.

A detailed genetic map of lettuce is being developed using RFLPs. DNA libraries from leaf cDNA or random genomic DNA selected for single or low copy sequences were constructed. Inserts were used as probes for polymorphism on nine restriction enzyme digests of four parental lines. The parental lines were chosen on the basis of their genetic distance calculated from isozyme studies. The relative frequency of polymorphism detected by each type of probe and each restriction enzymes was compared. The influence of CpG dimers in the recognition sequence on the frequency of polymorphisms was investigated. The segregation of the RFLPs has been extensively analyzed in a single cross to develop the linkage map. Linkage data to isozymes and disease resistance genes is being developed.

J58 SPECIES SPECIFIC SURFACE CARBOHYDRATE PATTERNS ON THE INFECTION STRUCTURES OF RUST FUNGI. Kurt Mendgen, Sibylle Freytag, Martina Lange and Karin Bretschneider, Universität Konstanz, Fakultät für Biologie, Lehrstuhl Phytopathologie, D 775o Konstanz, Fed. Rep. of Germany

The rust fungi develop a series of infection structures to infect the host plant: A germ tube that recognises the host cuticle, an appressorium that is located over the host stoma, an infection peg and a substomatal vesicle in the substomatal chamber of the plant. From this vesicle, infection hyphae develop to infect the leaf cells. The surface carbohydrates of these structures were probed with FITC-labelled enzymes and lectins. The amount of binding of the probes to the infection structures was measured with a microscope photometer. The germ tubes of all rust fungi have mainly chitin on their surface, whereas the structures of the rust fungi in the leaf, e.g. the substomatal vesicles and the infection hyphae, have mainly (1-3) ß-D-glucans on their surface. Minor amounts of other sugars were also detected. The amount of binding sites for the different carbohydrate probes was specific for each rust fungus tested: Puccinia graminis f. sp. tritici, Puccinia coronata f. sp. avenae, Uromyces appendiculatus and Uromyces fabae.

J59 DETOXIFICATION OF THE PHYTOALEXIN MAACKIAIN IS IMPORTANT FOR VIRULENCE OF NECTRIA HAEMATOCOCCA ON CHICKPEA, Vivian Miao, Matthew Lucy, and Hans VanEtten, Cornell University, Ithaca, NY 14853

Virulence of Nectria haematococca towards Pisum sativum appears to be dependent on its ability to detoxify the pea phytoalexin pisatin. Pisatin demethylation by this fungus is controlled by a family of independently acting PDA genes, possibly encoding a group of monooxygenase isozymes. N. haematococca is also a pathogen of chickpea, Cicer arietinum, and can detoxify the chickpea phytoalexins maackiain and medicarpin via apparent monooxygenase reactions. The role of phytoalexin degradation in this disease was examined by testing maackiain tolerance, medicarpin tolerance, maackiain hydroxylation and virulence towards chickpea in a collection of 120 N. haematococca field isolates, and in ascospore progeny derived from some of these. These four traits were strongly correlated, except that some phytoalexin-tolerant isolates were nonetheless low in virulence; thus detoxification of the chickpea phytoalexins may be required, but is not sufficient, for virulence on this host. In one cross all twelve tetrads examined showed a 1:1 segregation for maackiain metabolism, suggesting a single-gene difference in the parents. All the maackiain hydroxylating progeny of this cross were also able to demethylate pisatin and vice versa: these two reactions may be catalyzed by the same gene product or by two enzymes encoded by closely linked genes.

J60 THE PRODUCTION AND INVOLVEMENT OF ETHYLENE DURING THE DISEASE PROCESS OF ALTERNARIA STEM CANKER OF TOMATO, Vasiliana V. Moussatos and David G. Gilchrist, University of California, Davis, CA 95616

Treatment of F_9 isogenic AAL-toxin susceptible tomato leaves with AAL-toxin results in an increase in ethylene evolution 10-30 hours prior to the onset of AAL-toxin induced necrosis. Repression, inactivation or delay of this increase in ethylene evolution by AVG, an ACC synthase inhibitor, silver thiosulfate, an ethylene action inhibitor, or continuous exposure to 660nm light resulted in a repression or delay of necrosis induced by AAL-toxin. These data suggest a possible requirement for ethylene production and action in the Alternaria tomato stem canker disease process.

J61 **ANALYSIS OF RACE-SPECIFIC VARIATION IN FUSARIUM OXYSPORUM F. SP. PISI, Jens. B. Mullen, Donald J. Hagedorn, and Sally A. Leong, Department of Plant Pathology and USDA-ARS, University of Wisconsin, Madison, WI 53706.**

Races of Fusarium oxysporum f. sp. pisi are presently differentiated by the use of host cultivar differentials. We are currently exploring the development of alternative methods for identification of races 1, 2, 5, and 6 which do not require infection of the host. Data will be presented on isozyme patterns, 2-D PAGE patterns, and retriction fragment length polymorphisms of nuclear and mitochondrial DNA, demonstrating their utility in race differentiation.

J62 Double-stranded RNAs from hypovirulent strains of Endothia parasitica vary in banding pattern and homology, C.P. Paul and D.W. Fulbright, Michigan State University, E. Lansing, MI 48824.

Endothia parasitica causes chestnut blight on American and European chestnut. Hypovirulent strains of the fungus have been found on both continents. These strains are reduced in virulence when compared with virulent isolates. The presence of double-stranded RNA (dsRNA) in the fungus correlates with the hypovirulent phenotype. The hypovirulent phenotype and dsRNA can be transmitted to virulent strains through hyphal anastomosis. Hypovirulent strains have been used successfully to reduce pathogen pressure in Europe, while naturally occurring hypovirulent strains have allowed the survival of large chestnuts in Michigan.

Although there is a correlation between the presence of dsRNA and hypovirulence, the mechanism by which dsRNA may cause hypovirulence is unknown. DsRNA banding patterns on gels vary in number, size, and intensity of bands between strains. The dsRNA molecules from some American hypovirulent strains share sequence homology, while others do not. No homology has been found between American and European dsRNA. Studies of hypovirulence in Endothia may lead to insights into ways to reduce virulence in other fungi.

J63 ISOLATION AND PARTIAL CHARACTERIZATION OF TWO ANTIFUNGAL PROTEINS FROM BARLEY, Walden K. Roberts and Claude P. Selitrennikoff, University of Colorado Health Sciences Center, Denver, CO 80262

We have developed a simple assay for detecting antifungal compounds utilizing impregnated paper discs on agar to inhibit hyphal extension of an indicator organism, Trichoderma reesei. Using this assay we have isolated and purified to apparent homogeneity two antifungal proteins from barley grain. Both proteins are present at high concentrations: over 10 mg of each protein can be isolated per 100 g of seed. The first protein has a molecular weight of 30,000 and is identical to the 30 kDa ribosome-inactivating protein previously isolated from barley. This protein very effectively inactivates fungal ribosomes and this may explain its antifungal activity and biological role. The second antifungal protein has a molecular weight of 28,000 and is 20-fold more potent than the 30 kDa protein in inhibiting hyphal extension of Trichoderma (0.1 ug/disc of the 28 kDa protein is sufficient for inhibition). In addition to Trichoderma, the 28 kDa protein also efficiently inhibits growth of Phycomyces blakesleeanus, Alternaria alternaria and a protoplast-forming mutant of Neurospora crassa. The 28 kDa protein partially hydrolizes bacterial cell walls and crab chitin, suggesting that it is a chitinase. However, it differs from bacterial chitinase in its antifungal specificity.

J64 TRANSFORMATION AND MOLECULAR ANALYSIS OF THE FILAMENTOUS PLANT PATHOGEN COLLETOTRICHUM LINDEMUTHIANUM (GLOMERELLA CINGULATA), R. J. Rodriguez, G. Turgeon, and O. C. Yoder, Dept. Plant Pathology, Cornell University, Ithaca, NY 14853

A transformation system has been established for the filamentous fungus Colletotrichum lindemuthianum. This was accomplished using either of two selectable markers, the amdS gene of Aspergillus nidulans which encodes acetamidase and allows for growth on acetamide as the sole nitrogen source, or the hygB gene of E. coli, fused to a "putative promoter" isolated from cochliobolus heterostrophus, which encodes a phosphotransferase that permits growth in the presence of the antibiotic hygromycin. Protoplasts were generated with the digestive enzyme complex "novozyme 234" and were 90-95% viable. Transformation was performed by exposing protoplasts to plasmid DNA in the presence of calcium and 60% polyethylene glycol. Transformation occured by integration of plasmid DNA into the fungal genome producing phenotypes and genotypes which were mitotically stable on selective and nonselective media. Multiple copies of both amdS and hygB plasmids integrated into genomic DNA of all transformants tested. There was no evidence of autonomous replication. All single-conidial cultures derived from both amdS and hygB transformants had the respective transformant phenotype, indicating that the transformants were not heterokaryotic.

J65 PLASMID-LIKE DNAS FROM FUSARIUM SOLANI, D. A. Samac, S. A. Leong, USDA-ARS, University of Wisconsin, Madison, WI 53706

Two plasmid-like DNAs have been isolated from the pathogenic fungus Fusarium solani f. sp. cucurbitae race 1 (teleomorph: Nectria haematococca, mating population I). Of eleven strains examined, two contain plasmid-like DNAs (PL-DNAs) of approximately 8 and 10 k bp. Test crosses show both PL-DNAs are inherited maternally, hence they are probably associated with mitochondria. Exonuclease and restriction endonuclease digests indicate that the PL-DNAs are linear ds DNA molecules with blocked 5' termini. The PL-DNAs have a high degree of homology with the mitochondrial genome of race 1 isolates but less than 27% homology with the race 2 mitochondrial DNA. The PL-DNAs also show homology to a high molecular weight repeated nuclear sequence which is homologous to the nuclear rDNA repeat of Neurospora crassa. However, they do not share homology with either nuclear or mitochondrial-encoded rDNA of N. crassa or Podospora anserina, respectively. The presence of PL-DNAs does not significantly alter the pathogenicity or growth characteristics of the fungus.

J66 IN VITRO SELECTION FOR DISEASE RESISTANCE: INHERITANCE OF SELECTED PATHOTOXIN RESISTANCE TO RACE 2 FUSARIUM WILT IN TOMATO. Elias A. Shahin, Rosa Spivey and Louis DiNitto. ARCO PLANT CELL RESEARCH INSTITUTE, 6560 Trinity Court, Dublin, California and ARCO SEED CO. Brooks, Oregon 93705

Tomato plants resistant to the fungal pathogen, Fusarium oxysporum f. sp. lycopersicon race 2 have been obtained using in vitro selection techniques against pathotoxins. Protoplasts were isolated from cotyledonary tissue of tomato cv. "UC-82" (1), which is susceptible to Fusarium race 2. Protoplasts-derived cells were challenged with the pathotoxins. Plants regenerated from toxin-resistant calli were screened for resistance to the pathogen using Fusarium slurry inoculation technique. Seeds were collected from the surviving individuals, germinated and re-screened for resistance to the pathogen. Data obtained from this test showed a ratio of three resistance to one susceptible among S1 seedlings. Further analysis of the S2 progenies confirmed that the Fusarium-resistant plants were either homozygous or heterozygous dominant for the gene conferring the resistance. The nature of this single-dominant-gene type of resistance is under investigation. Interestingly, resistance to Fusarium race 2 is under the control of a single dominant gene (I-2), which was found in a hybrid between L. pimpinellifolium and L. esculentum (2).

(1) Elias A. Shahin. 1985. Totipotency of tomato protoplasts. Theor. Appl. Genet. 69:235-240.
(2) R.W. Stall and J.M. Walter. 1965. Selection and inheritance of resistance in tomato isolates of races 1 and 2 of the Fusarium wilt pathogen. Phytopath. 55:1213-1215.

J67 ERYSIPHE GRAMINIS MUTANTS WITH INCREASED VIRULENCE ON BARLEY. John E. Sherwood and Shauna C. Somerville. Michigan State Univ., E. Lansing, MI 48824

The interaction between Erysiphe graminis f. sp. hordei and barley (Hordeum vulgare L.), which leads to powdery mildew disease, is typical of host-parasite relations described by the "gene-for-gene" hypothesis. We are studying the specificty of this interaction by induction of pathogenicity mutants of E. graminis race CR3 capable of establishing a compatible relationship with barley line CI-16137, which is normally resistant to CR3. Pustules of race CR3 were mutagenized by exposing infected leaf segments of CI-16138 (susceptible congenic line of CI-16137) to ethyl methanesulfonate. Conidia were transferred to leaf sections of CI-16137 and pathogenic mutants were identified as those capable of establishing a pustule on the resistant host. Pustules with red conidia were found at the same frequency as pathogenicity mutants. Deposition of autofluorescent compounds indicative of the hypersensitive response in barley was delayed following inoculation of CI-16137 and CI-16138 with the mutant cultures. In addition, these mutants were tested for pathogenicity on 9 other congenic pairs of barley lines, each carrying a different disease reaction allele. No mutants lost the ability to infect susceptible barley lines while two of the mutants were able to infect previously resistant lines other than CI-16137.

J68 CONTROL OF PATHOGENICITY IN PYRICULARIA AND COLLETOTRICHUM BY BLOCKING POLYKETIDE MELANIN BIOSYNTHESIS, Hugh D. Sisler, Department of Botany, University of Maryland, College Park, MD 20742.

The polyketide pathway leading to fungal melanin biosynthesis plays a critical role in the pathogenicity of certain Pyricularia and Colletotrichum species. Chemical or genetic blocks in this pathway prior to the intermediate, vermelone, result in loss of ability of appressoria to synthesize black melanin as well as ability to penetrate plant epidermal walls. Melanin biosynthesis and penetration capacity can be restored by the addition of the intermediate, scytalone, to albino mutants which are blocked in melanin biosynthesis between acetate and 1,3,6,8-tetrahydroxynaphthalene. Melanin biosynthesis and penetration capacity of buff (tan) mutants or of wild type strains treated with specific melanin biosynthesis inhibitors such as tricyclazole, cannot be restored by addition of scytalone, but can be partially restored by addition of vermelone or 1,8-dihydroxynaphthalene. The latter two intermediates occur subsequent to the blocked site in the melanin biosynthetic pathway. Evidence suggests that infiltration of melanin into the appressorial wall or cross-linking of wall fibrils by a melanin precursor provide the wall architecture and rigidity necessary for epidermal penetration.

J69 STRUCTURE AND EXPRESSION OF A USTILAGO maydis GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE GENE. Timothy L. Smith and Sally A. Leong, USDA-ARS, University of Wisconsin--Madison. Madison, WI 53706

A gene encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been cloned from a Ustilago maydis genomic library by cross-hybridization with a cloned Saccharomyces cerevisiae GAPDH gene. A structural analysis of the gene and its regulatory signals will be presented. The response of GAPDH gene expression to different carbon sources will also be described. A chimeric selectable marker for use in transforming U. maydis is being constructed using the GAPDH promoter and terminator in conjunction with the hygromycin phosphotransferase gene.

J70 Transformation of filamentous fungi with the hygromycin B phosphotransferase gene Gillian Turgeon, Robert C. Garber and Olen C. Yoder, Department of Plant Pathology, Cornell University, Ithaca, NY 14853

We have developed a system for transformation of filamentous fungi using the prokaryotic hygromycin B phosphotransferase gene (hygB) fused to a fungal DNA sequence which enables the gene to function in fungal cells. We have used the technique to transform the maize pathogen Cochliobolus heterostrophus and several other previously untransformed filamentous Ascomycetes to resistance to the aminoglycoside antibiotic hygromycin B. We constructed a library of random 0.5-1.5 kb C. heterostrophus DNA fragments in a plasmid containing the hygB gene lacking translation initiation and transcription activation sequences. This "promoter library" was used to transform wild type C. heterostrophus protoplasts. Colonies resistant to hygromycin B arose at a low frequency. Southern hybridizations of DNA from resistant colonies showed the presence of chromosomally-integrated copies of the transforming plasmid. The transforming DNA was recovered, cloned in E. coli, and used to retransform C. heterostrophus. Resistant colonies arose at a frequency significantly higher than in the original transformation with the "promoter library". This procedure is attractive for use in genetically undeveloped organisms because it transforms wild type cells, eliminating the need to obtain mutants and transform by mutant complementation. In addition, we have shown that a single C. heterostrophus sequence placed upstream of the hygB gene enables the gene to function in a variety of filamentous fungi whose wild type cells are sensitive to hygromycin B.

J71 ISOLATION AND EXPRESSION OF A NECTRIA HAEMATOCOCCA GENE FOR PHYTOALEXIN DETOXIFICATION IN ASPERGILLUS NIDULANS, Klaus-M. Weltring, Patty S. Matthews, Gillian Turgeon, Olen C. Yoder, and Hans D. VanEtten, Cornell University, Ithaca, NY 14853

The PDA genes of Nectria haematococca confer the ability to demethylate the pea phytoalexin pisatin, tolerance to pisatin, and a potential for high virulence on pea. A genomic library of Nectria haematococca isolate T-9 (Pda+) was constructed in the cosmid vector pKBY2, containing the trpC gene of Aspergillus nidulans. This library was used to transform A. nidulans UCD1 (trpC⁻, argB⁻). Of 1250 Trp+ transformants screened, one transformant expressed the Pda+ phenotype. As in N. haematococca, the expression of the PDA gene in the transformant was glucose repressed. The rate of demethylation and the degree of pisatin tolerance were lower in the transformant than in N. haematococca T-9. The demethylation product was not further metabolized. The cosmid containing the PDA gene, designated pNhT9, was recovered by λ packaging of total DNA from the transformant. Upon transformation of A. nidulans UCD1 with pNhT9, 98% of the Trp+ transformants were Pda+. When Aspergillus was cotransformed with restriction enzyme digests of pNhT9 mixed with the plasmid pMT201, containing the Aspergillus argB gene as a selectable marker, 7 of 11 restriction enzymes tested gave rise to transformants with the Arg+/Pda+ phenotype, indicating that these enzymes do not cut in the PDA gene. Cotransformation with single fragments from these digests will be used to subclone the PDA gene from the 37 kb Nectria insert in pNhT9.

J72 PROLIFERATING CULTURES FROM SMUT GALLS, Herman Wenzler and Fred Meins,Jr., Friedrich Miescher-Institut, P.O. Box 2543, CH-4002 Basel, Switzerland

The smut fungus, Ustilago maydis, induces the abnormal proliferation and enlargement of maize cells. We inoculated maize plants with the fungus and found that gall tissues on the mature leaves proliferate in culture whereas surrounding tissues do not. Positions on the leaves where galls arise were mapped and the gall tissues were assayed for proliferative capacity in culture. Axenic cultures from the gall tissues can be established on a routine basis. This shows that the continued proliferation of gall cells does not require the presence of the inciting fungus. Immature cells at the leaf base also have the capacity for proliferation in culture. Therefore, depending upon the target of the fungus, galls could arise by the continued proliferation of immature cells or by the return of quiescent, mature cells to the proliferative state. To distinguish between these possibilities, we mapped the competence of tissues for proliferation in culture at the time of infection and the competence of the tissues for gall formation. We present evidence to favor the hypothesis that the fungus can induce the proliferation of mature cells.

J73 DELETION AND INSERTION EVENTS COMMON AMONG MUTANTS TO MALE FERTILITY AND TOXIN INSENSITIVITY IN T-CYTOPLASM MAIZE, Roger P. Wise (1), Alan G. Smith (1), Daryl R. Pring (2), and Burle G. Gengenbach (3), (1) Plant Pathology Department and (2) USDA-ARS, University of Florida, Gainesville, FL 32611, and (3) Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108.

An important approach in identifying the molecular basis of male sterility and disease toxin sensitivity in T-cytoplasm maize was obtained by the regeneration of plants from tissue culture. These plants displayed mutation to male fertility and insensitivity to two pathotoxins, Helminthosporium maydis race T-toxin and Phyllosticta maydis toxin. These traits are maternally inherited; analysis of chloroplast and mitochondrial DNAs revealed variation in mtDNA restriction patterns with no evidence of ctDNA variation. Eighteen of 19 lines examined have a 6.5 kb instead of a 6.7 kb XhoI mtDNA restriction fragment. One mutant, T-4, retains the intact 6.7 kb XhoI fragment, but is distinguishable from parental T mtDNA. A 188 bp AluI fragment in T that is internal to the 6.7 kb XhoI fragment is shifted to 194 bp in T-4. The alteration in T-4 is due to a five base pair insertion followed by a G→A transition in addition to a single C insertion six bases after the G→A transition. A more common mutant, T-7, which has a 6.5 kb instead of a 6.7 kb XhoI fragment, has undergone a 3 kb deletion. The insertion in T-4 occurs in sequences that are deleted in T-7. These sequences are in an area that is heavily transcribed. Transcription in this region is greatly reduced in T-7 or N; a normal, male-fertile cytoplasm. Transcription is unaltered in T-4 suggesting that an aberrant gene product(s) or loss of normal translational activity is associated with this mutant.

Bacterial Pathogens: Virulence Determinants and Mechanisms of Control

J74 ASSOCIATION OF HOST PLASMA MEMBRANE K^+ EFFLUX/H^+ INFLUX EXCHANGE WITH PATHOGENICITY OF PSEUDOMONAS SYRINGAE PV. SYRINGAE ON PHASEOLUS VULGARIS Merelee M. Atkinson and C. Jacyn Baker, Plant Pathology Lab., USDA, Beltsville, MD 20705.

Pseudomonas syringae pv. syringae causes bacterial brown spot disease on bean which is characterized by necrotic lesion formation on leaves with or without a chlorotic halo. We are investigating the molecular and genetic basis for pathogenicity of this bacterium. The present study involved P.s. pv. syringae Tn5 transposon mutants previously selected for the inability to induce the hypersensitive reaction and plasma membrane K^+ efflux/H^+ influx in tobacco. The K^+/H^+ response has been associated with hypersensitive cell death in tobacco cells inoculated with P.s. pv. pisi (Plant Physiol 79:843-847). When infiltrated into bean leaves over a range of $5x10^6 - 5x10^8$ viable bacteria/ml, wild type P.s.pv. syringae induced a strong but delayed K^+/H^+ response 6h or more after inoculation and multiplied rapidly. P.s. pv. pisi which is incompatible on beans also induced a strong K^+/H^+ response but this began at 1-2 hr after inoculation and the bacterium multiplied slowly. Mutant strains of P.s. pv. syringae ranged from moderately strong to no induction of the K^+/H^+ response with bacterial multiplication being proportional. These results suggest an association of host plasma membrane K^+ efflux/H^+ influx with both pathogenicity and incompatibility of P. syringae pathovars on bean. The rapidity with which the response is induced as well as its magnitude may be distinguishing characteristics of pathogenic, nonpathogenic and incompatible plant-bacterium interactions.

J75 TN5 MUTANTS OF PSEUDOMONAS SYRINGAE PV. SYRINGAE UNABLE TO INDUCE THE HYPERSENSITIVE RESPONSE OR STIMULATE K^+/H^+ EXCHANGE IN TOBACCO, C. Jacyn Baker, Plant Pathology Lab., USDA, Beltsville, MD 20705, Merelee M. Atkinson and Alan Collmer, Department of Botany, University of Maryland, College Park, MD 20742.

Stimulation of a net K^+ efflux/H^+ influx has been demonstrated to be closely associated with the hypersensitive response (HR) in tobacco (1985 Plant Physiol. 79:843). To further test this relationship, mutants of Pseudomonas syringae pv. syringae (P.s.s.) were tested for the lose of ability to induce the HR or stimulate K^+ efflux/H^+ influx. Mutants were constructed by using plasmid vector pGS9 to introduce transposon Tn5 into P.s.s. The streptomycin resistance phenotype of Tn5 was expressed in P.s.s. but not E. coli, permitting complete counterselection of both donor and recipient strains with streptomycin. Ability to induce the HR was assayed by infiltrating P.s.s. mutants, $5x10^7$ CFU/ml, into tobacco leaves. Stimulation of the K^+ efflux/H^+ influx was detected by incubating suspension-cultured tobacco cells with mutants at 10^8 CFU/ml for 18 hr and then testing for an increase in pH of the medium with a pH indicator. Colonies which failed to stimulate H^+ influx were assayed in a second assay which confirmed the absence of K^+ efflux from suspension cultured tobacco cells. All mutants which failed to induce the HR also failed to stimulate the K^+ efflux/H^+ influx and seven of these mutants were found to be prototrophic. One mutant with a single Tn5 insertion in a 17 kb. EcoRI fragment was selected for detailed study. The results from this study demonstrate a close relationship between K^+ efflux/H^+ influx and the HR.

J76 GENES ORGANISATION AT THE 3' END OF TOBACCO RATTLE VIRUS. Martine Boccara†, William Hamilton and David Baulcombe. Plant Breeding Institute Cambridge CB2 2LQ,England. †present adress INA P/G 16 rue Claude Bernard 75005 Paris France

To investigate the crop protection phenomena on plant viruses, we have undertaken a programm of introducing individual viral genes into plants. As a first step we are characterising the genes and their products of Tobacco Rattle Virus (TRV)RNA1 which carries the cross protection property.We have sequenced a cDNA clone of 2.2kb located in the 3' region of RNA1. We have identified three longppen reading frames on this sequence, two of which have significant homology with gene products in Tobacco Mosaic virus (TMV). The many similarities between TRV and TMV in viral morphology, gene organisation and sequence suggest that these two viruses share a common viral ancestor.

J77 INDOLE-3-ACETIC ACID SYNTHESIS BY AGROBACTERIUM TUMEFACIENS AND ITS ROLE IN CROWN GALL TUMOR FORMATION, Gerard A. Cangelosi, Noaman Hasan, Meii Lei Huang, and Eugene W. Nester, University of Washington, Seattle, WA 98195.

Agrobacterium tumefaciens synthesizes indole-3-acetic acid (IAA) from tryptophan via two pathways. One is a two step pathway specified by T-DNA (transferred DNA) genes on the Ti (tumor inducing) plasmid. Indoleacetamide is an intermediate. Mutations in these genes result in an altered tumor morphology. The second pathway is not specified by plasmid genes and is the predominant pathway for IAA production in free-living agrobacteria, since strains that are cured of the Ti plasmid synthesize as much IAA as the Ti plasmid-containing strain. Indolepyruvic acid is an intermediate in this pathway. α-keto acids are required for indolepyruvate and IAA formation in vitro, which suggests that the initial step in IAA synthesis via this pathway is the transamination of tryptophan. Mutants which synthesize less than 5% of the wild-type level of IAA were isolated after random mutagenesis of virulent bacteria with Tn5. These mutants do not differ from wild-type strains in the abilities to attach to freshly isolated Zinnia leaf mesophyll cells in vitro and to form tumors when inoculated onto wounded Kalanchoe leaves. Thus, a wild-type level of IAA production by free-living bacteria is not required for crown gall tumor formation.

J78 HEME BIOSYNTHESIS IN SOYBEAN NODULES, Barry K. Chelm, C. Robertson McClung, Prudence J. Hall, John E. Somerville and Mary Lou Guerinot, DOE-Plant Research Laboratory and Department of Microbiology, Michigan State University, East Lansing, MI 48824
The hemoprotein leghemoglobin is actively synthesized during the development of symbiotic nitrogen-fixing nodules. Other hemoproteins such as low oxygen bacterial cytochromes must also be synthesized. It is known that some of the bacterial enzymes responsible for heme biosynthesis are found at elevated levels in nodules. We have been studying the mechanism of this induction of bacterial heme biosynthesis and the importance of bacterial heme biosynthesis for nodule development and function. Utilizing a mutant strain of Bradyrhizobium japoniucm we find that the first committed step of heme biosynthesis, 5-aminolevulinic acid synthase, is non-essential for the induction of leghemoglobin containing, nitrogen-fixing nodules. This indicates that the exchange of heme, or intermediates in its synthetic pathway, can be exchanged from plant to bacteria. We will present data characterizing the phenotype of nodules incited by bacteria defective in heme biosynthesis and the regulation of the induction of bacterial heme biosynthesis during soybean nodule development.

J79 Dual control in the regulation of *Agrobacterium tumefaciens* Ti plasmid virulence gene expression. T.J. Close, R.C. Tait, J.A. Chimera, P. Rogowsky, T. Hirooka, J.J. Shaw and C.I. Kado. Department of Plant Pathology, University of California, Davis, CA, 95616.

A. tumefaciens carries a large plasmid (Ti) that is responsible for tumor induction on susceptible host plants. A region of the Ti plasmid contains virulence genes that are expressed within the bacterium. Many of these Vir genes are induced upon exposure of the bacterium to a plant wound environment. Using gene fusions constructed *in vitro* between several *vir* loci and marker genes for chloramphenicol acetyltransferase (*cat*) or bacterial bioluminescence (*lux*), we have determined that a dual regulatory control is involved in Vir gene expression. Corresponding to findings by other investigators, several *vir* loci are positively controlled by the pTiC58 *virG* locus, and respond to acetosyringone, which is abundant in the plant wound site. We have found also that a negative control is mediated by the *ros* locus on the bacterial chromosome. Mutations in *ros* alter the capsular material produced by the bacterium. The primary Vir region target of Ros repression seems to be the *virC* genes, which are homologous in nopaline and octopine type Ti plasmids. This evidence suggests that Ros signals a general physiological state within the bacterium, and VirG is keyed to specific compounds present in the plant wound site.

J80 MOLECULAR CLONING OF POTENTIAL PATHOGENICITY FACTORS FROM PSEUDOMONAS SOLANACEARUM
Timothy Denny and Mark Schell, University of Georgia, Athens, GA 30602.

Pectolytic and cellulolytic enzymes are likely to play a role in pathogenesis of the phytopathogenic bacterium Pseudomonas solanacearum. To isolate the genes that encode these potential pathogenicity factors we constructed a genomic library of P. solanacearum SM 178, a virulent race 3 strain, in E. coli JM 107 using the cosmid vector pLAFR3. The library was screened for E. coli clones expressing polygalacturonase genes (PG'ase) and for clones expressing cellulase genes (CMC'ase) using standard enzyme activity growth media. The cosmid pJE8, which contains a 30 kb insert, encodes two PG'ase isozymes (pI 8.9 and 8.1) that are found both intra- and extracellularly. The cloned pI 8.9 isozyme corresponds to the isozyme of major PG'ase activity excreted by SM 178, which also excretes isozymes with less activity that have pI's of 8.1, 7.6, and 4.7. One of the PG'ase genes has been subcloned on a 7.6 kb EcoRI fragment. The cosmid pHE3, which contains a 31 kb insert, encodes CMC'ase activity that is expressed intracellularly in E. coli. This CMC'ase activity is fully inhibited by antiserum prepared against a homogenous preparation of the major CMC'ase excreted by SM 178. Transfer of pHE3 into a spontaneous, avirulent, CMC'ase negative mutant of SM 178 restored wild type production of CMC'ase, but did not appear to increase virulence. A second CMC'ase gene on pHE3 was identified by subcloning and Tn5 mutagenesis. Preliminary evidence suggests that this second CMC'ase gene may share homology with the gene encoding the major CMC'ase of P. solanacearum.

J81 RHIZOBIUM MELILOTI NOD GENE PRODUCTS, Tom Egelhoff and Sharon Long, Stanford University, Stanford, Ca. 94305.

Rhizobium meliloti is a gram-negative bacterium that forms nitrogen-fixing nodules on the roots of Medicago sativa (alfalfa). Genetic mapping and DNA sequencing have indicated a cluster of four Rhizobium genes (nodDABC) which are involved in inducing the initiation of nodule development on the host plant (1,2). Mutations in nodA, B, or C abolish even the earliest detectable steps in nodule development, including root hair curling and induction of cortical cell divisions. NodD appears to be a regulatory gene, whose product is required for induction of nodABC by alfalfa root exudate (3). Protein products corresponding to all four genes have been identified in E. coli S30 and maxicell systems, and the nodA product has been purified to homogeneity from an overproducing E. coli strain and used to obtain antisera directed against this protein. Cell fractionation experiments, followed by western blotting, indicate that in wild type Rhizobium nodA is a membrane protein. Further experiments are in progress to localize the nodA product more precisely. 1) Jacobs et al. J. Bacteriol.(1984) 162:469-476. 2) Egelhoff et al. DNA (1985) 4:241-248. 3)Mulligan and Long P.N.A.S.(1985) 82:6609-6613.

J82 Biological Control of Crown Gall: Molecular Approachs to Improved Agrocin 84 Delivery Systems. S. K. Farrand, M. H. Ryder, J.-S. Shim and A. Kerr. Stritch Sch. Med., Maywood, IL 60153 and Waite Agr. Res. Instit., Glen Osmond, 5064, South Australia

Crown gall, induced by Agrobacterium tumefaciens, causes substantial losses to orchardists and ornamentalists. In 1972 a biological method to control this disease was introduced and found to be effective in many parts of the world. The method involves inoculation of plants with the avirulent A. radiobacter strain, K84. Strain K84 produces a fradulent adenine nucleotide, called agrocin 84, which selectively kills certain tumorigenic agrobacteria. Agrocin 84 production, and immunity to the agent, are encoded by a 48 kb plasmid, pAgK84. In at least one case, failure in control was linked to acquisition of pAgK84 by the virulent A. tumefaciens strains in the soil. Because these strains now express immunity, they are refractory to control. For this reason improved control strains should contain transfer-defective plasmids. Random Tn5 insertions allowed us to show that the plasmid is self conjugal, and to map agrocin production and conjugal transfer loci. The former occupies 20 kb and is divided into at least six transcription units, while Tra functions map to a 3.5 kb region. Replication and stability loci were mapped by deletion analysis and transposon rescue. Transfer-deficient insertion mutants were tested for their ability to control crown gall. In the biovar 2 host background they were as effective as the wild-type plasmid. Protection was much less effective when the control strains were of the biovar 1, NT-1 background. Results from recovery studies suggest that successful root colonization by the control strain plays an important role in effective biocontrol of the pathogen.

J83 THE ROLE OF IAA-LYSINE IN TRYPTOPHAN SECONDARY METABOLISM AND VIRULENCE OF PSEUDOMONAS SYRINGAE PV. SAVASTANOI. M. Louise Glass and Tsune Kosuge, Plant Pathology Department, University of California, Davis, CA 95616

The phytopathogen Pseudomonas syringae pv. savastanoi (P. savastanoi) incites the production of galls on olive and oleander plants. Gall formation is dependent upon the bacterial production of the phytohormone indoleacetic acid (IAA). Bacteria isolated from oleander plants are capable of further metabolizing IAA to 3-indole-acetyl- -L-lysine. IAA-lysine is not accumulated to an appreciable extent by olive isolates, although a fragment homologous to the oleander IAA-lysine synthetase gene is present. A 6.25kb Eco RI fragment bearing the IAA-lysine synthetase gene was cloned from an oleander isolate into pUC8 and subjected to TN5 mutagenesis. Eco RI fragments bearing TN5 inserts were ligated into the wide host range vector pLAFR3 and conjugated into P. savastanoi by a tri-parental mating system. IAA-lysine synthesizing capacity was restored to wild-type levels when constructs with TN5 inserts on either side of the IAA-lysine synthetase gene were introduced into P. savastanoi lacking IAA-lysine synthetase activity. A TN5 insert within the IAA-lysine synthetase gene had no activity when conjugated into P. savastanoi. The conversion of IAA to IAA-lysine may help to regulate IAA pool size in the bacterium, and therefore, may modulate virulence as assayed by gall size.

J84 MOLECULAR BASIS OF HOST RANGE IN AGROBACTERIUM TUMEFACIENS. Bernard Leroux, Martin Yanofsky, Steven Ziegler and Eugene Nester, University of Washington, Seattle, WA 98195

The factors contributing to the host specificity of a tumor-inducing plasmid of Agrobacterium, pTiAg162 which confers a narrow host range, have been investigated. Determinants both within the T-DNA and virulence regions contribute to host specificity. Within the T-DNA a defective cytokinin biosynthetic gene limits host range. Nucleotide sequence analysis revealed a large deletion in the 5' coding region of this gene when compared with the homologous gene from the wide host range tumor-inducing plasmid, pTiA6. Introduction of the wide host range cytokinin biosynthetic gene into the T-DNA of the limited host range strain expanded the host range and suppressed the rooty morphology of tumors incited by the limited host range strain. Two genes from the virulence region of the wide host range plasmid, designated virA and virC, must also be introduced into the limited host range strain in order to restore a wide host range phenotype. Although no significant homology can be detected between the WHR virA and virC loci and the LHR plasmid, it is possible to complement WHR virA mutants with a region of the LHR plasmid. The LHR virA locus has been localized by insertion mutagenesis and complementation on Nicotiana glauca leaf discs. Its nucleotide sequence will be presented and compared to WHR virA locus.

J85 EXPRESSION OF PSEUDOMONAS SYRINGAE PATHOGENICITY GENES IN ESCHERICHIA COLI MAXI CELLS. Dallice Mills, Frank Niepold and Mohammed Zuber*, Department of Botany and Plant Pathology and Laboratory for Nitrogen Fixation.* Oregon State University, Corvallis, OR 97331

A pathogenicity locus has been cloned by complementing a Tn5-induced Path strain, PS9021, using a cosmid library constructed of wild type DNA from Pseudomonas syringae pv. syringae, the causal agent of brown spot disease of bean. An 8.5kb segment from the cosmid is required to restore symptoms by complementation. Site-directed Tn5-mutagenesis of this region followed by marker-exchange of 13 independent insertions into the wild type strain, produced a series of mutants that were used in bioassays to map determinants of pathogenicity. At least two regions were identified within the 8.5 Kb segment that encode products important in disease expression. One region corresponds to the site of insertion of Tn5 in PS9021, and encodes a protein of approximately 85Kd from a transcriptional fusion vector, pMZ545, in Escherchia coli maxi cells. A second region identified by Tn5 insertion that affects the rate of symptom development, encodes a protein of approximately 79Kd. The role of these proteins in disease expression is currently under investigation.

J86 PLASMID-CHROMOSOME RECOMBINATION IN PSEUDOMONAS, Marilyn Ehrenshaft, Christopher Small, Janet Williams and Dallice Mills. Department of Botany and Plant Pathology. Oregon State University, Corvallis, OR 97331.
Strain LR700 of Pseudomonas syringae pv. phaseolicola, the etiologic agent of halo blight disease of bean, harbors an indigenous 150 Kb cryptic plasmid, pMMC7105. This plasmid has been observed to integrate into and imprecisely excise from the host chromosome by a process that leads to the formation of a variety of new plasmids. Repetitive sequences (RS) found on pMMC7105 and the chromosome, as well as a repetitive sequence that is present only on pMMC7105, act as sites for recombination. One of these sequences, RSII, present on both replicons, has been demonstrated to be involved in both integration and excision of pMMC7105. Restriction endonuclease mapping and Southern hybridization analysis have shown that it is highly conserved on five unique BamHI fragments and that its contour length is approximately 1200±300 nucleotides. Two other repetitive sequences, designated RSI and RSIII have been shown to act as sites only for recombination of plasmid DNA sequences, a process that leads to the formation of plasmids that are always smaller than pMMC7105. RSI appears to be less than 0.6Kb in size, whereas RSIII appears to be smaller than 2.0Kb. Nucleotide sequence analysis is in progress to determine whether these repetitive sequences conform to the properties of insertion sequence elements.

J87 A plant cell wall moiety inhibits A. tumefaciens binding and tumor formation. Nicole Neff and Christine Brandt, Dept. of Biology, University of Pennsylvania, Philadelphia, PA 19104

A pectin-enriched soluble cell wall fraction (CWF) prepared from suspension cultured tomato cells inhibits binding of A. tumefaciens to these cells (Plant Physiol. 77 35-42, 1985). On the basis of these and additional results it has been hypothesized that the CWF contains the plant surface binding site for A. tumefaciens. To demonstrate the physiological relevance of the the CWF to tumor formation, we examed its ability to inhibit tumor formation on potato slices using the quantitative assay of Pueppke and Benny (Physiol. Plant Path 18 169-179, 1981). When A. tumefaciens are incubated with the tomato CWF, subsequent tumor formation on potato slices is inhibited in a dose-dependent fashion. Furthermore, tumor formation and binding are inhibited to equal extents indicating that these two processes are directly related and that the attachment moiety is not species specific. These results and the fact that the CWF has no effect on A.tumefaciens viability strongly suggest that the inhibition of tumor formation is not due to a non-specific or toxic interaction of the CWF in the assay. Inactivation of the CWF by boiling reduces both its binding and tumor inbhibitory activities. Results from 2 types of experiments indicate that A. tumefaciens bind to a moiety in the CWF. Increasing numbers of bacteria saturate out the tumor inhibitory activity of CWF with no inhibition apparent at 1×10^8 bacteria/ml. Secondly, CWF inhibition of tumor formation can be eliminated or substantially reduced by prior incubation of the factor with A. tumefaciens. In summary, results on the tumor and binding inhibitory activity of the pectin-enriched CWF indicate that 1) a tomato cell wall fraction is enriched for the putative A. tumefaciens binding site, and 2) bacterial binding to this moiety is a pre-requisite to tumor formation.

J88 CLONING AND CHARACERIZATION OF THE INDOLEACETIC ACID OPERON OF PSEUDOMONAS SAVASTANOI, Curtis J. Palm and Tsune Kosuge, Department of Plant Pathology, University of California, Davis, CA 95616
Indoleacetic acid (IAA) production by the plant pathogen Pseudomonas savastanoi is essential for tumor formation on olive and oleander. In the bacterium, IAA is synthesized from tryptophan in reactions catalyzed by tryptophan monooxygenase and indoleacetamide hydrolase. In oleander isolates of the bacterium the IAA genes are located on a plasmid, pIAA. This work describes the construction and characterization of recombinant plasmids which contain the genes for IAA biosynthesis. Both genes are located within a 4 kb fragment of pIAA 1 DNA. E. coli strains carrying these recombinant plasmids excrete IAA into the culture media and crude cell extracts have both tryptophan monooxygenase and indoleacetamide hydrolase activity. In vitro coupled transcription-translation of plasmid DNA shows that this cloned fragment codes for proteins of 62 and 47 kd which correspond to tryptophan monooxygenase and indoleacetamide hydrolase. Insertion of a kanamycin resistance gene cartridge in the tryptophan monooxygenase gene has a polar effect on indoleacetamide hydrolase activity. This evidence, along with sequence data of these clones (Yamada,T., C.J.Palm, B.Brooks and T.Kosuge. 1985. Proc. Natl. Acad. Sci. USA 82:6522-6526), indicates that these genes are transcribed as an operon.

J89 PROTECTION OF SOYBEANS AGAINST DISEASE BY A RHIZOPLANE BACTERIUM. Jack D. Paxton, Department of Plant Pathology, University of Illinois, Urbana, IL 61801

An *Erwinia spp.* isolated from soybean roots has been used as a seed protectant for soybeans. In six years of field trials this bacterium has increased yields an average of 10%. Root weights of plants growing in pathogen-infested soil are increased on seed-treated plants. The bacterium appears to be an excellent colonist of soybean roots and may prevent colonization by pathogens such as *Phytophthora megasperma f.sp. glycinea*. Labeling the bacterium with markers such as rifampicin and naldixic acid resistance, has established it's ability to move from the treated seed at planting time to the root system. The bacterium will inhibit the growth of *Phytophthora megasperma f. sp. glycinea* in vitro, but it is not clear at this time that this has any role in plant protection. A patent on this organism is now being applied for by University Patents Inc.

J90 EFFICIENT T-DNA TRANSMISSION BY AGROBACTERIUM TUMEFACIENS REQUIRES TWO DISCRETE SEQUENCES: THE T-DNA RIGHT BORDER REPEAT AND OVERDRIVE, A T-DNA TRANSMISSION ENHANCER, Walt Ream, Ernest Peralta and Renate Hellmiss, Indiana University, Bloomington, IN 47405

T-DNA integration during crown gall tumorigenesis requires the right hand copy of the 23 bp direct repeats that occur at T-DNA ends. Several different border repeats exhibit similar activity. Our results also show sequences outside the border repeat stimulate its function substantially (Peralta & Ream, PNAS 82: 5112-16, 1985). Sequences normally lying within 40 bp right of the border repeat fully enhance its function. The sequence 5´-TAAPuTPy-CTGTPuT-TGTTTGTTTG-3´ lies within 40 bp right of two different T-DNA right borders. A deletion removing 15 bp from the 3´ end of this sequence destroys its ability to stimulate T-DNA transmission. We call this sequence OVERDRIVE because it puts T-DNA transmission in high gear. We synthesized the OVERDRIVE sequence and placed it 4 bp to the right of a synthetic pTiT37 right border repeat. The OVERDRIVE oligonucleotide greatly stimulates the ability of the border repeat to promote T-DNA transmission. Thus, the exact distance and sequence between a border repeat and OVERDRIVE can vary without affecting T-DNA transmission.

J91 COMPARATIVE STUDY OF TWO *AGROBACTERIUM RHIZOGENES* T-REGIONS. François Richaud, Françoise Boulanger, Arlette Combard, Claire Estramareix, Pascal Ratet and Oscar Reyes. Université Paris-Sud, Institut de Microbiologie, 91405 ORSAY, FRANCE

Agrobacterium rhizogenes induces root formation at the wound site of inoculation in plants and inserts fragments (T-DNA)of its plasmid (Ri) into the plant nuclear DNA. Two A. *rhizogenes* of biotype 2 have been studied: the agropine type strain A4 and the mannopine type strain 8196. The transformed plant cells obtained after infection with the A4 strain harbors two T-DNA (T_L and T_R). Homology has been shown to exist between the T_R and the *tms* (responsible for auxin synthesis) region of A. *tumefaciens*. However the 8196 strain T-DNA has been found partially homologous only to the T_L-DNA of strain A4.

We have mutagenized the T-DNA of the two strains using two transposable elements derived from the Mu phage. Nononcogenic mutants were obtained as single insertion mutant in strain 3196 while only double insertion mutant in strain A4 did confer the same phenotype.

A comparative study of our results will be presented.

J92 CYTOKININ PRODUCTION BY PSEUDOMONAS SAVASTANOI. Frank Roberto and Tsune Kosuge, Department of Plant Pathology, University of California, Davis, CA 95616

We have recently examined cytokinin production in a number of virulent and avirulent strains of Pseudomonas syringae pv. savastanoi (P. savastanoi), a phytopathogenic bacterium able to induce hyperplasias on olive and oleander plants.

The cytokinins trans-zeatin, ribosylzeatin, isopentenyladenine and isopentenyladenosine accumulated in culture filtrates at levels as high as 0.5 mg/l. Little or no trans-zeatin or ribosylzeatin was detected in avirulent strains.

Examination of avirulent, non-zeatin secreting mutants of an Italian isolate, PB213, which normally harbors 5 cryptic plasmids ranging in size from 33 to 73 kb, revealed that each mutant lacked a 41 kb plasmid, distinct from the plasmid bearing genes for indole-3-acetic acid (IAA) metabolism.

Southern blot hybridization analysis of PB213 wild type and mutant strains with a probe for P. savastanoi zeatin secretion genes (obtained in collaboration with R.O. Morris, Oregon State Univ.) showed hybridization with the same 41 kb plasmid in the wild type, and no detectable hybridization in mutant strains.

These data strongly suggest that cytokinins, in concert with IAA, act as virulence factors in the association of P. savastanoi with the host.

J93 REGULATION OF THE EX PLANTA EXPRESSION OF R. MELILOTI NITROGEN FIXATION (NIF) GENES, Wynne W. Szeto and Frederick M. Ausubel, Dept. Mol. Biol., MGH, Boston, MA 02114.

The nfrC gene from the symbiotic nitrogen-fixing procaryote Rhizobium meliloti was isolated. Growth characteristics of R. meliloti ntrC mutants indicate that the RmntrC product, like its counterpart in enteric bacteria, is required for the expression of a variety of nitrogen utilization pathways. The transcription of RmntrC is activated when the cells are grown in nitrogen-limiting media. Although nitrogen fixation, as measured by the acetylene reduction assay, cannot be detected in free-living R. meliloti under conditions where ntrC is activated, the ntrC product induces the ex-planta transcription of several R. meliloti nif genes, including a nif-specific transcriptional activator, nifA. In contrast to free-living cells, the ntrC product is not required for nif-gene expression during symbiotic nitrogen fixation and nodules elicited by ntrC mutants (ntrC⁻ nodules) are Fix⁺. Despite the Fix⁺ phenotype of RmntrC mutants, the ntrC product probably plays a role in the normal establishment of symbiotic nitrogen fixation because the development of full nitrogen fixing activity in ntrC⁻ nodules is delayed compared to wild type. The experiments reported here demonstrate that R. meliloti has separate regulatory pathways for activating nif-gene expression ex planta and during symbiotic nitrogen fixation.

J94 EXPRESSION OF GLUTAMINE SYNTHETASE IN LEAVES, ROOTS AND NODULES OF PEA, Scott V. Tingey Elsbeth L. Walker and Gloria Coruzzi, The Rockefeller University, New York, NY 10021.

Nitrogen-fixing root nodules provide a novel system in which to study bacterial-plant interactions. There is a delicate interaction between the host and the invading bacteria which leads to the induction of several host specific proteins (nodulins). One of these nodulins, glutamine synthetase (GS), is responsible for the primary assimilation of ammonia produced by nitrogen fixing bacteroids. We have recently isolated cDNA clones encoding GS from leaf, root and nodule cDNA libraries of P. sativum. Northern blot analysis has shown the presence in these tissues of a poly(A)-RNA 1500 bp long, which is present at 10-20 fold higher concentrations during nodule formation. Western blot analysis of two dimensional gels has identified three isoforms of GS, each present at different relative levels in the leaf, root and nodule. Southern blot analysis and DNA sequence analysis has shown the presence of at lease two genes encoding GS in pea. Present studies are directed towards isolating the genes encoding these various mRNAs, and identifying DNA sequence elements responsible for the induction of GS mRNA in developing nodules.

J95 TRANSPOSABLE ELEMENTS OCCURING IN IAA OPERON IN PSEUDOMONAS SAVASTANOI, Tetsuji Yamada, Pin-Du Lee and Tsune Kosuge, University of California, Davis, Davis, CA 95616

Two types of transposable elements, IS51 and IS52, were found that spontaneously insert into and inactivate iaaM which results in the loss of indoleacetic acid production, attenuation of virulence, and the loss of activities of the enzymes coded by iaaM and iaaH in Pseudomonas savastanoi. The nucleotide sequences of both IS elements have structural features common to other prokaryotic IS elements. IS51 is 1,311 base pairs (bp) long and has terminal inverted repeats of 26 bp. IS52 is 1,209 bp long and has terminal inverted repeats of 10 bp with a 1 bp mismatch. In insertion involving IS51, the trinucleotides sequence, CAG, is duplicated within iaaM sequences at the recombination junction; in those involving IS52 the tetranucleotides sequences, TTAG or CTAG, are duplicated within iaaM sequences at the recombination junction. No nucleotide sequence homology was found between IS51 and IS52.

Plant Viruses

J96 PARTIAL PURIFICATION OF CITRUS TRISTEZA VIRUS BY IMMUNOPRECIPITATION. Roger A. Acey and Christopher M. Smith, Department of Chemistry, California State University,Long Beach, Long Beach, CA 90840.

With today's rapidly increasing world population and subsequent demand on agricultural production, it is imperative that plant viruses and their diseases be studied extensively in an attempt to develop suitable control measures. One of the most important citrus viruses in California is citrus tristeza virus(CTV). Bark tissue from healthy and CTV infected trees (as judged by ELISA) was homogenized in 100mM Tris, 0.2% PVP, 0.1mM PMSF, pH 7.4, using a mortar and pestle. The homogenate was filtered through cheese cloth and centrifuged at 30,000xg for 30 minutes. Supernatants were incubated with increasing amounts of purified goat anti-CTV IgG and allowed to stand overnight at 4°C. Precipitates were collected, layered over buffered sucrose containing 150 mM NaCl and 1% Triton X-100, and centrifuged at 10,000xg for 10 minutes. Precipitates were washed with 20 mM Tris, 150 mM NaCl, pH 7.6, and dissolved in 1% SDS, 700 mM mercaptoethanol, 1 mM EDTA, 1 mM Tris, pH 8.1. Proteins were fractionated on 8-16% gradient SDS polyacrylamide gels and visualized by staining with silver. Several polypeptides ranging in size from 18 to 45 Kd were evident in diseased tissue. Extracts from healthy plants were completely devoid of these proteins. After immunoprecipitation of CTV, supernatants were assayed for CTV by ELISA and found to be negative. This study was supported by a contract from the California Department of Food and Agriculture.

J97 MOLECULAR GENETIC ANALYSIS OF REPLICATION SIGNALS IN BROME MOSAIC VIRUS. Roy French and Paul Ahlquist, Biophysics Laboratory and Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin 53706.

Brome mosaic virus (BMV) is a virus of grasses, including cereal crops, whose genome is divided among three single stranded messenger-sense RNAs. Appropriate full-length cDNA clones of the three BMV genomic RNAs are infectious following in vitro transcription, allowing use of recombinant DNA technology to engineer changes into the RNA genome of BMV for a variety of experimental purposes. For example, the BMV coat protein gene has been replaced with a bacterial chloramphenicol acetyl transferase (CAT) gene, yielding virus derivatives which replicate to high copy number and direct efficient expression of active CAT gene product in transfected monocot cells.

BMV RNA replication in protoplasts requires only the two largest viral RNAs (RNA1 and RNA2), suggesting that the smallest BMV component, RNA3, is replicated solely because it contains sequences recognized by the replication machinery induced by RNA1 and RNA2. The nature of such cis-acting replication signals in RNA3 has been investigated in vivo by systematic deletion analysis. Accumulation of RNA3 in inoculated protoplasts is not affected by removal of either of the two RNA3 coding regions. Surprisingly, in addition to expected requirements for terminal or near-terminal portions of the 5' and 3' noncoding sequences, RNA3 accumulation has a strong requirement for a 95-152 base portion of the intercistronic region separating the two coding sequences. Some of the required terminal sequences are involved in initiation of (-) strand RNA synthesis, and others may function in (+) strand initiation and/or RNA stability. The role of the required intercistronic sequence is under investigation.

J98 EXPRESSION OF GENES ENCODING THE TMV COAT PROTEIN AND 30 KD PROTEIN IN TRANSFORMED PLANTS. Roger N. Beachy, Patricia P. Abel, Richard S. Nelson, Carl M. Deom, Melvin J. Oliver, Philip Dube, Steven G. Rogers, and Robert T. Fraley, Department of Biology, Washington University, St. Louis, MO 63130, and Monsanto Company, St. Louis, MO 63017

Chimeric genes containing cDNAs encoding either the coat protein or the 30 kd protein of tobaccco mosaic virus, and one of several different transcriptional promoters (CaMV 35S, and 19S promoters, and petunia SSU promoter) were produced. Chimeric genes were introduced into tobacco leaf disc cells using disarmed Ti-plasmid and plants were regenerated. Multiple, independently transformed plants were selected by resistance to kanamycin, and the amount of TMV-related mRNA produced from the chimeric genes was assessed. The results indicated that the amount of mRNA that accumulates depends upon the promoter used; however, there were differences of 5 to 10 fold between transformants containing the same chimeric gene. The amount of coat protein in transformed plants reached the level of 0.1% of soluble cell protein. The 30 kd protein has yet to be detected in transformed plants. The transformed plants are being used to study the phenomenon of cross protection and to study virus mutants containing identified genetic lesions.

J99 GENES ORGANISATION AT THE 3' END OF TOBACCO RATTLE VIRUS. Martine Boccara†, William Hamilton and David Baulcombe. Plant Breeding Institute Cambridge CB2 2LQ, England. †present adress INA P/G 16 rue Claude Bernard 75005 Paris France

To investigate the crop protection phenomena on plant viruses, we have undertaken a programm of introducing individual viral genes into plants. As a first step we are characterising the genes and their products of Tobacco Rattle Virus (TRV) RNA1 which carries the cross protection property. We have sequenced a cDNA clone of 2.2kb located in the 3' region of RNA1. We have identified three long open reading frames on this sequence, two of which have significant homology with gene products in Tobacco Mosaic virus (TMV). The many similarities between TRV and TMV in viral morphology, gene organisation and sequence suggest that these two viruses share a common viral ancestor.

J100 STUDIES ON THE REPLICATION OF GEMINIVIRUSES AND CONSTRUCTION OF GENE VECTORS, Robert H.A. Coutts and Kenneth W. Buck, Imperial College, London, U.K.

A detailed study of the potential of geminiviral DNA in the construction of transient and integrated gene expression vectors has been initiated. Investigations on the replication of tomato golden mosaic virus (TGMV) DNA in whole plants, protoplasts and isolated nuclei indicates that a number of approaches are available to study transient gene expression. Studies on the deletion and replacement of viral genes with selectable chimeric DNA sequences in one or both of the genomic components of TGMV DNA will be reported. A helper system based on using both genomic components in combination with a defective sub-genomic DNA species derived from the smaller component will also be described, with specific reference to replication and encapsidation of the defective DNA and the expression of selectable chimeric DNA inserted into clones of this DNA.

J101 ISOLATION AND CHARACTERIZATION OF HYPERSENSITIVE RESPONSE-RELATED MESSENGER RNA FROM TMV-INFECTED TOBACCO, David D. Dunigan, Thomas E. Smart, Kenneth A. Gabard and Milton Zaitlin, Cornell University, Ithaca, NY 14853.

The protein products produced as a consequence of the hypersensitive response (HSR) of Nicotiana tabacum L. cv. Xanthi nc (genotype NN) to tobacco mosaic virus (TMV) infection have been investigated and compared to those of mock-infected Xanthi nc and TMV-infected Turkish Samsun (genotype nn) plants. Expression of the HSR in Xanthi nc is controlled by the single dominant N gene and is temperature sensitive: a systemic infection occurs at the non-permissive temperature of 31°C, whereas necrotic hypersensitivity occurs at the permissive temperature of 25°C. TMV-infected Xanthi nc grown at 31°C for 3 days post inoculation shows necrosis 8 hours after a temperature shift to 25°C, thereby enabling experimental regulation of the HSR. Both polyribosomal and total cytoplasmic poly (A) containing mRNAs were isolated from the top, middle and bottom leaves of infected and mock-infected Xanthi nc, and infected Turkish Samsun plants at various times following the temperature shift. The mRNAs were translated in vitro and the products analyzed by 1- and 2-dimensional PAGE to provide a preliminary identification of the leaf and temporal distribution of transcriptional and translational changes occurring during the onset of the HSR. At least five unique in vitro translation products have been observed for both the polyribosomal and total cytoplasmic poly(A)-containing mRNA derived only from hypersensitively-responding Xanthi nc, thus indicating de novo synthesis of their corresponding mRNAs. Neither the mock-infected Xanthi nc nor the TMV-infected Turkish Samsun plants show any symptoms of the HSR and the unique translation products were not observed.

J102 MOLECULAR GENETIC ANALYSIS OF REPLICATION SIGNALS IN BROME MOSAIC VIRUS. Roy French and Paul Ahlquist, Biophysics Laboratory and Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin 53706.

Brome mosaic virus (BMV) is a virus of grasses, including cereal crops, whose genome is divided among three single stranded messenger-sense RNAs. Appropriate full-length cDNA clones of the three BMV genomic RNAs are infectious following in vitro transcription, allowing use of recombinant DNA technology to engineer changes into the RNA genome of BMV for a variety of experimental purposes. For example, the BMV coat protein gene has been replaced with a bacterial chloramphenicol acetyl transferase (CAT) gene, yielding virus derivatives which replicate to high copy number and direct efficient expression of active CAT gene product in transfected monocot cells.

BMV RNA replication in protoplasts requires only the two largest viral RNAs (RNA1 and RNA2), suggesting that the smallest BMV component, RNA3, is replicated solely because it contains sequences recognized by the replication machinery induced by RNA1 and RNA2. The nature of such cis-acting replication signals in RNA3 has been investigated in vivo by systematic deletion analysis. Accumulation of RNA3 in inoculated protoplasts is not affected by removal of either of the two RNA3 coding regions. Surprisingly, in addition to expected requirements for terminal or near-terminal portions of the 5' and 3' noncoding sequences, RNA3 accumulation has a strong requirement for a 95-152 base portion of the intercistronic region separating the two coding sequences. Some of the required terminal sequences are involved in initiation of (-) strand RNA synthesis, and others may function in (+) strand initiation and/or RNA stability. The role of the required intercistronic sequence is under investigation.

J103 EXPRESSION OF Qβ BACTERIOPHAGE COAT PROTEIN BY E. COLI CONFERS A HIGH LEVEL OF RESISTANCE TO Qβ INFECTION. Rebecca Grumet and Stephen A. Johnston, Duke University, Durham, NC 27706 and John C. Stanford, Cornell Univ. Expt. Sta., Geneva NY 14556

Pathogen-derived resistance is a new approach to creating disease-resistance; genes from the pathogen are expressed by the host in a way that is detrimental to the invading pathogen. We demonstrate one example of this approach using a model host-parasite system of the bacteriophage Qβ, and its host E. coli. E. coli strains JM103 and GM1 were transformed with a high copy number plasmid (pGL101) containing the coding sequence for the Qβ coat protein fused to the lacZ promoter. Bacteria containing this plasmid are fully resistant to infection by Qβ at levels that completely lyse a lawn of non-transformed E. coli. This resistance can be overcome at high levels of infection. In contrast, JM103 or GM1 strains transformed with pGL101 plasmid that has a deletion of the coat protein sequence are as susceptible to Qβ infection as non-transformed bacteria. Growth rates of the strains with the two types of plasmids were comparable. Other bacteriophages were tested to determine if the Qβ coat protein could confer cross-protection. Qβ coat protein appears to produce partial resistance to the ss F-specific RNA phage f2, the ss DNA phage ST-1, and the ds DNA phage P1, but not to the ss F-specific DNA phage f1. The potential advantages of pathogen-derived resistance over conventional host-derived resistance will be discussed.

J104 TMV-INDUCED GENE EXPRESSION IN TOBACCO. Jeannine Horowitz and Robert B. Goldberg, University of California, Los Angeles, California 90024.

We have been investigating the effect of viral infection on plant gene expression. We are utilizing tobacco plants as a model system to dissect the molecular events controlling pathogen-induced gene expression. Innoculation of *Samsun NN* plants with TMV results in the formation of localized necrotic lesions at the infection sites and "resistance" to subsequent infection in uninnoculated leaves. Induced resistance is dependent upon the *N* gene because plants homozygous for the recessive *n* allele fail to show "resistance" and systemically respond to TMV infection. cDNA libraries were constructed for *Samsun NN* healthy, three-day infected, and three-day resistant leaves. Diffferential colony hybridization was used to identify cDNA clones reperesenting mRNAs induced by TMV infection. Selected cDNA clones were hybridized with gel blots containing *Samsun NN* healthy, infected, resistant, mock-infected, and wounded leaf mRNAs. In addition, gel blot studies were carried out with *Samsun nn* healthy and sytemically infected mRNAs, as well as with root mRNA from *Samsun NN* plants. No consistent hybridization pattern was observed; however, one clone (INF4) was shown to correspond to a message representing at least 0.1% of infected and resistant mRNA populations. INF4 mRNA was approximately 4-fold less prevalent in heavily wounded leaves, and at least 100-fold less prevalent in mock-infected leaves, roots from infected plants, and systemically infected *nn* leaves. We further showed that INF4 reacted with similar-sized mRNAs in healthy *NN* and *nn* leaves, but that these mRNAs were at least 100-fold less prevalent that that observed in infected and resistant NN leaves. Hybridization of INF4 and other cDNA clones with *NN* and *nn* DNAs produced identical gel blot patterns. Together, our findings suggest that the presence of the *N* gene enables weakly-expressed, pre-existing genes to be induced specifically in the leaf in response to TMV infection. Research supported by a USDA grant.

J105 REPLICASE AND REPLICATION OF CAULIFLOWER MOSAIC VIRUS, Joh-E Ikeda, Hiroshi Takatsuji and Hirohiko Hirochika, Natl. Inst. of Agrobiological Resources, Tsukuba Science City, Ibaraki 305, Japan

Cauliflower mosaic virus (CaMV) is one of a small number of plant DNA virus containing double stranded DNA, which is about 8 kb long. Recently, it has been reported that CaMV and hepatitis B viruses as well as the Drosophila copia-like element and the yeast TY element replicate through RNA intermediate with the help of reverse transcriptase. However, no afirmative evidence has been obtained to show the direct involvement of reverse transcriptase as a replicative enzyme in CaMV DNA replication. We have succeeded to clone and express the CaMV-ORF V gene which codes for a putative revese transcriptase in active form in yeast cells. To obtain the direct expression of the ORF V gene in yeast cells, we constructed the plasmid pAM.ORFV in which the entire ORF V was inserted to the immediately downstream from the acid phosphatase promoter of the yeast expression vector pAM82. We have also constructed the recombinant CaMV in which an intron 2 of genomic phaseolin gene (176 bp) is inserted into ORF VII region. 18 days of postinfection with the recombinant CaMV virus, all of the replicated viral DNA molecules isolated from the virus infected plants completely lacked the intron 2. The deletion of the intron 2 occured at the exact splicing site. From these results of *in vitro* and *in vivo*, it can be concluded that CaMV replicates through RNA as intermediate by the help of CaMV coded reverse transcriptase.

J106 **Integration and Expression of Viroid cDNAs in Plant Cells.** J. M. Jaynes[1], Y. C. Tian[2], J. Pickard[3], and H. E. Flores[3]. Academia Sinica, Beijing (2), Departments of Biochemistry (1) and Plant Pathology & Crop Physiology (3), Louisiana State University, Baton Rouge, LA 70803.

Single and multimeric cDNA copies of full length and defined regions of the Potato Spindle Tuber Viroid (PSTV) were fused to the Cauliflower Mosaic Virus 19s promoter found in the plasmid pMON237. These vectors were introduced into the disarmed *A. tumefaciens* strain B6S3SE via triparental mating techniques. Tobacco leaf disks were transformed with the above strains after the method of Horsch et al. (1985). Approximately 80 plants were recovered from shooting medium containing 500ug/ml kanamycin. Representative samples from the regenerated kanamycin resistant plants were screened for integration and expression of the various PSTV cDNAs by southern and Northern analysis. Results have been positive by both criteria in six out of six plants analyzed. Screening for PSTV cDNAs in various organs and in the progeny of the regenerated plants is currently underway.

J107 EXPRESSION OF BEAN GOLDEN MOSAIC VIRUS AND USE OF PROMOTER SEQUENCES IN CHIMERIC CONSTRUCTS, Jean C. Kridl, Calgene, Inc., Davis, CA 95616

Bean golden mosaic virus is a geminivirus with a two component, circular, single-stranded DNA genome. The complete nucleotide sequence of both components was previously determined (Howarth et al., 1985, Proc. Natl. Acad. Sci. USA 82,3572-3576). The sequence revealed six major open reading frames (ORFs), conserved in size and location with other geminiviruses, and arranged on both the viral strand and the complementary strand. Evidence for bidirectional transcription has been obtained using strand-specific probes for the ORFs. Five major viral-specific, polyadenylated transcripts have been identified; three transcripts specific to DNA 1, one in the viral sense orientation and two in the complement, and two transcripts specific to DNA 2, one in each orientation. The cap sites for the two transcripts in the viral sense orientation for both DNA 1 and DNA 2 have been located by S1 mapping and primer extension. 5'-upstream sequences from several of the ORFs have been shown to function as promoters in chimeric constructs utilizing the neomycin phosphotransferase II coding region from the bacterial transposon TN5 and a 3' polyadenylation signal from the T-DNA tml sequence. mRNA specific for the chimeric constructs has been detected in *Agrobacterium tumefaciens* induced galls on both bean (*Phaseolus vulgaris* var. Golden Wax) and tomatoes (*Lycopersicon esculentum*).

J108 CLONING OF ALFALFA MOSAIC VIRUS COAT PROTEIN GENE AND ANTI-SENSE RNA INTO A BINARY VECTOR AND THEIR EXPRESSION IN TRANSFORMED TOBACCO TISSUE, L. Sue Loesch-Fries, E. Halk, D. Merlo, N. Jarvis, S. Nelson, K. Krahn, and L. Burhop, Agrigenetics Corp., 5649 East Buckeye Road, Madison, WI 53716

A phenomenon, termed cross-protection, occurs with strains of many plant viruses. It does not always provide complete immunity; the challange virus may replicate to a limited degree, but symptoms are reduced or inapparent. For example, alfalfa mosaic virus (AMV)-infected plants are not susceptible to infection by a second (challange) strain of AMV. One or more viral gene products are likely to be responsible for cross-protection. The gene products of AMV are proteins of 126K, 90K, 32K, and 24K (coat protein). The message for coat protein is contained in a monocistronic subgenomic RNA (RNA 4). Capped transcripts of RNA 4 synthesized from the pSP65 vector were biologically active in tobacco and alfalfa protoplasts. Antisense RNA 4 transcripts synthesized from the pSP64 vector blocked virus RNA infection of protoplasts. Eukaryotic promoter and polyadenylation sites were cloned next to RNA 4 cDNA. Tobacco tissue was transformed by a binary vector containing the promoter-gene or antisense gene-polyadenylation site. Regenerated plants are being tested for the synthesis of sense and antisense RNA 4 and for expression of coat protein. The analysis of regenerated plants will allow us to determine the role of coat protein in cross-protection and symptomology of AMV.

J109 HETEROLOGIES BLOCK GENE CONVERSION IN TURNIP PLANTS, Ulrich Melcher, Valerie Vaden, XuShao Zhang and Richard C. Essenberg, Biochemistry Department, Oklahoma State University, Stillwater OK 74078

Cauliflower mosaic virus (CaMV) produces virion DNA in turnip plants by a process that includes reverse transcription. Nevertheless, CaMV DNAs in nuclei of infected cells exchange genetic information by general recombination. Among the manifestations of general recombination is gene conversion. The DNA of UM130, an infectious derivative of the cabbage S (CabbS) isolate of CaMV, differs from CabbS DNA in the replacement of a 2bp sequence in open reading frame (ORF) III with a 14bp sequence containing an EcoRI site. CaMV DNA extracted from diseased plants inoculated with a mixture of UM130 and CabbS DNAs or virions lacked the UM130-specific EcoRI site. Gene conversion at the EcoRI site was also detected when the CabbS DNA was given a competitive disadvantage by the introduction of a lethal mutation in ORF VI. In contrast, isolates W, CM4-184 and NY8153 whose DNA sequences are about 95% homologous with the sequence of CabbS DNA failed to convert the UM130-specific EcoRI site. Isolates that contained recombined DNA derived from CabbS and W DNAs also failed to convert the UM130-specific EcoRI site. Heterologous sequences may prevent gene conversion by interfering with branch migration during recombination.

J110 PLANT VIRUS-VECTOR INTERACTION: REGULATION OF WOUND TUMOR VIRUS GENE EXPRESSION IN CULTURED LEAFHOPPER CELLS, Donald L. Nuss and Andrew J. Peterson, Roche Institute of Molecular Biology, Nutley, NJ 07110 and Wadsworth Center for Labs and Res., Albany, N.Y. 12201

The interaction between a plant virus and its insect vector was studied at the molecular level by examining wound tumor virus gene expression in cultured cells (line AC-20) derived from its leafhopper vector. Infection of vector cells by wound tumor virus is noncytopathic having an acute (through day 5) and a persistent (beginning with the first cell passage) phase. Viral-specific polypeptide synthesis and viral genome RNA accumulation increased to a maximum level during the first 5 days following inoculation and then decreased to 5% of the level observed during the acute phase by passages 10-15. In contrast, viral specific mRNAs were present at approximately the same level in the acute phase and in the early stage (passage 10) of the persistent phase of infection. Viral transcripts isolated from infected cells at different times postinoculation exhibited identical electrophoretic migration patterns but different functional activities in cell-free translation systems. Transcripts isolated from persistently infected cells were inefficiently translated *in vitro* reflecting the situation observed in infected cells. The results suggest that the "down regulation" of viral polypeptide synthesis associated with the persistent phase of wound tumor virus infection is a consequence of changes in the functional activity of viral transcripts.

J111 EVALUATION OF A NOVEL GEMINIVIRUS FOR CROP IMPROVEMENT IN POTATOES.
Eulian J F Roberts, I T D Petty, R H A Coutts and K W Buck, Department of Pure and Applied Biology, Imperial College, London, England.

Investigation of a novel whitefly-transmitted virus isolated from potatoes grown in Venezuela has shown it to be a member of the geminivirus group. Mosaico amarillo de la papa (potato yellow mosaic virus, PYMV) has been mechanically inoculated onto *Lycopersicon sp.*, *Nicotiana sp.* and *Petunia sp.*. However, PYMV cannot be mechanically transmitted to potatoes. We have found that graft transmission from infected tobacco scions to healthy potato stocks can be used to initiate infection.

The genome of PYMV consists of circular single-stranded DNA which cross-hybridizes to the cloned DNA of another South American geminivirus, (TGMV). However, we have not found any serological relationship between TGMV and PYMV.

Double-stranded (ds) DNA forms have been isolated from PYMV-infected plants and are similar to those characterised in other geminiviral infections. We anticipate that gene vectors based on PYMV dsDNA species will facilitate the analysie of potato genes in homologous tissue culture systems.

J112 Host response to cauliflower mosaic virus (CaMV) in solanaceous plants is determined by a 496 bp DNA sequence within gene VI. J. E. Schoelz, R. J. Shepherd, and S. D. Daubert. Department of Plant Pathology, University of Kentucky, Lexington KY 40547.

Most strains of CaMV are limited to species of the cruciferae in host range. Some CaMV strains replicate in, and cause hypersensitive reactions on, a few solanaceous species. Recently an atypical strain, CaMVD4, has been found which causes systemic infections in Datura stramonium and Nicotiana bigelovii. In the present study, recombinant virus genomes were constructed between CaMVD4 and ordinary CaMV strains to determine which portion of the genome controlled the disease reaction on solanaceous plants. A 496 bp fragment consisting of the first half of the coding region of gene VI was found to control the type of host response (localized hypersensitivity versus systemic chlorotic mottle) in D. stramonium or N. bigelovii. A DNA sequence comparison between CaMVD4 and the incompatible strain CM1841 revealed that within the 496 bp DNA segment there were 17 sequence differences which resulted in amino acid exchanges.

J113 SMALL PATHOGENIC RNAS ASSOCIATED WITH TURNIP CRINKLE VIRUS. Anne Simon and Stephen Howell, Dept. of Biology C-016, University of California at San Diego, La Jolla, CA 92093.

Satellite RNAs are small pathogenic RNAs, infectious only in the presence of a helper virus. We are studying the satellite associated with turnip crinkle virus (STCV), which intensifies the symptoms of TCV when co-inoculated on turnip leaves. Several smaller RNA species which share homology with STCV are also present in infected leaf tissue. Two of these RNAs, RNA F and RNA D, are apparently also satellite RNAs which can replicate independently of STCV. The results from cloning and sequencing of STCV suggest: 1) STCV is a heterogeneous population of sequences approximately 356-359 bases. 2) nearly half of STCV is homologous (92%) with the 3' untranslated end of TCV and 3) since the intensification of symptoms due to STCV may be attributed to a dosage effect of TCV 3' homologous sequences since neither RNA D or F share homology with TCV nor alter symptom expression of TCV. We also present evidence on the successful infection of turnip with TCV and *in vitro* synthesized STCV RNA.

J114 IMMUNOBLOT DETECTION OF CITRUS TRISTEZA VIRUS SPECIFIC ANTIGENS FROM FIELD ISOLATES. Christopher M. Smith and Roger A. Acey, Department of Chemistry, California State University, Long Beach, Long Beach, CA 90840.

While citrus tristeza virus (CTV) has been detected in Southern California, little is known of the extent and/or severity of the disease. More importantly, a simple diagonstic test for distinguishing the common CTV complex (often symptomless) from the more virulent seedling yellow strain does not exist. However, serological characterization of the various strains of CTV should be critical in devising a simple and reliable assay for these pathogens. CTV was immunoprecipitated from field isolates with goat anti-CTV IgG and the proteins fractionated on 8-16% gradient SDS polyacrylamide gels. Subsequently, proteins were electrophoretically transfered to nitrocellulose (NC). The NC was blocked with 3% gelatin and incubated with rabbit anti-CTV IgG. Immune complexes were visualized by horseradish peroxidase conjugated goat IgG directed against rabbit IgG. Five distinct antigens were present in extracts from CTV infected tissue. The most prominent antigen had an apparent molecular weight of 23 Kd. Other antigens ranged in size from 18 to 50 Kd. Similar results were obtained with whole tissue homogenates or TCA precipitable material from whole homogenates. These antigens were not detected in extracts from healthy tissue or tissue known to harbor other viruses or viroids. Experiments in progress involve determination of the serological differences between the common and seedling yellow strains of CTV. This project was supported by a contract from the California Department of Food and Agriculture.

J115 REPLICATION OF CUCUMBER MOSAIC VIRUS SATELLITE-RNA IN VITRO, Nevin D. Young, Peter Palukaitis, and Milton Zaitlin, Cornell University, Ithaca, NY 14853.

The satellite-RNA of cucumber mosaic virus (CMV-Sat) is a small, single stranded RNA molecule of approximately 340 nucleotides which is completely dependent upon its helper virus for replication and encapsidation. We have developed an in vitro system, consisting of a crude, membrane-associated enzyme fraction, which is capable of template-independent synthesis of both CMV-Sat-specific replicative structures and those of the genomic RNAs of CMV.

The major in vitro-synthesized CMV-Sat-specific structures are: 1) a stable RNA species which comigrates (on non-denaturing agarose gels) with double-stranded (ds) monomeric CMV-Sat, 2) a rapidly turned-over RNA species which migrates to the position expected for ds-dimeric CMV-Sat, and 3) a heterodisperse RNA species which migrates between the putative ds-monomer and ds-dimer. Both the putative ds-dimer and the heterodisperse RNA species are converted to ds-monomer by treatment with RNase A.

We are currently analyzing these CMV-Sat replicative structures by denaturing and partially denaturing gel electrophoresis and by RNA-fingerprinting. In addition, we are pursuing experiments, based on preliminary results, which indicate that these replicative structures are capable of limited self-processing.

Herbicides: Modes of Action and Mechanisms of Resistance

J116 AN IMIDAZOLINONE AND SULFONYLUREA TOLERANT MUTANT OF CORN, Paul C. Anderson and Marcy Georgeson, Molecular Genetics Inc., 10320 Bren Road East, Minnetonka, MN 55343

The imidazolinones and sulfonylureas are potent new families of herbicides capable of controlling broad spectra of weeds in agronomic crops. Both families of compounds are active at low application rates and act by selectively inhibiting the activity of acetohydroxyacid synthase (AHAS), the first enzyme involved in the biosynthesis of leucine, valine, and isoleucine. However, these compounds cannot be used with some crops such as corn because of a high degree of crop sensitivity.

Cell culture selection techniques were used to identify a corn somaclonal variant expressing greater than 100 fold tolerance on an herbicide concentration basis. The selected cell line showed tolerance toward both the imidazolinones and sulfonylureas. The tolerance was accompanied by a decrease in the herbicide sensitivity of the AHAS activity of the cell line, suggesting an alteration of the herbicides' site of action. Feedback regulation of branched chain amino acid biosynthesis was not significantly altered.

Herbicide tolerance was shown to be expressed in regenerated plants and their progeny. Segregation of the tolerance trait in progeny of self pollinated and outcrossed plants indicated that it is encoded by a single dominant gene. Plants homozygous for the trait showed greater than 100 fold tolerance of the imidazolinones tested. The trait was found to be expressed well under field growth conditions.

J117 DETOXICATION OF THE XENOBIOTIC SALICYLIC ACID BY AN INDUCIBLE GLUCOSYLTRANSFERASE, Nelson E. Balke, University of Wisconsin, Madison, WI 53706

Phenolic acids, such as salicylic acid, are known to inhibit several physiological processes in plants leading to reduction in growth and to phytotoxicity. Previously, salicylic acid has been shown to inhibit the absorption of mineral ions by plant roots. This inhibition is concentration-, time-, and pH- dependent, so that the absorption of K+ is inhibited to varying degrees depending upon the amount of salicylic acid absorbed by the tissue. At nonlethal rates, salicylic acid induces a glucosyltransferase in oat (Avena sativa). This enzyme catalyzes the transfer of glucose from UDP-glucose to salicylic acid and thus is a UDPG:salicylic acid glucosyltransferase (EC 2.4.1.35). A lag phase of 5-7 hr precedes appearance of the enzymatic activity in excised roots. Induction is inhibited by cycloheximide or puromycin but not chloramphenicol. Transfer of tissue from solution containing salicylic acid results in decay of the glucosyltransferase activity. The enzyme can be re-induced at a faster rate upon transfer of the tissue back into solution containing salicylic acid. Our working hypothesis is that the induced glucosyltransferase detoxifies salicylic acid by glucosylating the compound. Currently, a sufficient quantity of the metabolite is being purified to test that hypothesis.

J118 MOLECULAR MECHANISMS INVOLVED IN THE APPEARANCE OF ATRAZINE RESISTANCE IN CHENOPODIUM ALBUM, P. Bettini, M. Sévignac, S. Mac Nally, H. Darmency, J. Gasquez and M. Dron Lab. Biol. Mol. Végét., Bât. 430, Uni. Paris Sud 91405. Orsay. France

Weeds resistant to Atrazine are now widely distributed among the world. Atrazine resistance is related to a single substitution in the chloroplast psbA gene of higher plants, always at the same position. These data have arisen questions about the selection of such a mutation.

We have used Chenopodium album plants for which resistance has appeared suddenly after selfing without any Atrazine selection pressure. Molecular analysis of the psbA gene of hese plants has demonstrated that the mutation can be selected without presence of the herbicide. We demonstrate also that the mutation is not sufficient to induce high levels of Atrazine resistance, other unknown mechanisms are surely necessary to obtain actual herbicide resistance.

We propose a model in which a recessive nuclear mutator will be responsible for chloroplast psbA mutation when plants are homozygous for it.

J119 GLYPHOSATE INDUCES ACTIVATION OF 3-DEOXY-D-ARABINO- HEPTULOSONATE 7-PHOSPHATE (DAHP) SYNTHASE IN POTATO CELLS, William E. Dyer, Jose E. B. P. Pinto, and Klaus M. Herrmann, Purdue University, W. Lafayette, IN 47907.

Glyphosate (N-phosphonomethylglycine) is a non-selective herbicide which inhibits 5-enolpyruvylshikimate 3-phosphate synthase, the penultimate enzyme of the shikimate pathway in higher plants and microorganisms. Carbon flow through the shikimate pathway of Escherichia coli is exclusively controlled by the activity of the first enzyme of the pathway, the DAHP synthase. The activity of this enzyme in cultured potato and carrot cells varies through the growth cycle (Suzich et al. (1984) Plant Physiol. 75, 369-371). The specific enzyme activity reaches a maximum in the linear growth phase, indicating a regulatable DAHP synthase. When potato cells were exposed to a sublethal dose of glyphosate, the specific activity of DAHP synthase increased about 3-fold within 24 h compared to the activity in untreated cells. Glyphosate treatment does not cause an increase in the second enzyme of the pathway, the dehydroquinate synthase. Western blots of extracts from glyphosate-treated cells show that the increase in specific activity is due to de novo enzyme biosynthesis. In separate experiments, potato cells were incubated with ^{32}P inorganic phosphate during glyphosate treatment. Enzyme activity eluted from phosphocellulose was associated with higher levels of ^{32}P labelling, indicating a posttrans iptional modification of the enzyme. We are currently investigating the molecular basis of the DAHP synthase activation.

J120 INTERACTION OF HERBICIDES AND QUINONE WITH THE Q_B BINDING PROTEIN IN THE DIURON RESISTANT CHLAMYDOMONAS reinhardtii MUTANT Dr2., Phil Haworth and Katherine E. Steinback, Zoecon Corp. Palo Alto, CA 94304 and Advanced Genetic Sciences, inc., Oakland, CA 94608.

Resistance to herbicides which inhibit photosynthetic electron transport has now been noted in a number of photosynthetic organisms. In every case, resistance has been correlated with changes in the structure of the 32-kD Q_B-protein; this protein binds a special plastoquinone which serves as the second stable electron acceptor on the reducing side of photosystem II. The form of resistance most extensively characterized to date is extreme triazine resistance in higher plants. Based on analysis of the chloroplast gene (psbA) encoding the resistance trait, it is known that a single amino acid change (ser 264 to gly) is responsible for triazine resistance. In the Chlamydomonas mutant, Dr2, a 17-fold resistance to diuron has been noted. A single amino acid change is also responsible for this resistance, but is remotely located on the 32-kD protein (val 219 to ile). Studies were carried out to establish a correlation between this amino acid change and the nature of herbicide resistance and cross resistance observed in this mutant. Phototrophic cells of Dr2 show approximately 50-fold resistance to diuron in liquid culture as compared to the wild-type strain (2137c+). Chloroplast membranes isolated from Dr2 demonstrate resistance to several herbicide classes. However, competitive binding studies with 14C-atrazine and diuron indicate that no change in the binding constants for either herbicide occurs in this mutant. The data strongly suggest that diuron resistance in Dr2 does not arise from an altered interaction between the herbicide and Q_B-protein, but rather from a change in the interaction between the herbicide and the plastoquinone at the qinone binding site.

J121 MANIPULATION OF ANTIOXIDATIVE SYSTEMS: CREATING HERBICIDE RESISTANCE IN PLANTS
Arno Schmidt and Karl J. Kunert, University of Konstanz, Konstanz, West Germany.

Peroxidation of polyunsaturated fatty acids is one of the most deteriorative reactions that can damage biomembranes of plant cells. Among the phytotoxic compounds that can initiate lipid peroxidation in higher plants in the light, herbicides, such as p-nitro-diphenyl ethers and paraquat, have been identified. Protection against the harmful oxidant reactions seems to be provided by antioxidants like the primary lipid-soluble antioxidant vitamin E that is regenerated by the water-soluble antioxidants vitamin C and glutathione. We expect improvment of resistance against herbicide-induced peroxidation by manipulation of either the concentration of reduced antioxidants present in plants or of the acceleration of enzymatic reduction of oxidized antioxidants.

An increased amount of vitamin C produced by biotransformation from L-galactono-1,4-lactone or reduced glutathione limits significantly herbicide-induced peroxidation and degradation of cellular phenolic compounds, such as the primary antioxidant vitamin E, in cotyledons of morning glory. The degree of herbicide-induced peroxidation in plants is further dependent on the ratio of reduced glutathione to the oxidized form that is controlled by the enzyme glutathione reductase. A higher level of the enzyme should significantly improve protection against peroxidative herbicides. First results about cloning and transformation of the enzyme will be presented.

J122 FUSION-MEDIATED TRANSFER OF TRIAZINE RESISTANCE: CHARACTERIZATION OF TOBACCO PLANTS WITH RESISTANT NICOTIANA PLUMBAGINIFOLIA PLASTIDS, Katherine E. Steinback, Laszlo Menczel, Lisa S. Polsby and Pal Maliga, Advanced Genetic Sciences, Inc., Oakland, CA 94608

A triazine resistant line of the weed species Nicotiana plumbaginifolia (TBR-2) obtained through a cell culture selection strategy (Mol Gen Genet, 200: 508, 1985) has reduced vigor and low fertility. It was therefore desirable to separate the plastid mutation that confers resistance from nuclear mutations that might affect fitness. A further goal was to introduce the plastid-encoded resistance trait into a crop species, N. tabacum. Protoplasts isolated from TBR-2 were x-irradiated, then fused with protoplasts from an albino plastome mutant (SR1-A15) of N. tabacum. Colonies were plated on RMOP medium; green colonies formed only if fusion between TBR-2 and SR1-A15 protoplasts had occurred and if TBR-2 plastids remained viable. Plants were regenerated from green colonies; those with a N. tabacum nuclear background were distinguished from somatic hybrids by morphology and chromosome number. In greenhouse tests, regenerated N. tabacum plants demonstrated extreme triazine resistance, whereas applications of 1 kg/ha atrazine proved lethal to control plants. Analysis of fluorescence induction in vivo and photosynthetic electron transport in vitro confirmed that the regenerates were triazine resistant to the same degree as TBR-2. However, the novel tobacco lines exhibited slower growth than herbicide sensitive tobacco plants containing either N. tabacum or N. plumbaginifolia plastids. The reduced vigor in triazine-resistant lines may therefore be due to a contribution from the plastid genome and is associated with the resistance trait itself.

J123 SOME FACTORS AFFECTING BIOLOGICAL DEGRADATION OF SOME HERBICIDES IN SOIL, IN EGYPT. Mohsen S. Tadros; Plant Protect. Dept., Fac. of Agric., Tanta Univ., Kafre El-Sheikh, Egypt.

Herbicide degradation in soil may be either chemical, and/or biological in nature. Since herbicides have mostly an indirect effect on soil organisms due to a probable direct effect on soil microflora, a study, that lasted for approximately one year, was conducted to evaluate the effect of six compounds on soil microfauna, soil microflora and earthworms. The degradation pathways of the applied compounds were discussed from a biological point of view and included, volatization, leaching, run-off, plant sorption, biological and chemical decomposition. Some other important ecological factors were also under consideration, i.e., soil type espically organic matter and moisture content, minerals and acidity and were found to affect the persistance of the applied compounds.

J124 CONTRIBUTIONS OF α-KETOBUTYRATE ACCUMULATION TO SULFONYLUREA HERBICIDE TOXICITY, Tina K. Van Dyk and Robert A. LaRossa, E. I. du Pont de Nemours and Company, Experimental Station, Wilmington, Delaware 19898 USA

The herbicide sulfometuron methyl (SM) is a potent inhibitor of acetolactate synthase (ALS), an enzyme of branched chain amino acid biosynthesis. In addition to limiting isoleucine, leucine and valine biosynthesis, SM inhibition of ALS in Salmonella typhimurium results in the accumulation of the ALS substrate α-ketobutyrate (AKB). The role of AKB accumulation in SM toxicity was examined by analysis of S. typhimurium mutants hypersensitive to SM. Three classes of SM-hypersensitive mutants were deficient in AKB consumption by enzymes other than ALS. Another mutant, which degraded AKB at a normal rate, lacked the enzyme aspartate aminotransferase. In this mutant, the SM-induced accumulation of AKB resulted in an aspartate limitation, possibly by limiting flux through glycolysis and the TCA cycle, thereby lowering the level of the aspartate precursor, oxaloacetate. These mutants demonstrate that AKB accumulation is an important element in sulfonylurea herbicide toxicity and that AKB accumulation affects pathways other than those of branched chain amino acid biosynthesis.

J125 ANALYSIS OF MAIZE AND PETUNIA HSP70 GENES IN TRANSGENIC PLANTS. Jill Winter, Dean Rochester, Robert Fraley, Dilip Shah, Monsanto Company, 700 Chesterfield Village Parkway, Chesterfield, MO 63198

Previously, we reported the cloning of two maize hsp70 genes, each containing an intron and each thermally induced. The thermal induction is 40-60 fold, which is particularly interesting due to the similarity between these maize genes and the non heat-inducible cognate genes from two other systems (*drosophila* and human). We have constructed a hybrid gene from the two maize genes and transferred the hybrid gene to petunia. The expression of this maize hsp70 gene in regenerated petunia plants will be discussed, as well as experiments to determine whether the transcript (which is the same size as that in maize) is initiated and processed in petunia as it is in maize. Additionally, we have a cDNA to the petunia hsp70 and are currently characterizing the hsp70 genes of petunia.

Controlling Insect Predation on Plants and Plant-Insect Interactions

J126 Root glycoproteins and Pseudomonas putida colonization, Anne J. Anderson, Pouran Tari, Frederick Albert and Beverley Graetz, Utah State University, Biology Department, Logan, UT 84322-4500.

Pseudomonas putida, a suppressant of certain root pathogens, is an aggressive colonizer of plant roots. Interaction of P. putida with two root surface factors is demonstrated. Colonization of bean roots by P. putida alters the isozymic composition of peroxidase detected in root washes. Prepartions from sterile bean roots yield an anodic pair of peroxidase bands upon native PAGE. Additional less anodic bands are observed in washes of P. putida-colonized roots. Another root surface glycoprotein agglutinates P. putida cells in the presence of Mg^{2+}. This agglutinin may promote attachment of P. putida cells to the root surface. Release of P. putida cells is enhanced 6-10 fold when 5 mM NaEDTA (pH 6.5) rather than water or 5 mM Na acetate (pH 6.5) is used to wash colonized roots. Tn^5 and Tn^9 mutants of P. putida, which were not agglutinable by the root glycoprotein, have reduced colonizable abilities. Other Tn^5 mutants that retain agglutinability colonized at parental levels.

J127 A BIFUNCTIONAL PLANT GENE: INSECTICIDE FUSED TO KANAMYCIN RESISTANCE

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We have found that the second half of the Bacillus thuringiensis toxin gene is dispensable for the expression of an active insecticide. Not only may it be deleted, the second half of the gene may be replaced by the codons of NPTII kanamycin resistance from Tn5, and both activities are expressed. When this gene fusion is made in the wrong reading frame, the kanamycin resistance is not expressed, indicating that read-through from the Bt toxin gene is responsible for expression in the in-frame fusion.

We have tailored transcriptional control signals from the T-DNA of pTiT37 to insert this fused gene in place of the nopaline synthase codons adjacent to the right border signal from T-DNA. In so doing we have retained, in a tandem, in-frame arrangement, the translational start signals from both the bacterial Bacillus thuringiensis gene and the nopaline synthase gene. The resulting gene expresses both insecticidal and kanamycin resistance activity (the latter to 200 ug/ml) in Agrobacterium tumefaciens. In plants, this gene should also express the insecticide and kanamycin resistance from the same promoter. Whether or not the two halves of the fused gene remain together at the level of gene product, selection for kanamycin resistance should ensure the expression of the insecticide, and could also be used to select for higher levels of expression.

J128 DESTRUCTION OF INSECT CYTOCHROME P-450 BY CYTOPLASMIC POLYHEDROSIS VIRUS, Lena B. Brattsten, E. I. du Pont de Nemours & Co., Experimental Station, 402/5237, Wilmington, DE 19898

A laboratory colony of Heliothis virescens was infected with cytoplasmic polyhedrosis virus. Cytochrome P-450 activities in the larvae decreased considerably and there was a loss in resistance to carbamates and other insecticides. Glutathione transferase activities were not affected and carboxyesterase activities decreased less than those of cytochrome P-450. Guts of infected larvae had an opaque appearance and electron micrographs showed disappearance of the endoplasmic reticulum membranes from their midgut columnar cells.

J129 PLANT PROTEIN QUALITY: ITS EFFECT ON INSECT GROWTH AND THE TOXICITY OF PLANT PROTEINASE INHIBITOR. Roxanne M. Broadway, Gary W. Felton and Sean S. Duffey, University of California, Davis, CA 95616.

The chemical aspects of the interaction between plants and insects has, primarily, been examined by identifying and/or quantifying phytochemical(s), and then determining the effect(s) of these phytochemical(s) on insects. It is implicitly assumed that individual phytochemicals act independently, according to their quantity, upon insects. This assumption ignores the potential interactivity between phytochemicals, which may profoundly modify biological activity. We present evidence that the quality of plant protein is a factor which significantly influences the antinutritional effect of plant proteinase inhibitors against certain phytophagous insects. When comparing the growth of larval Spodoptera exigua (Lepidoptera: Noctuidae) on diets containing soy protein, corn gluten, corn gluten/zein or casein (control), larval weight gain is significantly affected by the type of plant protein ingested. When soybean trypsin inhibitor is co-ingested with a protein by the larvae, the antinutritional effect of the inhibitor is, in general, inversely related to the nutritional value of the protein (i.e. a more nutritious protein reduces the effect of the inhibitor to a greater extent than a less nutritious protein). Thus, in theoretical terms, the quality of plant protein may be a central factor in determining the toxicity of a variety of other phytochemicals to phytophagous insects.

J130 How Heliothis spp. Complex Achieves Major Pest Status in Cotton

Bock G. Chan, Noreen E. Mahoney and Jack B. Fraser
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Heliothis spp. are polyphagous, generally feeding on plant reproductive organs. Heliothis zea and H. virescens became major economic pests on cotton after heavy usage of insecticides against cotton pests decimated predaceous insect populations. Heliothis/cotton interactions were studied with isolated and identified allelochemicals from cotton. They are gossypol and its analogues, cyclopropenoids and flavonoids (flavanols, flavonols and condensed tannins). Their distribution and concentration were measured in various reproductive organs of different developmental stages. Biological activities were measured in ED_{50} for antibiosis by incorporation into an artificial diet and FD_{50} for feeding deterrence with our cotton cotyledon preference assay.

Cotton contains a large array of defense chemicals. A model is offered for how Heliothis spp. overcame the cotton defense. Avoidance mechanism is crucial to their success, i.e. the most vunerable first instar larvae feed on the floral primordial tissues which are generally devoid of secondary plant products. Later instar larvae, which are more tolerant to allelochemicals, feed less discriminately. The significance of a defense compound is therefore dependent on its presence in the proper concentration at the feeding site of a susceptable life stage of the pest.

J131 POLYPHENOL OXIDASE MODULATION OF CHLOROGENIC ACID TOXICITY TO INSECT LARVAE. G.W. Felton, R.M. Broadway & S.S. Duffey. University of California, Davis, CA.

We are attempting to utilize o-dihydroxyphenolics as an antibiotic basis of resistance of the tomato plant against specific insect pests. A general assumption about chemical defense of plants against insects is that toxicity of a given phytochemical is independent of the chemical milieu. We present evidence that interaction of plant polyphenol oxidase (PPO) and plant protein with these phenolics profoundly affects their potential to serve as a basis of host-plant resistance.

Chlorogenic acid (CHA), an o-dihydroxyphenolic, when added to diet at levels found in foliage, inhibits the growth of several larval insects. However, tomato foliage also contains high levels of PPO activity which can rapidly oxidize CHA to chlorogenoquinone when the leaf is damaged by insect feeding. This quinone is an alkylator of free primary amines (e.g. ε-NH_2 of lysine); this alkylation can result in reduced nutritive quality of protein. We found that when CHA is co-ingested with PPO, the toxicity of CHA to a larval insect is dependent upon the quantity of protein (total # of free NH_2's) but also upon protein quality (# of NH_2's/mole of protein). Low quality and/or quantity resulted in increased CHA toxicity; whereas increased quantity and/or quality of protein alleviated CHA toxicity.

Our results suggest that the chemical context of CHA must be specified before its toxicity can be predicted. Hence, in breeding for resistance to insects based on CHA, multiple sets of interactive plant factors must be considered. These results should be extrapolatable to other plant/insect crop systems.

J132 AgNPV Replication in Cell Cultures and Physical Map of its DNA. Maruniak, J.E., Johnson, D. and Gowan, D. Univ. of Florida, Gainesville, FL 32611.

Anticarsia gemmatalis nuclear polyhedrosis virus was serial plaqued three times in IPLB-Sf21 cells. When the DNAs from the plaque-purified isolates were compared with PstI and HindIII, six different genotypic profiles were detected. The prototype isolate, AgNPV-2 comprised one-third of the 24 original plaques.

The extracellular virus of the six genotypic variants was titered in microtest wells using four cell lines: TN368, IPLB-Sf21, IAL-PiD and Hz-AM. The AgNPV variants replicated to the highest titer in IPLB-Sf21 cells. They replicated poorly in Hz-AM cells. There were mostly minor changes in titers within a cell type for the AgNPV variants. However, there was up to 1.5 logs difference of infectious virus between AgNPV-1 and AgNPV-2 in IAL-PiD cells.

AgNPV-2 DNA was restricted with HindIII, KpnI, EcoRI, BglI, BamHI, BglII, and PstI and electrophoresed in a 0.75% agarose gel. The genome size was about 110 kilobases. A physical map of the BamHI, BglII, BstEII, and PstI fragments was generated. The map was constructed by the criss-cross hybridization method, probing DNA fragments on blots with labeled individual fragments and by double digestion of selected fragments. To determine the probable location of the AgNPV-2 polyhedrin gene, cloned AcNPV-2 PstI-D, containing the polyhedrin gene, was labeled, and it hybridized to AgNPV-2 BamHI-B, BglI-B, BstEII-A, and PstI-C. Therefore, since the polyhedrin gene is conserved in baculoviruses, the AgNPV-2 polyhedrin gene is likely within these fragments.

Late Additions

J133 PROCESSING OF T-DNA DURING AGROBACTERIUM TUMEFACIENS INFECTION. Lisa M. Albright, Deqin Ma, Bernard Leroux, Martin F. Yanofsky, Milton P. Gordon, and Eugene W. Nester, Dept. of Microbiology, University of Washington, Seattle, Wa. 98195.

During infection by Agrobacterium tumefaciens, a portion of its Ti plasmid, the T-DNA, is transferred to its plant host. The mechanism by which this occurs is unknown. We have investigated processing of the T-DNA of pTiA6, using the technique of "Southern" hybridization with restriction enzyme-digested Agrobacterium DNA. Upon cocultivation of strain A136(pTiA6) with Nicotiana tabacum suspension culture cells, double-stranded cleavages of the Ti plasmid are seen. The locations of the cleavages that have been mapped are near the 25 base pair repeats of the T-L DNA. We are investigating the involvement of the Ti plasmid-encoded virulence functions in the cleavage events, and are also mapping the cleavage sites more precisely.

J134 A NOVEL STRATEGY FOR DEVELOPMENT OF NEMATODE RESISTANT TOMATOES, Willie H.-T. Loh, Stephanie A. Kut, and Herculano P. Medina-Filho, DNA Plant Technology Corp., 2611 Branch Pike, Cinnaminson, New Jersey 08077

The association between a variant isozyme pattern and resistance to root-knot nematode has been exploited as an early screening system in tomato breeding. Seedling populations are routinely screened for an acid phosphatase allele ($Aps\text{-}1^{1}$) closely linked with field resistance to Meloidogyne spp. Evidence for Mendelian segregation of both acid phosphate alleles is presented here.

A somaclonal variant, homozygous ($Aps\text{-}1^{1/1}$) for nematode resistance, was regenerated from tissue culture of heterozygous ($Aps\text{-}1^{1/+}$) plants. The variation may have been resulted from tissue culture induced mitotic recombination. A method to make small genetic changes in plants regenerated in vitro may be exploited as part of a breeding strategy to fine tune elite breeding lines for improved agronomic performance.

J135 MOLECULAR BASIS OF SPONTANEOUS RACE CHANGE MUTANTS IN XANTHOMONAS CAMPESTRIS PV. VESICATORIA, Jean Swanson[+], Brian Kearney[++], Douglas Dahlbeck[+] and Brian Staskawicz[+], Departments of Plant Pathology[+] and Genetics[++], University of California, Berkeley, CA 94720

Genetic resistance of pepper (Capsicum annuum) to strains of Xanthomonas campestris pv. vesicatoria (Xcv) containing the dominant avirulence gene ($avrBs_1$) is specifically controlled by the monogenic dominant locus Bs_1 in pepper (1). The $avrBs_1$ gene resides on a 200 kb self-transmissable copper plasmid (2) and was identified and cloned from isolated plasmid DNA in the wide host range cosmid vector, pLAFR3. The cloning and characterization of this avirulence gene provides definitive proof for the existence of a gene-for-gene system in this plant-bacterial interaction.

The durability of the Bs_1 locus is often jeopardized by races of Xcv containing the $avrBs_1$ gene that can spontaneously mutate to overcome genetic resistance at a high frequency. Spontaneous race change mutants were isolated from the Xcv strains E3-C and 81-23 by inoculating the near-isogenic resistant pepper cultivar 10R and isolating stable virulent mutants from individual lesions that appeared in four to five weeks. The introduction of the wild type $avrBs_1$ clone into these mutants resulted in complementation for avirulence, suggesting that the mutation was at this locus. The mutants were then analyzed by Southern analysis employing the $avrBs_1$ clone as a probe and genomic alterations were observed in some of the mutants. DNA from one mutant, 81-23 M-1, that displayed a genomic alteration in the avirulence gene locus, was isolated and a genomic library was constructed in pLAFR3. Two clones were isolated that hybridized to the wild type $avrBs_1$ clone and were shown to be the same genomic alterations as revealed by previous Southern analyses. Preliminary characterization of these clones suggests that a 1.5 kb insertion sequence is responsible for spontaneous race change mutants that overcome single gene resistance in pepper. We are currently further characterizing these mutants and testing the hypothesis that the insertion is a transposable element that inactivates avirulence by inserting into this locus at a high frequency.

1. Cook, A. and R. E. Stall, 1963. Inheritance of resistance in pepper to bacterial spot. Phytopathology 53:1060-1062
2. Stall, R. E., D. C. Loschke, and J. B. Jones. 1986. Copper resistance and avirulence loci are linked to a self-transmissable plasmid in Xanthamonas campestris pv. vesicatoria. Phytopathology (in press).

J136 GENETIC ENGINEERING AND HERBICIDE RESISTANCE IN PSII, Dexter Chisholm, J Williams, H Pakrasi, W Vermaas, B Diner and CJ Arntzen, Du Pont Experimental Station, Central Research and Development, Wilmington, DE 19898

Most of the herbicides that inhibit PSII function compete with plastoquinone for binding at the Q_B site, believed to reside on the 32-kdal D1 polypeptide. Sequence homologies between the D1 and D2 polypeptides of PSII and the L and M subunits of the bacterial reaction center, for which the crystal structure has been determined, suggest similar functions in binding of the reaction center chlorophylls and the electron carriers Q_A and Q_B. We are using genetic transformation and oligonucleotide-directed mutagenesis to alter certain amino acid residues in the D1 and D2 polypeptides of PSII in the cyanobacterium Synechocystis 6803. Several of these mutations are expected to affect the affinity of PSII for herbicides, and they should prove useful in elucidating the nature of the herbicide-binding site.